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► **Cover picture:** Pixtal

7th Tannin Conference (Presymposium) and 58th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Date/Location: **29th August – 2nd September 2010, Berlin, Germany**

Chairman: Professor Dr. Matthias F. Melzig
Professor Dr. Herbert Kolodziej

The 58th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research as well as the 7th Tannin Conference offered as presymposium will be held this year in Berlin, Germany. The congress venue is going to be the Henry Ford Building of the Freie Universität Berlin, which is well equipped to host such an important scientific event.

The objective of the 7th Tannin Conference (presymposium) is to promote further collaborations between chemists, biologists and human health related disciplines and to focus on expanded possibilities of polyphenols for their application in human health, nutrition, and the food industry. This meeting provides an opportunity for the members of the "tannin family" to discuss ideas with experts on herbal medicines and natural product chemistry and an exciting venue for GA participants to exchange scientific information.

The specific objectives of the 58th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research will be to promote dialogue and the exchange of medical practices and resources of modern and traditional nations. Alexander von Humboldt – born in Berlin and the foremost natural scientist of the early 19th century – was one of the pioneers of international scientific exchange. In 1828, he organised the first international scientific conference in Berlin, Germany, attended by about 600 participants from all over the world. This unique meeting was a model for many similar reunions in various countries in the following years. The focus was on the scientific exchange across borders – also addressed in the 58th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research.

Some selected main topics of the congress are:
– New analytical methods
– Authentication of plants and drugs/DNA-Barcoding/PCR profiling
– New targets for herbal medicines
– Enzyme inhibitors from plants
– Natural products for the treatment of infectious diseases
– Indigenous knowledge of traditional medicine and evidence based herbal medicine
– Biopiracy and bioprospecting

The programme of the Congress is offering nine invited lectures to be delivered by distinguished scientists, 60 short lectures which will be in parallel sessions and more than 650 poster presentations. In addition, seven workshops will be held on specific topics. As Presidents of the Congress, we are very happy that the scientific programme attracted so many scientists from approximately 70 different countries. Many thanks are most sincerely extended to Georg Thieme Verlag KG for the proper processing of the huge number of abstracts and to the agency CTW – Congress Organisation Thomas Wiese GmbH for organizing this Congress. We also express our gratitude to all members of the Scientific Committee who acted as reviewers and contributed to the good level of scientific quality of this abstract volume.

We hope that everybody will enjoy their stay in Berlin, an attractive venue with international atmosphere and culture.

Prof. Dr. Herbert Kolodziej
Co-ordinator of the 7th Tannin Conference and
President of the 58th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Prof. Dr. Matthias F. Melzig
President of the 58th International Congress and Annual Meeting
of the Society for Medicinal Plant and Natural Product Research

7th Tannin Conference

Lectures

TC-1 Stereochemical structure determination of complexes of tea catechins and caffeine

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Catechins and caffeine are included in the leaves and buds of the tea plant as major ingredients, and have various bioactivities. Interestingly, it is known that catechins form complexes with caffeine, especially in black tea and coffee. We have investigated stereochemical structures of the complexes of various tea carechins and caffeine by X-ray crystallography analysis. (−)-Epicatechin (EC) of the non-gallated catechin and (−)-epicatechin-3-*O*-gallate (ECg) of the gallated catechin, which are included in Japanese green tea as the major catechins, formed a 1:1 and 2:4 complexes with caffeine, respectively. The serious difference between the forming modes of the two complexes occurred owing to the presence of the galloyl group. The π-π complexation site of EC with caffeine was only the A ring, whereas that of ECg was the all aromatic rings, the A, B, and B′ rings.

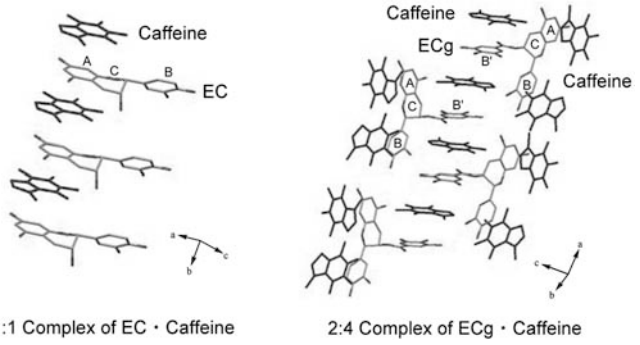

Fig. 1

TC-2 Tannin conformation in solution: effects of tannin structure, solvent quality and oxidation

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Condensed tannins play an important part in the colour and taste of plant-based food. Their main properties derive from their oxidizability and their capacity to develop interactions with other biopolymers. Due to their chemical reactivity, tannins are not stable once extracted from plants. Various chemical reactions take place, leading to structural changes of the native structures to give so-called derived tannins and pigments. These changes likely have an impact on tannins physicochemical properties, including their propensity to interact with other biopolymers. These past years, we have focused on tannin characterization using scattering techniques (light, X-rays) to get information on native tannins structures and changes induced by their oxidation. Indeed, monomeric structures are now well identified, but less is known about the macromolecular structures (dimensions, conformation in solution) of more polymerized species. We found that native tannins in good solvents can be described as thick and relatively flexible chains, provided they are long enough. Upon oxidation, new (macro)molecules were formed. When oxidation was performed at high concentration (e.g. 5 gL^{-1}), the weight average degree of polymerization determined from SAXS increased. This shows that some reactions occurred between two macromolecular chains. SAXS intensity patterns also evidenced some structural changes of the macromolecules: at long oxidation times the tannins gave intensity patterns that were characteristic of branched macromolecules. Conversely, when oxidation was done at low concentrations (e.g. 0.1 gL^{-1}), we observed no change in molecular weight, indicating that the reaction was intramolecular; yet the conformations were different.

TC-3 A novel approach towards the syntheses of proanthocyanidins and biflavonoids

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Oxidative condensation of tetra-O-methyl-3-oxocatechin and tetra-O-methylcatechin with silver tetrafluoroborate readily affords procyanidin B-3 analogues with the 3,4-cis diastereomers predominating. The 3-oxo-group deactivates the 8-position and no self condensation or oligomerisation was observed [1]. This condensation suggests the oxidative biosynthesis of proantocianidins from flavan-3-ol precursors [2].

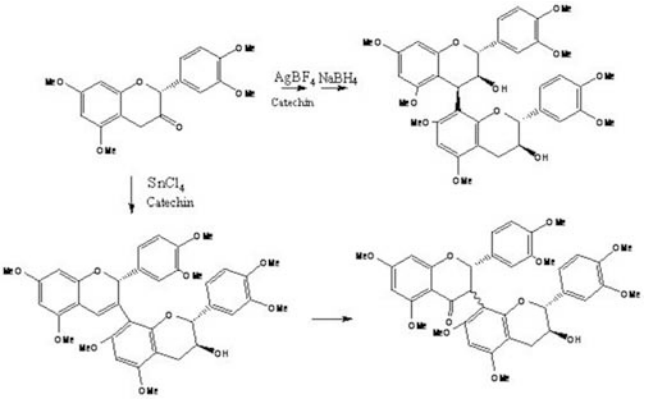

Fig. 1: Lewis acid catalysed condensation of tetra-O-methyl-3-oxocatechin and tetra-O-methylcatechin with tin chloride readily affords the first synthetic access to optically active 3-coupled biflavonoids.

Acknowledgements: 1. Mimosa Central Co-Op, 2. THRIP. **References:** 1. Achilonu, M. et al. (2008) Org. Lett.10 (17): 3865 – 3868. 2. Weinges, K. et al. (1968) Annalen 711:184 – 186.

TC-4 Proanthocyanidin/polyphenol research: trials and thrills

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The 5-deoxyproanthocyanidin pools of plants are of exceptional complexity. This is mainly due to extensive variation in hydroxylation pattern as well as several regio-/stereo-chemical and conformational issues. Such structural complexities also complicate purification and structure elucidation especially via NMR techniques where 1 H and 13C spin systems are often broadened and/or multiplied due to the restricted conformational mobility of the interflavanyl bond.

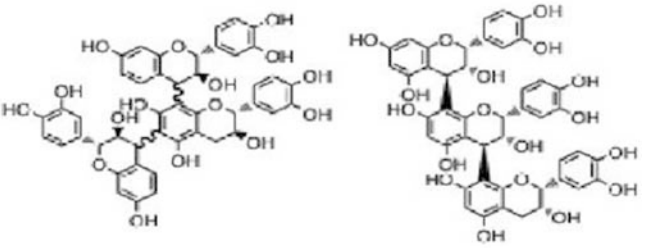

Fig. 1

Our investigations to comprehend the intricate structural, configurational, and chemical features commenced in the late 1970's when we designed a synthesis protocol to unambiguously define the linkage mode and the absolute configuration of the constituent flavanyl moieties. We will discuss some key issues that emanated from these studies, e.g., (1) the principles that control the regio- and stereo-chemistry of the interflavanyl bond formation process, (2) the development of an electronic circular dichroism method to define the absolute configuration at C-4 of the chain extension unit and corroboration of the results via. theoretical calculation of ECD spectra, (3) the enantioselective total synthesis of potential monomeric proanthocyanidin precursors, (4) the chemical manipulation of some crucial bonds in the proanthocyanidin

architecture, and (5) a unique fragmentation of the biaryl bond of Pomegranate ellagitannins.

TC-5 Towards a molecular interpretation of astringency. Synthesis, 3D-structure, colloidal state and human saliva protein recognition of procyanidins

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Astringency is a mouth-feel character determining red wines quality. This feeling is the result of an interaction between tannins and saliva proteins, mainly PRP, leading to the formation and precipitation of the complex. A dry, rough, and pucker sensation is then perceived in the mouth. To get an insight into astringency at a molecular level, were investigated: (i) an efficient and iterative method for 4 – 8 procyanidins synthesis that gives rise to all the possible procyanidins up to the tetramer with a total control of the oligomerization degree and the stereochemistry of the interflavan link. (ii) Their 3D-structure determination, which takes into account their internal movements, using 2D NMR and molecular modeling. (iii) Their self-association process in water or hydro-alcoholic solutions using Diffusion NMR spectroscopy that gives the active proportion of tannins able to fix proteins. (iv) The comprehensive description of the PRP- procyanidins complex formation to get information about stoichiometry, binding site localization and affinity constants for different procyanidins. The data collected suggest that the interactions are controlled by both procyanidins conformational and colloidal state preferences. All these results shine a new light into the molecular interpretation of tannins astringency.

TC-6 Metabolites of ellagitannins and their antioxidant activity

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Various biological activities such as antioxidant, antiviral and antitumor activities were reported for different types of ellagitannins [1,2]. Nevertheless, there are little definitive studies on the absorption and metabolism of ellagitannins. We describe the characterization of urinary and intestinal microbial metabolites in rats after ingestion of geraniin, which is a typical ellagitannin isolated from *Geranium thunbergii*, an anti-diarrheic in Japan. Seven metabolites (**M1**-**M7**) were isolated from the suspension of rat intestinal microflora and rat urine samples. The structures of **M1** (urolithin A), **M2** (3,8,9-trihydroxy-6*H*-dibenzo[*b,d*]pyran-6-one), **M3** (3,8-dihydroxy-9-methoxy-6*H*-dibenzo[*b,d*]pyran-6-one), **M4** (3,9-dihydroxy-8-methoxy-6*H*-dibenzo[*b,d*]pyran-6-one), **M5** (3,4,8,9,10-pentahydroxy-6*H*-dibenzo[*b,d*]pyran-6-one), **M6** (3,8,9, 10-tetrahydroxy-6*H*-dibenzo[*b,d*]pyran-6-one), and **M7** (3,8,10-trihydroxy-6*H*-dibenzo[*b,d*]pyran-6-one) were determined based on spectroscopic data. [3]. Furthermore, four major metabolites (M1-M4) were evaluated for antioxidant activity. These metabolites showed more potent antioxidant activity than intact ellagitannins such as geraniin in the ORAC assay, suggesting that the metabolites may contribute to the health benefits of ellagitannins as an antioxidant in the body. **References:** 1. Okuda T. et al. (2009) Chemistry and biology of ellagitannins – An underestimated class of bioactive plant polyphenols-, World Scientific, Singapore: 1 – 54. 2. Yoshida T. et al. (2009) Chemistry and biology of ellagitannins – An underestimated class of bioactive plant polyphenols-, World Scientific, Singapore: 55 – 93. 3. Ito, H. et al. (2008) J. Agric. Food Chem. 56:393 – 400.

Short Lectures

O-1 Catechin derivatives from *Parapiptadenia rigida*: biological studies and conformational analysis

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Parapiptadenia rigida (Benth.) Brenan (Fabaceae), popularly known in Brazil as "Angico vermelho", is a perennial tree native in South America. Preparations from its bark are used in traditional medicine because of its astringent, expectorant, antiseptic and antihemorrhagic properties [1 – 2], but no detailed phytochemical analysis has yet been performed. To increase our knowledge on the biologically active compounds from *P. rigida*, the ethanolic extract of its bark was phytochemically analysed and 10 catechin derivatives were isolated and identified by 1D and 2D NMR (^{1}H, ^{13}C, COSY, HSQC, HMBC, NOESY, 2D-ROESY) and ESI-MS spectroscopy analyses. 3′, 4′-O-methyl-apocynin (**1**), 3″,4′-*O*-methyl-apocynin-B (**2**), epigallocatechin-(4β-8)-4′-*O*-methyl-gallocatechin (**3**), 4′-*O*-methyl-gallocatechin-(4α-8)-4′-O-methyl-gallocatechin (**4**) and (−)-epigallocatechin-3-*O*-ferulate (**5**) have been found for the first time and 4′-*O*-methyl-(−)-epigallocatechin-3-*O*-gallate (**6**) for the first time from natural sources, in addition to the four known compounds 4′-*O*-methyl-gallocatechin (**7**), 4′-*O*-methyl-epigallocatechin (**8**), 3′-*O*-methyl-(−)-epicatechin (**9**) and (−)-epigallocatechin-3-*O*-gallate (**10**). Comprehensive conformational analyses were performed for the dimeric procyanidins **3** and **4**. Absolute configuration of **2** was determined by CD analysis. The extract and some of the catechin derivatives were studied for their wound healing effect in the scratch assay and gave promising results which suggest that plant preparations from *P. rigida* and their effective ingredients may have beneficial effects as a wound healing remedy. **Acknowledgements:** Government of Baden-Württemberg (Zukunftsoffensive IV). Brecht, V., Dept. of Pharm. Med. Chem., Uni Freiburg, Germany. **References:** 1. Souza, G.C. et al. (2004) Rev. Bras. Pl. Med. 6:83 – 91. 2. Souza, G.C. et al. (2004) J. Ethnopharmacol. 90:135 – 143.

O-2 Ellagitannins from *Phyllanthus muellerianus*: geraniin stimulates keratinocytes differentiation and collagen synthesis of skin dermal fibroblasts – new concept for improved wound-healing

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Leaves from *Phyllanthus muellerianus* (Kuntze) Exell. are traditionally used for wound healing in western Africa. Aqueous extracts of dried leaves recently have been shown to stimulate proliferation of human keratinocytes and dermal fibroblasts [1]. Within bioassay-guided fractionation the ellagitannins geraniin, corilagin, furosin, the flavonoids quercetin-3-O-β-D-glucoside (isoquercitrin), kaempferol-3-O-β-glucoside (astragalin), quercetin-3-O-rutinoside (rutin), as well as gallic acid, methyl gallate, caffeic acid, chlorogenic acid, 3,5-dicaffeoylquinic acid and caffeoylmalic acid (phaselic acid) have been identified in *P. muellerianus* for the first time. Geraniin was shown to be the dominant component of an aqueous extract (5.5%, m/m, related to the dried leaves). Geraniin and furosin increased the cellular energy status of human skin cells (normal human dermal fibroblasts, NHDF, HaCaT keratinocytes), triggering the cells towards higher proliferation rates, with fibroblasts being more sensitive than keratinocytes. Highest stimulation of NHDF by geraniin was found at 5 μM, and of keratinocytes at 50 to 100 μM. Furosin stimulated NHDF at about 50 μM, keratinocytes at about 150 to 200 μM. Toxicity of geraniin, as measured by LDH release, was observed at 20 μM for NHDF and 150 μM for keratinocytes. Toxicity of furosin – less than that of geraniin – was observed at > 400 μM. Furosin and ger-

aniin stimulated the biosynthesis of collagen from NHDF at 50 µM and 5 – 10 µM respectively. Geraniin at 105 µM significantly stimulated the differentiation in NHEK while furosin had a minor influence on the expression of involucrin and cytokeratins K1 and K10. **References:**
1. Agyare et al., (2009). *J. Ethnopharmacol.* 125:393 – 403.

O-3 Thiolytic screening method for exploring condensed tannin variation in a unique sainfoin germplasm bank

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This EU funded 'HealthyHay' project established a sainfoin (Onobrychis viciifolia) germplasm bank at NIAB, Cambridge, with 360 accessions from around the world. A screening method was developed to characterise tannins by thiolytic degradation [1] directly in green plants for the first time. The method was validated by separate analysis of unextractable, extractable and purified tannins using thiolysis, HPLC-GPC and MALDI-TOF MS. Most tannins (58 to 73% of the total) could be recovered after Toyopearl HW50 fractionation with water, aqueous methanol and acetone. The greatest losses during purification occurred amongst larger molecular weight tannins with mean degree of polymerisation (mDP) > 18. The composition of water-, aqueous methanol- and acetone-soluble tannins differed considerably in their mDP and trans/cis ratios, but not in their prodelphinidin/procyanidin (PD/PC) ratios. Direct thiolysis revealed that the condensed tannin contents in this germplasm collection ranged from 0.6 to 2.8 g/100 dry weight; mDP ranged from 12 to 84; PD/PC ratios from 53/47 to 95/5; and trans/cis ratios from 12/88 to 34/66. Detailed analysis of leaves and stems of the Perly variety, which was grown at INRA near Clermont-Ferrand (France) and harvested at three phenological stages between 2 June to 16 July, demonstrated a 2-fold higher tannin content and a 3-fold lower mDP in the less mature compared to mature plants. There was little change in PD/PC or trans/cis ratios. Leaf and stem tannins differed particularly in their mDP and PD/PC ratios. This screening method will underpin future breeding programmes to improve the nutritional and veterinary properties of sainfoin for animals and to reduce greenhouse gas emissions (NOx and CH4) from ruminants. **Acknowledgements:** EU Marie Curie Research Training Network (MRTN_CT-2006 – 035805). **References:**
1. Guyot, S. et al. (2001) In: 'Flavonoids and Other Polyphenols'. Methods in Enzymology 335, 57 – 70.

O-4 Proanthocyanidins and ellagitannins: new insights into the use for antiadhesive prophylaxis against viral and microbial pathogens and as skin active compounds

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The study deals with molecular investigations on potential targets for proanthocyanidins and ellagitannins used as antiadhesive compounds for antiviral, antimicrobial and wound-healing activity. Antibacterial and antiviral effects of defined proanthocyanidins and ellagitannins were investigated against Herpes simplex virus I (HSV-1) and Porphyromonas gingivalis, the major pathogen for periodontitis. Geraniin, procyanidin B2 and 3,3′-digalloylated B2 exhibited strong antiviral activity. Galloylation strongly increased the activity. Activity was due to an inhibition of viral adhesion to host cells; penetration and replication were not influenced. The digalloylated dimer interacts with the major viral surface gD-adhesin, which was oligomerized, forming a rigid structure, not able to initiate further adsorption process. Antiadhesive effects of oligomeric proanthocyanidins against P. gingivalis were due to inhibition of the major bacterial adhesins (Lys- and Arg-gingipaine). Additionally the expression of bacterial virulence factors is inhibited significantly, leading to a diminished pathogenic activity. The role of procyanidin-enriched extracts within clinical development products is discussed. Positive effects of tannins on skin are traditionally described, while the mode of action is unknown. Therefore the influence on geraniin on cell physiology of skin cells was investigated. Geraniin stimulated cell differentiation of keratinocytes (involucrin, cytokreatin 1, 10) and proteins responsible for formation of extracellular matrix (collagen). Potential pathways how tannins act on skin physiology are discussed. Summarizing it is shown that tannins act quite specifically on defined targets. Effects on target proteins are not as unspecific as often claimed. Therefore the medical use of tannins has to be investigated in more detail.

O-5 *In vitro* transport studies of Hawthorn procyanidins by Caco-2 monolayers: transport and efflux

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Extracts from Hawthorn (*Crataegus* sp.) are considered as a rational based phytomedicine for declining cardiac performance, with flavonoids and procyanidins as active compounds. Especially oligomeric procyanidins (OPC) are assessed to have the most marked pharmacodynamic effects. Detailed investigations on the bioavailability of procyanidins after oral ingestion are not available. The current study was designed to investigate the absorption of OPC from different fractions/extracts of Crataegi folium cum flore using validated monolayers of the human Caco-2 cell line grown in Transwells. Respective concentrations of distinct OPC clusters were determined in the basolateral and apical compartments and in cell lysates of Caco-2 cells. Quantitation was performed by HPLC on diol stationary phase with fluorescence detection. No significant absorption of OPCs with degree of polymerization (DP) ≥ 2 into the basolateral compartments was observed after apical application of 125 – 250 µg/mL, but OPC with DP 6 to 9 were detected in the respective cell lysates. Interestingly, transport of procyanidin B2 in the basolateral → apical direction was higher than that in the apical → basolateral direction, indicating efflux transporters carrying out OPCs after initial absorption to the apical side. This hypothesis was clearly proven by use of verapamil, a P-glycoprotein inhibitor in the absorption assay together with OPC as shown with B2: data from these experiments proved an increased apical → basolateral absorption of B2. These data clearly prove the suitability of the Caco-2 system for transport studies of polyphenols and indicate that intestinal OPC absorption is subjected to an efflux competition.

O-6 Chemical characterisation and sensory evaluation of Bordeaux wines. Correlation with wine age

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Quality evaluation of a red wine is based on wine-tasting. Chemical analyses are performed to understand what compounds influence sensory properties and how they affect them. Quantitative determination of certain chemical compounds is a criterion of wine valuation origin [1] and authenticity [2]. Our study concerning wine quality was carried out with 24 vintages of Cabernet-Sauvignon (CS) and with 7 vintages of Merlot (M) produced by Bordeaux wine-growing areas. Proanthocyanidin monomers and oligomers were identified and quantified by HPLC-UV-Fluo. Galloylation (%G) and prodelphinidins percentage (%P), mean degree of polymerization (mDP) were determined [3]. Total phenolic compounds, total anthocyanins, total tannins, hue, IC′ (color intensity), total acidity, ethanol level and pH were evaluated. Sensory analysis concerning astringency and bitterness intensity was also performed. Total phenolic compounds, anthocyanins, tannins, tannin monomers, hue, IC′,% G,% P, mDP and astringency intensity differentiate both wines according to vintage. Correlation between wine age and: mDP, hue, astringency, phenolic compounds, tannin monomers, total tannin levels are obtained. The qualitative wine tannin characterization is established between astringency and mDP ($R^2 = 0.509$, $p = 0.051$, CS; $R^2 = 0.780$, $p = 0.000$ M). mDP is a vintage marker ($R^2 = 0.796$, $p = 0.000$; CS and $R^2 = 0.946$, $p = 0.000$; M). Scale patterns between wine mDP and both ageing and tannin perception are proposed. M wines are characterized

by lower levels of total phenolic compounds, tannins, anthocyanins, mDP,% G,% P% levels as well as by lower astringency intensity than CS wines. **References:** 1. Forina, M. et al. (1986) Vitis 25: 189 – 201. 2. Arvanitoyannis, I. S. et al. (1999) Trends Food Sci. Technol. 10: 321 – 336. 3. Drinkine, J. et al.(2007) J. Agric. Food Chem. 55: 6292 – 6299.

O-7 Fluorescence lifetime imaging microscopy (FLIM) to demonstrate the nuclear binding of flavanols and (−-epigallocatechin gallate

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The use of light microscopy and DMACA staining strongly suggested that plant and animal cell nuclei act as sinks for flavanols [1, 2]. Detailed uv-vis spectroscopic titration experiments indicated that histone proteins are the likely binding sites in the nucleus [2]. Here we report the development of a multi-photon excitation microscopy technique combined with fluorescent lifetime measurements of flavanols. Using this technique, (+) catechin, (−) epicatechin and (−) epigallocatechin gallate (EGCG) showed strikingly different excited state lifetimes in solution. Interaction of histone proteins with flavanols was indicated by the appearance of a significant τ2-component of 1.7 to 4.0 ns. Tryptophan interference could be circumvented in the in vivo fluorescence lifetime imaging microscopy (FLIM) experiments with 2-photon excitation at 630 nm. This enabled visualisation and semi-quantitative measurements that demonstrated unequivocally the absorption of (+)catechin, (−)epicatechin and EGCG by nuclei of onion cells. 3D FLIM revealed for the first time that externally added EGCG penetrated the whole nucleus in onion cells. The relative proportions of EGCG in cytoplasm: nucleus: nucleoli were ca. 1:10:100. FLIM experiments may therefore facilitate probing the health effects of EGCG, which is the major constituent of green tea. **Acknowledgements:** The Science and Technology Facilities Council provided facility access time **References:** 1. Feucht W. et al. (2004) Plant Cell Rep 22: 430 – 436. 2. Polster J. et al (2003) Biol. Chem. 384: 997 – 1006.

O-8 Fractionation and characterization of high molecular weight proanthocyanidin from persimmon fruit by thiolysis-HPLC, size-exclusion chromatography, MALDI-TOF/MS, and NMR

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High molecular weight proanthocyanidin (condensed tannin) from persimmon pulp was fractionated on Toyopearl TSK-HW-50-F. The crude tannin and the three fractions were characterized by thiolysis-HPLC-ESI-MS, GPC, MALDI-TOF-MS and 13C-NMR. Thiolysis-HPLC-ESI-MS showed that the proanthocyanidin terminal units were catechin and epigallocatechin gallate, and extender units were epicatechin, epigallocatechin, (epi)gallocatechin-3-O-gallate, and (epi)catechin-3-O-gallate. The crude tannin had a very high prodelphinidin content (65 – 80%) and a high degree of 3-O-galloylation (72%). The composition of the fractions and the unfractionated tannin was similar, but the fractions were distinguished by degree of polymerization. Thiolysis suggested that the persimmon tannin was comprised of polymers ranging from 13kD to 20kD (degree of polymerization 30 – 50), but sizes estimated by GPC were much smaller. MALDI-TOF-MS revealed the presence of a heteropolyflavanol series including (epi)catechin and (epi)gallocatechin repeating units, and suggested that the persimmon proanthocyanidin contained some A-type interflavan linkages. The crude material was gently chemically degraded with acid to yield products that were amenable to NMR analysis, which was used to confirm the A-type linkages. **Acknowledgements:** Financial support was provided by the National Natural Science Foundation of China (No.30972398), and the Key Project of Chinese Ministry of Education (No.109115) to Huazhong Agricultural University, and by Agricultural Research Services Specific Cooperative Agreement Number 58 – 1932 – 6-634 with Miami University.

58th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Lectures

L-1 Sustainable drugs and global health care

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The global population has now reached 7 billion, and forests and other resources around the world are being irreversibly depleted for energy, food, shelter, material goods, and drugs to accommodate population needs. For the developed world, efforts have been initiated to make drug production "greener", with milder synthetic reagents, shorter reaction times, and more efficient processing, thereby using less energy, becoming more atom efficient, and generating fewer by-products. However, for most of the world's population, plants, based on many well-established systems of medicine, in either crude or extract form, represent the foundation of primary health care for the foreseeable future. Contemporary harvesting methods for medicinal plants are severely depleting these critical indigenous resources. However, maintaining and enhancing the availability of quality medicinal agents on a sustainable basis is an unappreciated public health care concept. To accomplish these goals for future health care, and restore the health of the Earth, a profound paradigm shift is necessary: all medicinal agents should be regarded as a sustainable commodity, irrespective of their source. Several approaches to enhancing the availability of safe and efficacious plant-based medicinal agents will be presented including integrated strategies to manifest the four pillars (information, botany, chemistry, and biology) for medicinal plant quality control. These integrated initiatives involve information systems, metabolomics, biotechnology, nanotechnology, in-field analysis of medicinal plants, and the application of new detection techniques for the development of medicinal plants with enhanced levels of safe and reproducible biological agents.

L-2 Plant molecular systematics: prospects for identifying species and for analysing bioactive compound evolution

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DNA-based approaches to unravel plant evolutionary relationships, to gain insights into patterns and processes of speciation, and to unravel the diversity of genotypes within species have revolutionized plant biology. Phylogenetic hypotheses now include all major lineages of flowering plants and the inclusion of more and more genera and species into phylogenetic trees is on their way. This offers a unique opportunity to reconstruct the evolution of secondary compounds and their biosynthetic pathways (macroevolution). For example, in the genus Hypericum (St. John's Wort) the evolution of bioactive compounds such as hypericin and hyperforin correlates with certain phylogenetic lineages. If there is an up to date taxonomic information source that connects genetic and phenotypic data for organisms to the respective taxon names, sequence data can be used to identify even small fragments of plant materials. Although this is a promising application, it should be noted that the required comprehensive monographic treatments so far exist for only a small fraction of flowering plant genera.

L-3 Infectious diseases and herbal medicines

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For many years the Laboratory of Pharmacognosy and Pharmaceutical Analysis has been involved in collaborative projects with research institutes in developing countries, where traditional medicine still plays an important role in local health care systems, for economic as well as cultural reasons. The aim of our work is twofold: Firstly, to provide a scientific basis for the therapeutic use of medicinal plants in these countries (or to discourage their use if not), and to sustain the development of standardised herbal medicinal products, the quality of which can be controlled, with proven safety and efficacy; secondly, to characterise lead compounds that can be used to develop new therapeutic

agents. During the past years our attention has mainly been focused, from a geographical point of view, on Tanzania, DR Congo and Guinea-Conakry; and from a medicinal point of view, on malaria. A biological screening programme of plants used in Tanzania against infectious diseases led to the selection of *Elaeodendron schlechteranum* (Celastraceae) and *Ormocarmpum kirkii* (Papilionaceae) for further investiagtion, based on their antibacterial and antiplasmodial properties, respectively. From *E. schlechteranum* a quinone-methide triterpene, 22β-hydroxytingenone or tingenin B, with a pronounced activity against a range of microorganisms was isolated, although a high cytotoxicity was observed as well. From *O. kirkii* a series of (3 – 3′)-biflavonoids was obtained, including several new ones, showing antiplasmodial activity to a various degree, which allowed to establish some structure-activity relationships. In a screening programme of plants traditionally used in DR Congo against malaria, *Nauclea pobeguinii* (Rubiaceae) was selected for further investigation. The main constituent of *N. pobeguinii* was the alkaloid strictosamide, and an HPLC method was developed and validated for the quantification of this compound in an 80% EtOH stem bark extract. The antimalarial activity of this standardised extract was established *in vivo*, and clinical studies in DR Congo have been initiated in order to provide a herbal medicinal product with proven safety and efficacy for non-severe malaria for the local market.

L-4 Validating the antimicrobial activity of African traditional medicines – Reflecting on a decade of research

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Unique and diverse botanical resources make traditional healing an integral part of the African cultural heritage. Plants have been used for centuries as anti-infective agents and the need to validate the traditional use in the last decade is addressed with respect to past challenges, latest developments and future recommendations. Microbiological methods (disc diffusion, minimum inhibitory concentration, time-kill and interactive assays) will be reviewed with practical examples given from some of the most widely used indigenous African medicinal plants. An extensive review of Artemisia afra will be given, encompassing antimicrobial screening, geographical variation, major compound analysis, comparison with commercial essential oils and the use in combination with other plants. Furthermore, the potential use in formulations is demonstrated where preservative efficacies within a cream formulation indicated bactericidal activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Pathogen specific studies will be presented where some of the most commonly used plants to treat sexually transmitted infections demonstrate activities as low as 0.2 mg/ml (*Hypericum aethiopicum* and *Polygala fruticosa*, tested against *Gardnerella vaginalis*). Also, the five most active plants tested against anti-diarrhoeal pathogens in the remote area of northern Maputaland include *Psiduim guajava* and *Garcinia livingstonei* (MIC values of 0.01 and 0.08 mg/ml respectively against *Bacillus cereus*), *Gymnosporia senegalensis* and *Syzygium cordatum* (0.13 mg/ml against *Enterococcus faecalis*) and *Sclerocarya birea* (0.13 mg/ml against *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella flexneri* and *Staphylococcus aureus*). These antimicrobial studies play an important role in the understanding of traditional healing and advancing the phytotherapeutic application of medicinal plants.

L-5 Herbal medicine research in Taiwan

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In a collective effort to upgrade and integrate Traditional Chinese Medicine (TCM) research, development and application, various national research centers and program projects were set up in Taiwan and reasonable successes have been achieved. A brief introduction and snap shots of these programs will be presented. As an example of TCM R&D in Taiwan, the research program at Academia Sinica will be examined in more detail. Cross-talk, collaborating research laboratories have since established theme projects and defined experimental systems for anti-inflammation and immuno-modulation studies. These include investigations on T-cells, dendritic cells, tumor cell-related immunomodulatory, and anti-inflammatory bioactivities in response to phytocompounds/botanical substances extracted from Chinese or Western medicinal plants including Anoectochilus, Echinacea, Bidens and Wedelia plants. Potential chemoprevention and anti-tumor activities of these phytoextracts/phytochemicals (e.g., shikonin, [BF(S+L)Ep], cytopiloyne, Wedelia Chinensis) have been investigated in breast and prostate tumor systems obtaining encouraging results. Functional genomics, proteomics and metabolomics studies have also yielded significant and interesting findings. Experimental approaches using clinically-relevant in vivo and ex vivo study systems are being evaluated for translation of research findings into medical and biotechnological applications. With TCM and medicinal plant research infrastructure outlined above, our highest priority for future R&D in Taiwan is to initiate, establish and optimize international research collaboration: research foci of such interest will be contemplated. **Acknowledgements:** Agriculture Biotechnology Research Center, Academia Sinica, Taipei, Taiwan. National Science Council, Taiwan.

L-6 South Africa's medicinal flora – abundant opportunities and daunting challenges

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South Africa is considered to be one of the most biodiverse areas in the world and harbours close to 10% of the world's flora. This diversity has provided abundant opportunities for the development of traditional healing practices and it is estimated that 60% of South Africa's population still rely on herbal medicines, often as a source for primary healthcare. Ironically, despite this access to unlimited botanical resources and the longstanding use of medicinal plants, very few medicinal plants have been developed into commercial products. Basic research underpins any commercial development, yet for many important ethnomedicinal plants aspects relating to quality, efficacy and safety remain poorly explored. The paper will provide a succinct overview on the uses, chemistry and biological properties for some of the most important medicinal species indigenous to South Africa including; Aloe ferox (Cape aloes), Pelargonium sidoides, Sutherlandia frutescens (cancer bush), Hoodia gordonii, Agathosma betulina (buchu), Mesembryanthemum tortuosum, Aspalathus linearis (rooibos tea), Lippia javanica, Siphonochilus aethiopicus (African ginger) etc. The challenges encountered when researching South Africa's medicinal flora such as chemical variation, the need for quality control protocols, indigenous knowledge systems and benefit sharing will also be highlighted.

L-7 Medicinal plants and natural products from Latin America – Subjects of international scientific exchanges

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Latin American countries possess part of the world' s biodiversity. Of the 17 megadiverse countries in the world six are in the neotropics. The percentage of Amerindian groups is high in this region. There is widespread use of medicinal plants among these groups. The number of monographs on Latin American plants in different pharmacopoeias varies from 5% in ESCOP, 12% in WHO and up to 15% in European Pharmacopoeia. Results of some collaborative programs such as the Iberoamerican Program of Science and Technology for Development (CYTED) and Organization of American States (OAS), which have fostered scientific exchanges in this region, will be presented. Case studies of research on selected medicinal plants used in traditional medicine will be highlighted. An overview of research on Panamanian medicinal plants a source of bioactive compounds will also be presented. **Acknowledgements:** OAS and CYTED Program.

L-8 Traditional uses and scientific evidence for selected native medicinal plants from Jordan: a critical evaluation

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Traditional medicine is part of the Jordanian culture. Both rural and urban Jordanian societies depend on traditional medicine; using plants

that are mainly locally grown. The occurrence of about 2500 plant species is recorded. A fifth of these are classified as medicinal plants and are used for the treatment of common mild diseases in addition to the treatment of chronic and/or incurable diseases. Although the practice of traditional medicine is based on years of belief and observation, scientific knowledge for most is very limited. To contribute to the existing, although limited, pool of data, our prime interests are native plants with antidiabetic and anticancer activities. Phase one of our investigation involved screening crude extracts of plants with claimed hypoglycemic activity in *in vitro* and *in vivo* experiments [1]. In parallel, the systemically collected medicinal plants were screened for their antiproliferative activity using different carcinoma cell lines [2]. Once efficacy and safety were established, in phase two, biologically active and safe plants were further evaluated in *in vitro* mechanistic assays [3, 4]. Results were compared to clinically used drugs. In phase three, the active plants were phytochemically investigated. Findings of our investigations are aiming, where possible, to link the traditional use with scientific evidence. **References:** 1. Hamdan, I. I., Afifi, F. U. (2004) J Ethnopharmacol 93: 117 – 21. 2. Abu-Dahab, R., Afifi, F. (2007) Sci Pharm 75:121 – 36. 3. Raghavan, G. et al. (2007) Planta Med 73: 427 – 32. 4. Wang, C. C. C. et al. (2008) Basic Clin Pharmacol Toxicol 102: 491 – 7.

L-9 Genomic mining – a concept for the discovery of new bioactive natural products

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Natural products continue to represent an important source for lead structures for drug discovery, but with discovery rates of novel structural classes in decline, the need to explore alternate sources of chemical diversity is evident. Genomic mining represents hereby a complementary strategy to address these issues and to access a tremendous source of new, biologically active metabolites. The base for this claim is provided by the outcomes of the recent genome sequencing projects which revealed the presence of numerous biosynthetic gene clusters for which the corresponding metabolites are currently unknown [1]. Importantly, it has been observed that the numbers of such orphan biosynthetic loci far outnumbers the quantity of the gene clusters directing the synthesis of known compounds of most of the considered organisms [2]. Apparently, only a fraction of natural products has been analyzed and the majority await discovery. Considering this discrepancy and that several hundreds of sequencing programs are ongoing, the huge potential of the genomic mining approach for natural product discovery becomes apparent. The potential of this untapped resource can be expanded even further by the exploitation of not only genomic, but also metagenomic DNA. After outlining the rationale and methods [1,3] of genomic mining, the success [2], but also the limits of this fascinating and interdisciplinary strategy will be illustrated by selected examples from plant and microbial genomes. **References:** 1. Gross (2007) Appl. Microbiol. Biotechnol. 75:267 – 277. 2. Gross (2009) Curr. Opin. Drug Discov. Devel. 12:207 – 219. 3. Gross et al. (2007) Chem. Biol. 14:53 – 63.

WS I: Workshops for Young Researchers

Cellular and molecular mechanisms of action of natural products and medicinal plants.
Chairs: Th. Efferth, D. Tasdemir, A. Hensel

WS I IL Impulse Lecture: Elucidation of the molecular basis of anti-inflammatory natural compounds from traditional medicinal plants

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Although many traditional medicinal plants are used in the therapy of inflammatory disorders, the bioactive ingredients and/or the molecular basis underlying the anti-inflammatory action are often unclear. Numerous plant-derived natural products with anti-inflammatory properties have previously been reported to reduce the biosynthesis of eicosanoids, i.e., prostaglandins (PGs) and leukotrienes (LTs) and this has essentially been attributed to an interference with the respective key enzymes cyclooxygenase (COX) and 5-lipoxygenase (5-LO). The dual inhibition of 5-LO and of microsomal PGE2 synthase-1 (mPGES-1), which forms pro-inflammatory PGE2 from COX-2 derived PGH2, is a novel and promising strategy for the therapy of inflammation being superior over single inhibition in terms of higher anti-inflammatory efficacy but also may cause fewer side effects. We have investigated selected natural products for which either anti-inflammatory efficacy in vivo or inhibition of PG biosynthesis was described. Here we report that many of these compounds (e.g., hyperforin, epigallocatechin gallate, garcinol, curcumin, myrtucommulone, arzanol) are poor inhibitors of COX enzymes but instead efficiently inhibit mPGES-1. Hence, the previously observed reduced PGE2 levels are rather due to inhibition of mPGES-1 than COX-1/2. Of interest, the mPGES-1 inhibitiory effect is often associated with suppression of 5-LO. Finally, analysis of selected compounds in experimental models of inflammation confirm inhibition of PGE2 and LT biosynthesis in vivo and suggest a valuable therapeutic potential for intervention with inflammatory diseases.

WS I-1 *Eruca sativa* Mill.: Phytochemical profile and antimicrobial properties of rocket leafy salads

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In the last decade salad species consumption is becoming increasingly important worldwide, encouraged from the positive link between eating fresh raw materials and absorption of health-promoting phytochemicals [1]. Rocket salads are well-known in the traditional medicine for their therapeutic properties as astringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient and stimulant [2]. However, the antimicrobial activity of rocket salad species has been poorly investigated. Our study was designed to characterize the phytochemical profile of Eruca sativa Mill. and to evaluate additionally their in vitro antimicrobial activity upon a representative range of pathogenic bacteria and fungi. Different plant extracts were prepared and tested in order to compare the activity of individual groups of phytochemicals. LC-MS and HPLC/DAD analyses led to the identification of glucosinolates and flavonoids, respectively. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all the extracts were determined using broth dilution methods in 96 wells micro plates. The plant extract showed very significant activity against Gram +ve bacteria with MIC and MBC values ranging from 0.125 – 1 mg/ml and 1 – 4 mg/ml, respectively. A lower activity was observed against Gram -ve and fungi. However, the plant extract containing GLSs had no effect on any of the Gram +ve and Gram -ve bacteria at any of the doses used. These results suggest that the greater antimicrobial of Eruca sativa leaf extract is not related to the GLS content but to other phytochemicals, and might be useful in controlling human pathogens through the diet. **Acknowledgements:** This work was supported by Foundation "Prof. Antonio Imbesi" (Messina, Italy). **References:** 1. Vermeulen, M. et al. (2006) J Agric Food Chem 54:5350 – 5358.

2. Perry, L. M. a. M., J., Medicinal plants of SE Asia. Attributed properties and uses. The MIT Press: Cambridge, Massachusetts and London.

WS I-2 Glucosinolates and their respective enzymatic hydrolysis products are not involved in the in vitro antioxidant properties of rocket salad species

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Epidemiological and experimental studies provide some evidence that chronic diseases could be prevented by high consumption of certain vegetables [1]. Oxidative stress is involved in the pathogenesis of these diseases, and may be reduced by improving physiological antioxidant defences through dietary interventions [2]. Dietary patterns rich in plant foods are the most likely to protect against oxidative stress by absorption of a wide range of naturally occurring antioxidants, that may act as synergists to reduce reactive oxygen species levels [3]. Cruciferous vegetables are widely studied for their health benefits due partly to the high content of antioxidants, like vitamin E and C, carotenoids, polyphenols, as well as characteristic phytochemicals, known as glucosinolates (GLSs). The role of GLSs and their enzymatic hydrolysis products, namely isothiocyanates (ITCs), as natural antioxidants is debated [4 – 6]. In this study, the antioxidant properties of bioactive compounds obtained from Eruca sativa Mill. were investigated using in vitro systems. The primary antioxidant properties by DPPH test and reducing power assay, and the secondary antioxidant ability by ferrous ion (Fe2+) chelating activity were evaluated. The plant extract showed a significant primary antioxidant activity in both DPPH test and reducing power assay, and a strong Fe2+ chelating ability. However, neither GLS fraction nor respective pure ITCs had antioxidant effects using the same experimental methods at all doses tested. The results suggest that these phytochemicals are unlikely to account for the direct antioxidant effects of Eruca sativa extract. **References:** 1. Pomerlau, J. et al. (2005) J Nutr 135:2486 – 2495. 2. Rahman, K. (2007) Clin Interv Aging 2:219 – 236. 3. Podsedek, A. (2007) Food Sci Technol 40:1 – 11. 4. Martìnez-Sànchez, A. et al. (2008) J Agric Food Chem 56:2330 – 2340. 5. Plumb, G.W. et al. (1996) Free Rad Res 25:75 – 86. 6. Valgimigli, L., Iori, R. (2009) Environ Mol Mutagen 50:222 – 37.

WS I-3 Bioactive fatty acids and cerebrosides from the TCM drug *Arisaema* sp.

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In this study active compounds from the TCM drug *Arisaema* sp. [1] were characterized by bioassay-guided isolation. Extracts and fractions of *Arisaema* sp. were tested for agonistic activity towards peroxisome proliferator-activated receptor-α and -γ (PPAR) and for activation of the AMP-activated protein kinase (AMPK). These proteins are therapeutical targets in treatment of metabolic disorders [2,3]. An apolar fraction strongly activated PPAR-α and -γ and had positive effects on AMPK activity *in vitro*. Among the main compounds identified by GC-MS were *n*-hexadecanoic acid, 9,12-octadecadienoic acid, 9-octadecenoic acid, octadecanoic acid, 13-phenyltridecanoic acid and pentadecanoic acid. Since cerebrosides from *Arisaema* with antihepatotoxic activity reported by Jung *et al* [4], were found to bind PPAR-α and -γ *in silico*, isolation and activity studies on these glycosphingolipids were continued. From a polar fraction, with moderate agonistic effect on PPAR-α and -γ *in vitro*, cerebrosides I-VI were isolated. Their structures were elucidated by NMR, ESI-MS-MS and matrix free LDI-TOF-MS-MS. In conclusion, in the present activity and analytical studies chemical constituents of *Arisaema* sp. that showed *in vitro* activity on important anti-diabetic targets were revealed. These findings affirm the great value and rich source of Chinese herbal drugs for natural product research. **Acknowledgements:** *Sino-Austria Project, supported by the Austrian Federal Ministry of Science and Research and Federal Ministry of Health, Women and Youth. This project was also supported in part by the Austrian Science Fund [NFN S 10704-B037] and the Austrian Federal Ministry for Science and Research [ACM-2009 – 01206].* **References:** 1. Bensky D. et al (2004) Chinese Herbal Medicine Materia Medica. Eastland Press. Seattle. 2. Kersten, S. et al (2000) Nature 405:421 – 424. 3. Winder W.W. et al (1999) Am. J. Physiol. Endocrinol. Metab. 277:1 – 10. 4. Jung, J.H. et al. (1996) J. Nat. Prod. 59:319 – 322.

WS I-4 Isolation of differencially expressed genes from *Psoralea corylifolia* by DD-PCR

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According to the world health organization (WHO) 80% of the world's population uses medicinal plants for the treatment of diseases. In recent years medicinal plants are primary health source for pharmaceutical industry. *Psoralea corylifolia* (*Psoralea* seed) is used in treatment of many skin diseases in Pakistan and India. Development of resistance in fungi to commonly used antifungal drugs diverted the attention of researches towards medicinal plants. In the present study we focused our research towards isolation of differenzially expressed genes from seedlings of *Psoralea corylifolia* after induction with a fungus, *Fusarium solani*. RNA was isolated from control (non-induced) and fungal induced seedlings. Quantity and integrity of RNA was checked by agarose gel electrophoresis and spectroscopy. cDNA was formed from RNA by reverse transcription using oligo-dT primers. All PCR reactions contained the same T_{11}MN primer, and an arbitrary primer. Different arbitrary primers (HAP 25 – 32) were tried for each cDNA. The amplified products were resolved on 6% denaturing polyacrylamide gel electrophoresis and detection was carried out with silver staining. Differenzially expressed genes were isolated from the induced samples after comparing with the controls after sequencing.

WS I-5 Natural coumarins and furanocoumarins as positive gabaergic modulators

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Coumarins and furanocoumarins are natural compounds commonly found in plants of the Apiaceae and Rutaceae family. Many of these plants are used as spasmolytic and sedative agents in traditional medicinal systems worldwide [1]. Thus, the effects of various (furano)coumarins on human recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors were investigated using the voltage-clamp technique according to [2]. From 18 substances tested (100 μM), only 2 potentiated the GABA induced chloride current above + 100%. The present study suggests that prenyl residues are essential for the postitive modulatory activity of this substance class. Furthermore it can be concluded that coumarins are more potent than furanocoumarins and that geranyl side chains or other bulky residues diminish the observed effect in both groups. The most potent substances were osthole (7-methoxy-8-(3-methylbut-2-enyl)chromen-2-one) and oxypeucedanin (4-[[(2S)-3,3-dimethyloxiran-2-yl]methoxy]furo[3,2-g]chromen-7-one) with a mean potentiation (± s.e.) of 125% (± 11%) and 110% (± 12%), respectively.

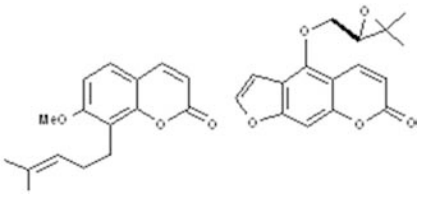

Fig. 1: Osthole and oxypeucedanin

Acknowledgements: This project was supported by the University of Vienna as part of the Initiativkolleg "Molecular Drug Targets" **Refer-**

ences: 1. Murray et al. (1982). The natural coumarins: occurrence, chemistry and biochemistry. Wiley. London. 2. Baburin I. et al. (2006). Pflugers Arch. 453: 117 – 23.

WS I-6 Anti-inflammatory potency of the traditionally used antimalarial plant *Fagraea fragrans*

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Fagraea fragrans Roxb. (Gentianaceae) was selected following an ethnopharmacological survey in Cambodia to treat fever or malaria. Little work has been done so far on this plant; only the presence of secoiridoids (gentiopicroside [1], [2], fagraldehyde, sweroside and swertiamarin [3]) has been described. Methanol and dichloromethane extracts have been prepared from bark and leaves to assess *in vitro* anti-plasmodial assays. The bioguided chromatographic fractionation against *Plasmodium falciparum* led to a spread of activity in different fractions. Multiple minor compounds acting in synergy could explain the activity provided in the crude CH_2Cl_2 extracts of the bark and the leaves. On the other hand, fever symptom is not only displayed during malaria disease but also in various illnesses including inflammatory reaction. Thus, the different fractions of *F. fragrans* have been tested on inhibition of Nitric Oxide (NO) overproduction on LPS-stimulated murine macrophages. The best anti-inflammatory potency (10 µg/ml = 80% inhibition) was provided by a fraction coming from the leaf apolar extract. Further investigations will be carried out in our laboratory to identify the active compounds inhibiting NO. **Acknowledgements:** the Belgian National Fund for Scientific Research (FNRS) (grant N° 3452005) and the Samuel de Champlain convention between France and Quebec (n° 62 103) are greatly acknowledged. **References:** 1. Wan, A.S.C., Chow, Y.L. (1964) J.Pharma.Pharmacol. 16: 484 – 486. 2. Natarajan, P.N. et al. (1974) Planta Med. 25: 258 – 260. 3. Jonville, M.C. et al. (2008) J. Nat. Prod. 71: 2038 – 2040.

WS II: Workshops for Young Researchers

Lead finding from Nature – Pitfalls and challenges of classical, computational and hyphenated approaches.
Chairs: J. M. Rollinger, A. R. Bilia, J-L. Wolfender

WS II IL Impulse Lecture: The potential of natural products in drug discovery – What is the challenge in academia?

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For more than a century bioactive natural products have served as molecular tools for biochemists and as inspiration for pharmacologists and medicinal chemists. Many prescribed drugs have directly or indirectly been discovered in natural product research1. However, with the rise of high-throughput screening technologies and the demand for huge synthetic compound libraries, as well as the more recent indroduction of biologics (e.g. therapeutic monoclonal antibodies), the interest in natural product research has declined in industry2. At the same time, the number of new chemical entities has declined too. While there are still some potentially interesting bioactive plant and animal natural products the chemical diversity of microorganisms (both terrestrial and marine) remains largely unknown. In the last decade, new academic initiatives have been realized to study natural products as potential lead structures or e.g. also to validate traditional herbal medicines3. Thus, academia is increasingly taking over natural product research. Academic research with natural products has led to thousands of scientific papers, describing actual or potential therapeutic uses. Unfortunately, only very few have triggered the development of innovative biochemical tool compounds or new drugs. The reasons for this are manyfold. In order to be successful, natural product research in academia should take advantage of the new developments in industry (high-content screening, promising targets, chemoinformatics, molecular library design, etc.) and combine the strength of "academic freedom" with the scrutinity of industrial selection. **References:** 1. D.J. Newman & G.M. Cragg, Natural products as sources of new drugs over the last 25 years. J. Nat. Prod,. 2007, 70: 461 – 77. 2. J.V. Li & JW Vederas, Drug discovery and natural products: end of an era or an endless frontier? Science, 2009, 325: 161 – 5. 3. J. Gertsch. How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. J. Ethnopharmacol. 2009, 122:177 – 83.

WS II-1 Bioactivity-guided isolation of potential anti-inflammatory constituents from *Betonica officinalis*

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Betonica officinalis (Lamiaceae) has been used in Austrian traditional medicine since ancient times against inflammatory disorders. The aim of this study was to investigate the anti-inflammatory properties of extracts, derived fractions, and isolated pure compounds of this plant by assessment of their effect on genes (E-selectin, IL-8) that are induced by inflammatory stimuli (TNF-α or LPS) in endothelial cells [1,2]. The plant material (herb) was extracted with dichloromethane (DCM) using an accelerated solvent extractor. Chlorophyll was separated by liquid-liquid-partition between DCM and a mixture of MeOH-H2O 1:1, in order to increase the concentration of the active compounds. Since the purified DCM extract showed strong activity in the mentioned assay, a bioactivity-guided fractionation was carried out. Subfractions were obtained by solid-phase extraction using C18 cartridges eluted with 30%, 70%, and 100% MeOH. The 30% and the 70% subfractions, which showed highest activity, were further fractionated by HPLC in order to identify and investigate their active constituents, whose structures were elucidated by HPLC-MS, 1D, and 2D NMR spectroscopy. Besides of some known polymethylated flavonoids (e.g. salvigenin), particularly the iridoid 8-O-acetylharpagide and two new diterpenoids were found to inhibit between 46% and 99% the LPS-stimulated induction of E-selectin at the concentration of 10 µg/ml, evidencing a considerable potential as new anti-inflammatory agents. **Acknowledgements:** This work is funded by the Austrian Science Fund, NFN: S10704-B037. **References:** 1. Chang et al. (2005) Exp Cell Res. 309(1):121 – 36. 2. Kadl et al. (2002) Vascul Pharmacol. 38(4):219 – 27.

WS II-2 Characterization of anti-inflammatory triterpene acids from rose hip powder (*Rosa canina* L.)

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The standardized rose hip powder LitoMove® (*Rosa canina* L.) is a widely used herbal remedy. Clinical trials have revealed that consumption of rose hip powder can reduce pain in patients suffering from osteoarthritis [1,2]. Synovial inflammation mainly mediated by macrophages has been reported to be involved in the pathology of osteoarthritis [3,4]. Therefore, the anti-inflammatory activity of crude extracts of standardized rose hip powder (LitoMove) was investigated and active principles isolated using the human monocytic cell line Mono Mac 6 as a model for inflammation. Incubation of Mono Mac 6 cells with a crude dichloromethane extract of rose hip powder significantly inhibited the lipopolysaccharide (LPS) induced interleukin-6 (IL-6) release in a concentration dependent manner. Through bioassay-guided fractionation this anti-inflammatory effect was correlated to a mixture of three triterpene acids; oleanolic, betulinic and ursolic acid (IC50 21 ± 6 µM). Investigation of the anti-inflammatory activity of each of the three triterpene acids revealed that oleanolic and ursolic acid was able to inhibit the LPS induced release of IL-6, in contrast to betulinic acid. Interestingly, combination of either oleanolic or ursolic acid with betulinic acid enhanced the anti-inflammatory effect of both oleanolic and ursolic acid. **Acknowledgements:** Hyben Vital International ApS is thanked for financial support. **References:** 1. Chrubasik, C. et al. (2006) Phytother. Res. 20:1.3. 2. Christensen, R. et al. (2008) Osteoarhritis Cartilage 16:965 – 972. 3. Farahat,

M. et al (1993) Ann. Rheum. Dis. 52:870 – 875. 4. Bondeson, J. et al. (2006) Arthritis Res. Ther. 8:R187.

WS II-3 Research of antifungal compounds from the Amazonian biomass by a bio-inspired approach

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Our research aims at understanding the chemical resistance mechanisms of durable woods against fungi. Our ultimate goal is to isolate and identify antifungal compounds from these woods that could be used for the treatment of human fungal diseases. We therefore screened highly durable Amazonian wood selected from technical databases [1] and demonstrated that bioactive secondary metabolites responsible of the natural durability of the woods [2] can also be used to treat mycoses. This screening has given a very high percentage of positive hits (30%) for 70 extracts tested, therefore validating the bio-inspiration hypothesis.

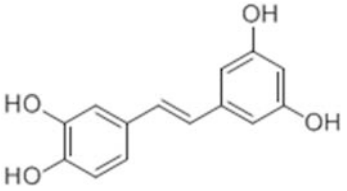

Fig. 1: Structure of piceatanol (**1**), isolated from *S. longifolia*

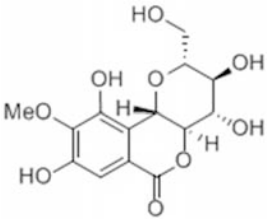

Fig. 2: Structure of bergenin (**2**), isolated from *H. balsamifera*

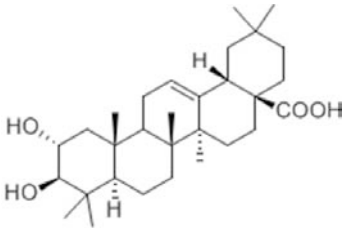

Fig. 3: Structure of maslinic acid (**3**) isolated from *H. balsamifera*

The bioguided isolation of the substances responsible for the antifungal activity has been pursued for 2 species: *Spirotropis longifolia* (Fabaceae) from which we isolated 5 active compounds (isoprunetin, piceatanol (**1**), resveratrol, genistein and 1 triterpen), and *Humiria balsamifera* (Humiriaceae) from which we isolated bergenin (**2**) and 3 triterpens including maslinic acid (**3**). Piceatanol (**1**) and maslinic acid (**3**) showed good antifungal activities against 7 human pathogenic fungi (3 dermatophytes and 4 *Candida* spp) with MIC values between 2 and 32 µg/mL, while (**2**) is active against yeasts only. **References:** 1. Scheffer, T. C., and J. J. Morrell. (1998). Natural durability of wood: a worldwide checklist of species. Coordinating ed., T. C. Scheffer and J.J. Morrell. Oregon State University, Corvallis, OR. 2. Schultz, T. P. et al. (1995) Holzforschung 49:29 – 34.

WS II-4 Antifungal components from Amazonian long lasting heartwood

Rodrigues A[1], Salmen Espindola L[2], Beauchêne J[1], Stien D[1]
[1]UMR ECOFOG, Université des Antilles et de la Guyane Cayenne, France; [2]Laboratorio de Farmacognosia, Faculdade de Ciências da Saude, Universidade de Brasilia, 70910 – 900 Brasilia, Brazil

About thirty Andira species have been described in America and Africa but most of them are found in Amazonian Rain Forest [1]. In French Guiana and Brazil *A. surinamensis*, *A. coriaceae* and *A. inermis* timbers (all named Saint-Martin Rouge in French) are commercialized for residential construction because of their excellent resistance to decay in ground contact [2]. In this study *A. surinamensis* heartwood was extracted with solvent of increasing polarities and extracts were evaluated against wood rotting fungi and human pathogens. Ethyl acetate extract proved strongly antifungal, showing that durable heartwood is a promising source of active metabolites for wood treatment and human health applications. Bioguided chemical fractionation allowed us to isolate five isoflavonoids including biochanin A [3]. These substances were described for the first time in this species and displayed submicromolar activities of human pathogenic fungi growth inhibition (dermatophytes and yeasts). Our study demonstrates that evolution selected antifungal phytoalexins may inspire research of new antifungal agents against human infections.

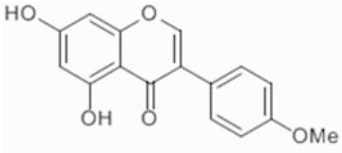

Fig. 1 biochanin A

References: 1. Silva et al. (2006) Quim. Nova 29(6): 1184 – 1186. 2. Detienne et al (1989) Revue Bois et Forêts des Tropiques n°219: 125 – 143. 3. Dakora et al. (1996) Physiol Mol Plant Pathol 49: 1 – 20.

WS II-5 Novel indole alkaloids from *Raputia simulans*

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Raputia simulans is a neotropical Rutaceae growing in the Amazonian basin. Investigation of the dichloromethane extracts of the stem bark and roots revealed the extraordinary abundance of non polar indole secondary metabolites and resulted to the isolation of several new natural products bearing unconventionally substituted indole moieties. The isolation procedure involved FCPC fractionation and successful isolation of minor indole components in one step from the original extracts, as well as traditional MPLC and HPLC techniques. More than 15 indole metabolites were isolated, comprising of 8 new monomers and 7 new dimers. The new indole monomers are exclusively substituted in position 5 of the indole ring, characteristically lacking substitution in position 3, with oxidated sidechains of open-type or forming furan or dihydrofuran closed rings. Among the new dimers, the 4 novel raputindoles (A-D) possess two indole moieties bridged via a fused cyclopentyl unit whereas the 3 new caulindoles bear an unusual MOM substitution at the terminal methyl groups, found for the second time in a natural product. [1],[2]. The novel raputindoles A-D were tested for their inhibition against CDK2, GSK-3β and DYRK1 and found to have moderate activity (IC_{50}> 10 µ*M*).

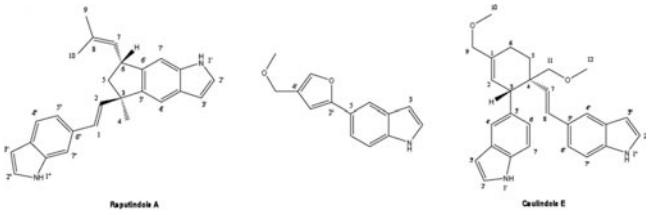

Fig. 1: Indole metabolites from *Raputia simulans*

References: 1. Makangara, J. et al. (2003) Phytochemistry 65: 227 – 232. 2. Wu, Y. et al. (2009) J Nat Prod 72: 204 – 209.

WS II-6

In silico strategy for the identification of cyclooxygenase inhibitors from the Thai medicinal mixture Prasaplai

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Prasaplai is a medicinal plant mixture that is used in Thailand to treat primary dysmenorrhea [1] which is characterized by painful uterine contractility and caused by a significant increase of prostaglandin release [2,3]. Cyclooxygenase (COX) represents a key enzyme in the formation of prostaglandins. Former studies revealed that extracts of Prasaplai inhibit COX-1 and COX-2 [4]. The major aim of this study was to predict which compounds of the complex mixture of natural products might be responsible for the COX inhibitory activity. Therefore, a comprehensive literature survey for known constituents of Prasaplai was performed. A multiconformational 3D database was generated comprising 683 molecules. Virtual parallel screening using a set of six validated pharmacophore models for COX inhibitors [5] was performed resulting in a hit list of 166 compounds. 46 Prasaplai components with already determined COX activity were used for the external validation of this set of COX pharmacophore models. 57% of these components were predicted correctly by the pharmacophore models, i.e. virtual hits with inhibitory activity on COX as well as inactive non-hits. The findings of this theoretical study confirm that the virtual approach provides a helpful tool for the fast identification of novel COX inhibitors [6]. **Acknowledgements:** This work was granted by the Austrian Science Foundation (B89-B03). **References:** 1. National Drug Committee (2006) List of Herbal Medicinal Products A.D. 2006; Chuoomnoom Sahakorn Karnkaset Publisher. Bangkok. 2. Connolly, T.P. (2004) Clin. Med. Res. 1:105 – 110. 3. Hayes E.C., Rock J.A. (2002) Obstet. Gynecol. Surv. 57:768 – 780. 4. Nualkaew, S., Tiangda, C., Gritsanapan, W., Bauer, R., Nahrstedt, A. (2005) 53 rd Annual Congress of the Society for Medicinal Plant Research, Florence, p 359. 5. Schuster, D. et al. (2010) Mol. Inf. 29:75 – 86. 6. Waltenberger, B. et al. (2010) submitted.

WS II-7

HPLC- based activity profiling for new antiparasitic leads: *In vitro* and *in vivo* antitrypanosomal activity of cynaropicrin

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In a medium throughput screen of 880 plant and fungal extracts for antiplasmodial, antitrypanosomal and leishmanicidal activity, the dichloromethane extract of *Centaurea salmantica* (*Asteraceae*) showed strong inhibition against *Trypanosoma brucei rhodesiense,* the parasite causing African sleeping sickness. HPLC-based activity profiling of this active extract [1] led to the characterisation of cynaropicrin, a guajanolide sesquiterpene lactone as the active constituent. Cynaropicrin had an IC_{50} of 0.3 µM (± 0.35) against *T. brucei rhodesiense in vitro*. It was ten and fifteen times less active against *P. falciparum* (IC_{50}: 2.99 ± 0.28) and *T. cruzi* (IC_{50}: 4.43 ± 0.036). A series of similar natural and semi-synthetic guaianolides were tested similarly for preliminary structure activity studies. Mice infected with *T. brucei rhodesiense* were treated intraperitoneally with cynaropicrin (10 mg/kg/d). After 4 days, a 98% decrease of parasitaemia was observed compared to the untreated controls. The treated test animals had 100% survival until day 14 after infection, whereas the control animals died within 12 days. Cynaropicrin is a promising new antitrypanosomal lead and the first natural sesquiterpene lactone to show activity against *T.b. rhodesiense in vivo*.

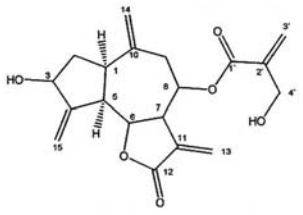

Fig. 1

References: 1. Adams, M. et al. (2009) Nat. Prod. Commun. 9: 1377 – 1381.

WS III: Permanent Committee on Regulatory Affairs of Herbal Medicinal Products

The importance of a risk-benefit analysis for the marketing authorization and/or registration of (traditional) herbal medicinal products (HMPs).
Chairs: A. Vlietinck, S. Alban

WS III-1

Taking the risk of the benefit: the unbalanced situation of herbal medicinal products

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Risk-benefit analysis is related to quality, safety and efficacy of the herbal in a specific context. In this context 3 variables can be considered: the patient, the intervention and the interpretation of the outcome. A careful characterisation of the patient brings us to constitutional (childhood, pregnancy and aged) and contextual (pathological) risks. EMA herbal monographs cover 92 indications (of which 21 for well established use). Only one (skin disorders and minor wounds, Avenae fructus) does not have any age restriction. It is also the only one allowing the topical use in case of pregnancy. On the other hand, it remains difficult to fix upper limits as age is concerned. Therapeutic interventions with herbals are more complex as several preparations of the same plant species can be made. The Herbal Medicinal Product Committee of EMA is restrictive on accepting herbal preparations. This contrasts with the wide range of herbal ingredients in food supplements. The question can be asked how far such preparations can move from traditional approaches: e.g. Exolise® (an 80% ethanolic dry extract of Camellia sinensis standardized at 25% catechins) had to be taken from the market after cases of severe hepatitis were linked to its use. With adverse events as an outcome, clear factual evidence is needed: drug, event, source and patient must be well-defined before an investigation can start. Mostly one or several of these elements are failing. Causal relationship should be dressed according to validated methodology (e.g. Austin Bradford-Hill criteria or the Naranjo scale).

WS III-2

National practice: Experiences with the safety assessment for (traditional) herbal medicinal products

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Herbal medicinal products (HMPs) are highly accepted in many Member States of the European Union, even though their specific regulatory status may be different. In contrast to other medicinal products HMPs have particular characteristics, which should be additionally considered in assessment of safety. Generally there are no exceptions in the European legislation with respect to the preclinical safety evaluation of HMPs.

Nevertheless, when applying the legislation a reasonable adaptation to cover the particulars of HMPs (e.g. testing strategies) is inevitable. The Committee on Herbal Medicinal Products (HMPC) was established at the European Medicines Agency (EMA) to generate a harmonised view an all issues concerning HMPs. Within this work the HMPC adopted a series of guidance documents referring to the safety of HMPs (e.g. general guidance on safety assessment, papers on specific issues like toxicity of defined natural products etc.). With respect to the traditional use of herbal medicinal products there is an option to reduce the set of tests to be performed in the context of preclinical testing. Nevertheless, experiences of the German national competent authority considering more than 300 applications in the field of traditional herbal medicinal products showed that consideration of the particulars of herbal medicinal products does not only open the chance to reduce the preclinical testing adequately but also provokes some additional questions which should be addressed case by case.

WS III-3 European practice: experiences with a risk-benefit analysis of HMPs for the elaboration of ESCOP monographs

Steinhoff B
Co-Chairperson of the ESCOP Scientific Committee, Auf dem Forst 53 Bonn, Germany

ESCOP, the European Scientific Cooperative on Phytotherapy, was founded in 1989 as a European umbrella organisation of national associations of phytotherapy. During the past 20 years, ESCOP has been working on more than 100 monographs on efficacy and safety in order to contribute to the scientific harmonisation process. A monograph comprises therapeutic indications, dosage recommendations, information on potential risks and pharmacological properties. During the preparation of monographs, benefit and risk of preparations of the respective herbal drug and its preparations are evaluated on the basis of pharmacological and clinical data as well as of toxicological data and clinical safety. The assessment results in a decision about which therapeutic indications and dosage recommendations are justified and which information on potential risks is required. In case the risks outweigh the benefit, or in case a therapeutic use cannot be proven, a monograph will not be prepared. Thus the issue of a benefit-risk analysis has to be addressed during the elaboration of each monograph. After publication of the 2nd Edition with 80 monographs in 2003, a Supplement containing 35 revised and new monographs was published in 2009. It can be ordered from the book trade or from the ESCOP Secretariat (www.escop.com).

WS III-4 European practice: Well-established use of herbal medicinal products and clinical research

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Dr. Wilmar Schwabe GmbH & Co KG, Willmar-Schwabe-Str. 4, 76227 Karlsruhe, Germany

When a clinical trial is about specific efficacy of an active substance the randomised placebo-controlled trial (RCT) is the gold-standard. This is the background of the well-known hierarchy of evidence of Evidence Based Medicine (EBM) also. Well-established use (WEU) of a drug as defined by European law requires proven efficacy by definition. But, when the modalities of the WEU of a specific product are assessed at a certain point during the product's lifetime many other questions contribute to the total body of evidence relevant for this assessment. The talk outlines the background of this situation and shows in which different ways clinical research may contribute. In this broader view the RCT design is only one option among others depending on the research question. The assessment of WEU needs a broader perspective, beyond of what an RCT can deliver.

WS IV: Permanent Committee on Biological and Pharmacological Activities of Natural Products

Use of polyphenols against cardiovascular diseases. Chair: V. Butterweck

WS IV-1 Challenges for research on dietary polyphenols in the prevention of cardiovascular diseases – New propositions for a structure-function analysis

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Much evidence supports the role of dietary polyphenols in the prevention of cardiovascular diseases. However polyphenols are not all equal. Their bioavailability and biological properties differ widely according to their fine chemical structures, and the exact nature of the most protective compounds is still largely unknown. New tools are clearly needed to explore the links between intakes of individual polyphenols and the risk of cardiovascular disease. We have built a new comprehensive database on polyphenol contents in foods called Phenol-Explorer (http://www.phenol-explorer.eu/) that includes more than 36,000 composition data for 502 polyphenols in 452 foods. This database is being further developed to include data on polyphenol metabolites and their concentrations in plasma and urine. The data is being used in cohort studies to estimate intake of the 502 polyphenols and the resulting concentrations of polyphenol metabolites. This will allow exploration of the links between polyphenols and disease risk with an unparalleled precision.

WS IV-2 Flavonoids and cardiovascular disease risk factors – a meta-analysis and recent findings from prospective cohort studies

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University of East Anglia, School of Medicine, Norwich, United Kingdom

Our recent meta-analysis of randomized clinical trials provides a snapshot of the current state-of-the-art in relation to the relative effectiveness of the different flavonoid subclasses in modifying biomarkers of cardiovascular (CVD) risk and highlights areas in which limited data exists. The available data suggests there may be clinically relevant effects of some flavonoid subclasses on cardiovascular risk factors, and to date the effects of flavonoids from soy and cocoa have been the main focus of attention. Chocolate/cocoa increased FMD acutely (3.99%; 95% CI: 2.86, 5.12; six studies) and chronically (1.45%; 0.62, 2.28; two studies) and reduced both systolic blood pressure (−5.88 mm Hg; −9.55, −2,21; five studies) and diastolic blood pressure (−3.30 mm Hg; −5.77, −0.83; four studies) following chronic intake. Only soy protein isolate, but not whole soy or soy extracts, reduced diastolic blood pressure (−1.99 mm Hg; −2.86, −1.12; nine studies) and LDL cholesterol (−0.19 mmol/L; −0.24, −0.14; thirty-nine studies). For many subclasses (anthocyanins and flavanones) there was insufficient evidence to draw conclusions about efficacy. We are currently updating the review, including all studies conducted through until Jan 2010 with a view to also examine potential structure-activity relationships. Recent data from ongoing collaborations with the Harvard cohorts (Nurses Health Study and Helath Professionals Follow-up Study) on flavonoid sub-class intake and cardiovascular health endpoints will also be discussed.

WS IV-3 Vascular protection of tea and grape-derived polyphenols: in vitro and in vivo evidence

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Faculty of Pharmacy, CNRS UMR 7213, 74, route du rhin, 67401 Illkirch, France

Epidemiological studies have indicated that regular intake of polyphenol rich sources such as vegetables, fruit, red wine and tea is associated with a decreased risk of coronary diseases. The protective effect of polyphenols on the vascular system has been attributable in part to their direct action on endothelial cells resulting in an enhanced formation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), two factors playing a major role in the control of vascular homeostasis. Indeed, tea and grape-derived polyphenols cause pronounced endothelium-dependent relaxations of isolated arteries, which are mediated by NO and, also in some arteries, by EDHF. The increased formation of NO is initiated by a pro-oxidant response in endothelial cells, which triggers

activation of Src kinase with the subsequent activation of the PI3-kinase/Akt pathway resulting in the activation of endothelial NO synthase following its phosphorylation on Ser1177. Intake of red wine polyphenols in the drinking water is also able to prevent the development of an endothelial dysfunction and hypertension induced by infusion of angiotensin II to rats, and also of an endothelial dysfunction in ageing. The protective effect of polyphenols involves their ability to prevent the excessive oxidative stress, the arachidonic acid-derived formation of endothelium-dependent contracting factors, and to regulate the local angiotensin II system in the pathologic artery. Thus, polyphenols induce a great variety of effects on both endothelial cells and vascular smooth muscle cells to protect blood vessels.

WS IV-4 Crataegus extract WS®1442 in patients with congestive heart failure class NYHA II-III: The SPICE trial

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Median Clinics Bad Krozingen, Herbert-Hellmann-Allee 38, 79189 Bad Krozingen, Germany

WS®1442 is registered for treatment of early stages of congestive heart failure (CHF) in several countries, e.g. Germany. As there was no study available regarding efficacy and safety of WS®1442 in more severely impaired patients already treated with optimal therapy, SPICE (Survival and Prognosis: Investigation on Crataegus Extract WS®1442) was performed. SPICE is a double-blind mortality trial conducted in 156 centres in 13 European countries. Patients were eligible if their left ventricular function was markedly impaired (WMI≤ 1.2). These patients already receiving optimal pharmacological therapy (83% ACE-inhibitors, 64% β-blockers, 56% glycosides, 39% spironolactone, 85% diuretics) were randomised to WS®1442 or placebo. Treatment duration was 2 years; primary endpoint was the time until first cardiac event (sudden cardiac death (SCD), death due to progressive heart failure, myocardial infarction, hospitalization; FCE). 2681 patients (mean age 60 yrs, 84% males, 44% NYHAIII, mean LVEF 24%) were randomised and evaluated. At all times, WS®1442 was superior to placebo regarding FCE, but without reaching statistical significance. For the first 18 months of WS®1442 treatment, deaths due to cardiac cause were reduced significantly (−20%; p = 0.046) and WS®1442 postponed cardiac deaths by four months. This effect was even more pronounced in patients with LVEF≥ 25% (−33% reduction; p = 0.044) and also holds true for total mortality and SCD up to 24 months. In conclusion, the study confirms the safety of WS®1442 in CHF patients with a mean LVEF of 24% and being treated with optimal pharmacological therapy. More important, WS®1442 may postpone deaths due to cardiac cause in this patient population.

WS V: Permanent Committee on Manufacturing and Quality Control of Herbal Remedies

The use of hyphenated techniques in the quality control of herbal medicinal products.
Chair: C. Erdelmeier

WS V-1 LC-NMR/MS and LC-SPE-NMR/MS: technical realization, possibilities and limitations

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The hyphenation between chromatographic and spectroscopic techniques is well established and approved in various fields, e.g. pharmaceutical or natural products analysis. It combines commercially available analytical HPLC equipment with an NMR spectrometer and a mass spectrometer using specially developed interfaces according to the type of application. LC-NMR/MS directly transfers the separated peak into an NMR flow probe and via split into the ion source of a mass spectrometer. Chromatographic separation is done using mixtures of protonated organic and deuterated aqueous mobile phase, resulting in strong background resonances from the organic solvent which needs to be suppressed in order to detect small signals originating from the analytes. Chromatographic peaks can be send either directly to the NMR or stored in loops for subsequent transfer into the NMR probe. This direct coupling is especially used for polar and/or labile compounds present in high to moderate concentrations. Post column solid phase extraction was first introduced 1998 by Griffiths and Horton: peaks eluting from a reversed phase column are diluted by aqueous phase and trapped on small SPE cartridges packed with reversed phase material. Elution into the NMR flow probe or tube can be done with deuterated organic solvent preferably after drying of the cartridge with nitrogen gas in order to remove residual protonated solvent. This indirect coupling can be used for medium polar to unpolar compounds down to very low concentrations due to the possibility of multiple peak trapping. In combination with cryogenically cooled NMR probes highest NMR sensitivity can be achieved.

WS V-2 HPLC-SPE-NMR in studies of medicinal plants and herbal products

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Hyphenated NMR spectroscopy is a state-of-the-art method for rapid and rigorous determination of natural product structures in plant extracts without isolation of the individual components in the classical sense. Among hyphenated NMR techniques, HPLC-SPE-NMR is the most convenient and superior in terms of quality of data, the most serious limitation being problems with solid-phase extraction of highly polar but nonionic species. The technique is particularly important in projects aimed at discovery of new chemical entities in medicinal plants, e.g., in connection with natural product discovery of novel pharmacologically active compounds. Moreover, studies related to standardization and uniformity of herbal products, performed either using metabolomic techniques or traditional methods, can benefit from support by hyphenated NMR experiments. HPLC-SPE-NMR work can be performed either using a flow NMR probe or a microtubes, and will benefit from increased sensitivity of cryogenically cooled probes. Examples of use of HPLC-SPE-NMR in analysis of medicinal plants and herbal products will be shown.

WS V-3 Hyphenated analytical techniques and phytoindustrial reality: do we need Ferraris to pull trailers?

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In recent years multi-hyphenated methods coupling NMR spectroscopy (as the most powerful method for structure elucidation of natural compounds) with HPLC or SPE (solid phase extraction) have been described and used by several high performance working groups. In principal those methods allow a rapid identification of a multitude of plant constituents avoiding the classical and complicated isolation and characterisation procedure. Within the presentation it is discussed if multihyphenated analytical techniques might be used during analytical development and routine analysis of herbal preparations and herbal medicinal products. In most cases the methods seem to be oversized for the analysis of traditional or well established HMPs, extracts and plants. However multihyphenated techniques may play a role during the basic research on medicinal plants, the development of new plant extracts, the screening on active or analytical markers, and for a holistic characterisation of plant extracts and bioanalytical profiling. As the techniques are based on special equipment and dedicated personal a broad industrial use seems unlikely. Corresponding problems should be solved within cooperations of the industry and the specialised labs.

WS V-4 Choice of analytical assay methods from a pharmacopoeial point of view

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The purpose of the European Pharmacopoeia is to promote public health by the provision of recognised common standards for use by healthcare professionals and others concerned with the quality of medicines. Such standards are to be appropriate as a basis for the safe use of medicines by patients and consumers. Their existence:
- facilitates the free movement of medicinal products in Europe;
- ensures the quality of medicinal products and their components imported into or exported from Europe.

European Pharmacopoeia monographs and other texts are designed to be appropriate to the needs of:
- regulatory authorities;

– those engaged in the control of quality of medicinal products and their consitutents;
– manufacturers of starting materials and medicinal products.

In this context, the author will provide background information on which grounds the choice of assay methods is made by the groups of experts and the European Pharmacopeia Commission. In the particular he will address the advantages and difficulties linked to the introduction of hyphenated assay techniques to monographs and the potential implications for the users of the monographs.

WS VI: Permanent Committee on Breeding and Cultivation of Medicinal Plants

Biotechnology in breeding and cultivation of medicinal plants. Chair: Ch. Franz

WS VI-1 Metabolic engineering for improved heterologous terpenoid biosynthesis

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Terpenoids belong to the largest class of natural compounds and are produced in all living organisms. The isoprenoid skeleton is based on assembling of C5 building blocks, but the biosynthesis of a great variety of terpenoids ranging from monoterpenoids to polyterpenoids is not fully understood today. Terpenoids play a fundamental role in human nutrition, cosmetics, and medicine. In the past 20 years, many metabolic engineering efforts have been undertaken in plants but also in microorganisms to improve the production of various terpenoids like artemisinin and paclitaxel. Recently, inverse metabolic engineering and combinatorial biosynthesis as main strategies in synthetic biology have been applied to produce high-cost natural products like artemisinin and paclitaxel in heterologous microorganisms. Artemisinin is an important antimalarial drug and its demand can hardly been covered by plant cultivation and harvesting herbal material for extraction. Therefore additional biotechnological approaches have been applied to solve the problem of sufficient demand and cultivation independent of ecological conditions. Today most of the combinatorial biosynthesis studies have been carried out in *E. coli* or *S. cerevisiae*. This presentation describes the recent progresses made in metabolic engineering of the terpenoid pathway and the early artemsinin pathway in *Xanthophyllomyces dendrorhous* as new industrial host. *X. dendrorhous* has been developed as terpene factory because of its ability to biosynthesise high quantities of different terpenoid classes (e.g. mono-, di-, sesquiterpenoids). Early genes like amorphadiene synthase (ADS), Cyp450 71AV1 and DHAA Reductase (RED1) have been assembled and expressed successfully. With particular focus on fundamental aspects as knock out strategies, vector design, and metabolic profiling assembly of the early artemisinin biosynthesis towards dihydroartemisinic acid will be discussed. **References:** 1. Tsuruta H, Paddon CJ, Eng D, Lenihan JR, Horning T, Anthony LC, Regentin R, Keasling JD, Renninger NS, Newman JD (2009) 4: e4489. 2. Muntendam R, Melillo E, Rydén A, Kayser O (2009) Appl Microbiol Biotechnol. 84:1003 – 19. 3. Rydén A, Ruyter-Spira C, Quax W, Osada H, Muranaka T, Kayser O, Bouwmeester H (2010) Planta Medica, in press.

WS VI-2 Generation of novel medicinal plants by metabolic engineering

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Plants produce a plethora of valuable natural products, many of which are used by humans as nutrients, colorants, flavors, fragrances, and pharmaceutics. For numerous natural products the dependency on natural occurring resources strongly limits their availability due to their occurance in eventually endangered species, due to the minute amounts of metabolites present in plant tissue, and due to the reluctance of many medicinal plants to propagation and cultivation in sufficient quantities. During the last decades an enormous amount of knowledge has been generated regarding the biochemistry and genetics of numerous complex natural product biosynthesis pathways in plants, like those leading to morphinans, terpenoid indole alkaloids, or lignans. This steadily increasing knowledge know opens up opportunities to engineer these pathways in heterologous hosts but also for metabolic engineering of those pathways to significantly enhance the yield of the desired products in planta. Additionally, novel catalysts like the human cytochrome P450 monooxygenases can be engineered into plants, presenting unique options to manipulate plant metabolism (1,2). Current achievements as well as future perspectives of metabolic engineering of medicinal plants will be evaluated in this presentation. **References:** 1. Warzecha, H. et al. (2007) Plant Biotech.J. 5: 185 – 191. 2. Warzecha, H. et al. (2010)J. Biosc. Bioeng. 109: 288 – 290.

WS VI-3 Plant breeding strategy for plants used for the purification of natural products

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Meanwhile the pharmaceutical industry has largely moved away from plant-derived natural products because of the advent of a huge variety of organo-chemical and bio-technological methodologies. These methodologies enable a shorter timeframe for the scale up of a substance from early development to launch and ensure a more robust supply. Herbal raw materials contain between about 0.01 up to some per cents of the natural product needed. This content is the basic default for the amounts needed for supply. An increase of this content has a direct input in the efficiency of the extraction process. Next to the screening of its distribution in the different plant parts the processability of the extraction from these plant parts has to be evaluated. After the decision which plant part should be used, the development of the extraction process should lead to the evaluation of possible "impurities" which cause a laborious separation. Next to an increase of the natural product the reduction of these substances is the focus of the breeding program. Quality management tools like e.g. "Quality function deployment" (QFD) should be installed to ensure a systematic approach. Further more measures to carry out the botanical rating should be specified in clear and reproducible dimensions. Project plans with clear defined mile stones and decision trees used to decide how to continue if an experiment fails are even valuable tools to ensure the success of each project. A systematic and strict management opens up the possibility to let natural products come back to the forefront.

WS VI-4 DNA-Barcoding: the master key to authenticate medicinal plants?

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DNA-barcoding is an initiative to define a standard DNA-fragment capable to assign a specimen to a known species. But so far no single DNA-fragment was identified working equally well for all plant species. Therefore, a combination of DNA-fragments is now the proposed assessment to identify a wide range of plant species [1]. The approach to identify medicinal plant specimens by a DNA-barcode seems convincing. However, some peculiarities of routine should be taken into account:

– Time and costs of routine analysis
– Resolution of difficult genera ('barcoding gap'): sometimes the evolutionary differences between related species ('barcoding gap') are rather small and need careful and intensive method development.
– Hybridisations: for DNA-barcoding mostly uniparentally inherited chloroplast markers are in use, hybridisations may therefore go undetected. Biparentally inherited nuclear markers should be preferred in cases of known species hybridisations.
– DNA degradation in processed drug material: Some processing steps will lead to DNA degradation. Therefore the identification methods need to be adapted to processed materials.

Molecular methods are already a good complementation meeting the demands of routine analysis in being reliable, fast and affordable. For the presence, it would be a great advantage to use all available systems complementarily, because they balance in many cases the weaknesses of individual approaches. Molecular approaches, however, have the potential to become the single authentication method in the future fuelled by an enormously fast progress in technology development. **References:** 1. Chase, M.W., Fay, M.F. (2009) Science 325:682 – 683.

Special Session: Opportunities and challenges in the exploitation of biodiversity – Complying with the principles of the convention on biological diversity.
Moderation: M. Simmonds (UK)

S-1 Impact of the convention on biodiversity on bioprospecting in phytomedicine

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The Convention on Biological Diversity (CBD) provides the legal framework for biodiversity conservation worldwide. The CBD has three main objectives: (1) the conservation of biodiversity; (2) the sustainable use of biodiversity; (3) the sharing of benefits from the use of genetic resources Despite being in force more than 17 years the number of successful access and benefit sharing schemes (ABS) based on CBD principles are limited. Based on a review of 22 studies of ABS globally we analysed positive and negative impacts of the CBD on the global phyto-medicines industry and common problems facing implementation? Just what is the resource in question? A plant, an idea, a DNA fingerprint – plant science and genetic engineering has rapidly progressed since 1993 when the CBD was drafted. Who really owns what? In cultures where communal ownership, traditional knowledge and oral history predominate and where national boundaries have limited meaning is westernjurisprudence appropriate to answer this question? Prior Informed Consent. Who represents "the people" in such negotiations? The proliferation of organisations claiming to "represent" owners of bioresources makes it sometimes difficult to identify who is consenting to what. Equitable benefit sharing arrangements. There are no international norms concerning how big, in what form and how long CBD benefit sharing arrangements should exist. Who is the arbitrator of fairness in this arena? Growth or equity? ABS negotiations often spend too much time debating potential profit sharing arrangements instead of ensuring that projects succeed and prosper; yet success rates in bioprospecting projects is low. **References:** 1. Denzil Phillips International (Editor), Plants, People and Nature (2009) AAMPS Publishing, Mauritius.

S-2 The implications of South Africa's bioprospecting legislation (NEMBA, Act 10 of 2004): local lessons for global benefit

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South Africa, as a megadiverse country has, since 1 April 2008, regulated bioprospecting, access and benefit-sharing activities in accordance with its obligations as a ratifying Party to the CBD. The context and process of key legislation developments in South Africa are discussed, prior to presenting a critique[1] which emphasises the practical impacts, especially on drug discovery, arising from the newly introduced systems. The subsequent effects on existing bioresource-based industries within South Africa, together with current as well as future bioprospecting activities are assessed. It is clear that various practicalities of bioprospecting methodology have been poorly accommodated, resulting in the development of impracticable and unnecessarily restrictive regulations, albeit well-intentioned. In particular, it is difficult for bioprospectors to establish broad-scale screening programmes given their user insecurity, legal uncertainty, and cost-inefficiency. Existing bioresource-based industries within South Africa also face potential closure in view of onerous bioprospecting permit application requirements. Further, the regulations have impacted negatively on basic biological research underway both in-country and internationally. Consideration is made of time and financial cost implications, as well as unworkable bureaucracy, which may discourage international partners from valuable and often essential collaborations in this sphere. An alternative, practical, CBD-compliant model on which to base urgently required legislative reforms is presented. The South African experience is contextualized in relation to other biodiverse countries of the developing world that that have sought to service their CBD obligations through the introduction of national legislation. We find that as a case study South Africa provides local lessons for global benefit. **References:** 1. Crouch, N. et al. (2008) S.A. J. Sci. 104:355 – 366.

S-3 Access and benefit sharing and the ethical sourcing of biodiversity

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The fair and equitable sharing of benefits is a central pillar of the conservation and sustainable use of biodiversity, as recognized by the Convention on Biological Diversity (CBD). Yet access and benefit sharing (ABS) remains little known in many of the sectors researching and working with biodiversity-based ingredients, including cosmetics, food and beverages. Lack of clarity on the scope of ABS and limited implementation in national legislation means companies are not aware of how ABS is relevant or how it could be put in practice – even as they often do engage in sustainable development initiatives with local partners. As awareness of biodiversity and calls for ethical practices increase, however, equitable benefit sharing is becoming more and more important. A new international regime on ABS will likely be finalized in 2010. This is where the work of the Union for Ethical BioTrade (UEBT), which promotes the "Sourcing with Respect" of biodiversity, plays an important role. Indeed, the fair and equitable sharing of benefits derived from the use of biodiversity is at the core of Ethical BioTrade. ABS principles are included in the Ethical BioTrade standard and the UEBT third-party verification system, through its assessment of company policies and their implementation, can determine gaps and issues to be addressed in regards to ABS. In addition, UEBT provides technical advice and support on ABS issues, including through practical tools and workshops. By addressing ABS in its outreach activities, UEBT is also helping to raise awareness of ABS within industry.

S-4 ProBenefit – Conclusions and lessons from an ABS project in Ecuador

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The UN Convention on Biological Diversity (CBD) gives all countries the sovereign rights over their genetic resources. Many countries now develop regulations for access and benefit sharing (ABS), among them Ecuador. However, so far no well-documented model procedures for ABS exist that respect both the needs of local and indigenous communities and of small and medium-sized pharmaceutical companies that seek access to medicinal plants. The goal of ProBenefit was to develop a suitable procedure for equitable benefit-sharing for the use of biological resources and the associated indigenous knowledge in line with the principles of the CBD. To this end the project partners, together with the Ecuadorian government, the local Indian organisations and other relevant groups in society, as well as interested non-governmental organisations, explored new models for sustainable use of biodiversity in the Ecuadorian Amazon region. A participatory approach was chosen to analyze stakeholders' needs and perceptions and to create a consultation process for indigenous communities in the project region. An agreement on collaboration between the project and the major regional indigenous organization FONAKIN was signed. A training course on biological, pharmaceutical, economic, legal and political aspects of ABS was organized for indigenous representatives in the project region. In the end, no access contract was agreed upon within the framework of the project. **Acknowledgements:** The reasons were analyzed within the research project and conclusions for further work on an ABS policy regime were drawn. Legal and socio-political analyses, scenario development, participatory approaches, ethnobotanical studies and pharmaceutical testing made up the methodological set-up of the interdisciplinary project. ProBenefit started in June 2003 and had a duration of five years. It was funded by the German Federal Ministry of Education and Research (BMBF) as part of the program BioTeam.

Short Lectures

SL-1 Phylogeny as selection tool for exploring CNS-activity in the Amaryllidaceae

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To discover whether DNA-based phylogenies can be used predictively to identify species for bioprospecting, we analysed 37 taxa of the tribe Haemantheae (Amaryllidaceae). DNA sequences from the nuclear ribosomal internal transcribed spacer (ITS) and the plastid trnL-F regions were used. Maximum parsimony analyses divided the Haemantheae into two main clades, A corresponding to the genera Clivia and Cryptostephanus, and B corresponding to the genera Apodolirion-Gethyllis-Haemanthus-Scadoxus. Acetylcholinesterase inhibitory activity was detected in all investigated clades except the Apodolirion-Gethyllis clade. No alkaloids were detected by GC-MS in extracts of the Gethyllis species, which could explain the lack of acetylcholinesterase activity. Within the Haemantheae, dose-dependent affinity to the serotonin transporter was restricted to the genus Haemanthus. The GC-MS profiles indicated that Haemanthus species contain alkaloids of a different type than alkaloids in other members of the tribe. In conclusion, the phylogeny produced in this study can be used to predict in which clades compounds with CNS activity can, or cannot, be found – and thus where to search for candidate species with potential for drug discovery.

SL-2 ^{1}H NMR-based metabolic profiling for the quality control of *Thymus vulgaris* raw plant material

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Conventional chromatographic methods for the characterization of *Thymus vulgaris* raw plant material comprise a combination of GC and LC, respectively employed for the analysis of monoterpenoids and phenolic constituents. Herein we demonstrate the suitability of ^{1}H NMR-based metabolic profiling for the quality control of *T. vulgaris* aerial parts of commercial and field culture origin. Extracts of 146 different *T. vulgaris* aerial parts samples were obtained by direct extraction with DMSO-d_6 and were subsequently analyzed by ^{1}H NMR spectroscopy. The preliminary analysis of the acquired 600 MHz ^{1}H NMR spectra was performed by Principal Component Analysis (PCA). A PCA model explaining 95.2% of the total variance using 5 principal components showed clear discrimination of thymol, carvacrol, and linalool chemotypes and enabled quick identification of interesting samples among the large number of samples analyzed. The use of anthracene as internal standard allowed quantification of five monoterpenoids (thymol, carvacrol, linalool, *p*-cymene, and γ-terpinene) and rosmarinic acid in the DMSO-d_6 crude extracts, employing the same spectral dataset. The NMR method was validated in terms of linearity, precision, accuracy and robustness. The quantitative results were compared to those obtained with conventional chromatographic methods such as GC and HPLC and were found to be in reasonable agreement. The simultaneous analysis of monoterpenoids and rosmarinic acid, difficult to achieve with one single chromatographic technique, makes ^{1}H NMR an attractive method for the quality control of *T. vulgaris* raw plant material. **Acknowledgements:** This work was financially supported by Bionorica research GmbH, 6020 Innsbruck, Austria.

SL-3 Potential of UHPLC for crude plant extract analysis: profiling, dereplication and metabolomics

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Ultra High Pressure Liquid Chromatography (UHPLC) systems operating at very high pressures and using sub-2 µm packing columns have allowed a remarkable decrease in analysis time and increase in peak capacity to HPLC [1]. UHPLC has rapidly been accepted and is gradually applied to various fields of plant analysis such as quality control, profiling and fingerprinting, dereplication and metabolomics. The shorter analysis time and the sharper peaks produced by UHPLC require detectors with sufficiently high acquisition rates. In this respect, UV/DAD, evaporative light scattering detector (ELSD) and time-of-flight mass analyzers (TOF-MS) are particularly well adapted [2]. Examples of fast fingerprinting (high throughput) and precise profiling of crude plant extracts (high resolution) will be presented, and compared with classical HPLC methods. Gradient transfer methods and applications of high temperature will be discussed as well. UHPLC coupled to MS represents a powerful platform for the rapid on-line identification of natural products. Through the high mass accuracy of the TOF-MS measurements, molecular formula provides a universal way to characterize natural products (NPs). However, for an efficient cross search in databases, an instrument-independent retention parameter is needed. In this respect, attempts to standardize the chromatographic dimension of the LC-MS datasets have been made. A protocol to extract online the log P parameter in specific profiling conditions [3] from the retention behavior of NPs, as standards or in extracts, will be presented. **References:** 1. Guillarme, D. et al. (2007) J. Chromatogr. A. 2007, 1149, 20 – 29. 2. Wolfender, JL. et al. (2009) Planta Med. 75, 719 – 734. 3. Henchoz, Y. et al. (2008) J. Med. Chem., 51, 396 – 99.

SL-4 Metabolomic analysis of *Ranunculus* spp. as potential agents involved in the aetiology of Equine Grass Sicknes

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Introduction: Equine grass sickness (EGS) is a polyneuropathy affecting the central, peripheral and enteric nervous systems of grazing horses (esp. in Great Britain). It is very likely that EGS has a multifactorial aetiology, including intoxication with *Clostridium botulinum* type C, but the causes of the disease are still unknown. Our recent research has shown that a range of edaphic and botanical factors are strongly associated with EGS outbreaks including the regular occurrence of *Ranunculus* spp. on EGS-sites. Aim: To determine if the metabolomic variability of *Ranunculus* spp. could be linked to increased risk of EGS outbreaks. **Methods:** *Ranunculus* samples from twelve farms with EGS outbreaks and nine controls were extracted with methanol in triplicate. Metabolic profiling using 1 H-NMR spectroscopy and multivariate data analysis in combination with principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and partial least squares (PLS) was used. **Results:** Metabolomic differences were found between different *Ranunculus* spp. PCA on *R. repens* methanol extracts from all Equine Grass Sickness and control sites showed a cluster of control samples, whereas there was a higher variation among the EGS samples. Seasonal variation and correlations with elevated metal levels from soil samples from the same sites were also found. **Conclusion:** The metabolomic composition of *Ranunculus* spp. on EGS and non-EGS sites differ. High levels of iron, nitrate or chromium may have an impact on the variation in the extracts' metabolomic profile. A toxic metabolite – protoanemonin (a hydrolisation product of ranunculin) may provoke lesions in the horses' gastrointestinal tract.

SL-5 Genetic diversity revealed by ISSR molecular marker in different species of *Ocimum*

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Genomic DNA assays of different *Ocimum* species were performed with inter simple sequence repeats primers (ISSR). The genetic diversity among four *Ocimum* species namely *O. basillicum var. basillicum*, *O. americanum*, *O. basillicum var. thysiflora*, and six different accessions of *O. tenuiflorum* collected from various region of Maharashtra were studied. *Ocimum* species are well-known for their medicinal benefits and are commonly used in indigenous system of medicine. The results obtained from ISSR study showed that *Ocimum* species and *O. tenuiflorum* accession shows high genetic difference. For this study DNA was isolated using modified CTAB protocol. The collected *Ocimum* species with some exception shows 100% polymorphism while collected *O. tenuiflorum* accessions showed genetic diversity that vary from as low 50% to as high as 89%. The collected Ocimum accessions with some exceptions were grouped according to geographical location and also according to altitude at which they grow. The phylogenetic map shows genetic diversity among variety where Jaccard's coefficient varies from 0.22 to 0.80 and it varies from 0.55 to 0.86 among accessions. Jaccard's coefficient was used as the measure of similarity or distance. The results obtained from this study will help in conservation of genetic diversity in *Ocimum* genotype and also for future crossing of *Ocimum* for their high yielding marker compound. This project also revealed the suitability of ISSR-PCR for assessing genetic relationship among *Ocimum* genotype. **References:**
1. Zietkiewicz, E., Rafalski, A., Labuda, D. (1994) Genomics 20:176 – 183. 2. Yao, H., Zhao, Y., Chen, DF et al., (2008) Biologia Plantarum 52(1): 117 – 120.

SL-6 Pharmacophore-guided elucidation of the active principle from natural compounds

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Pharmacophore-based virtual screening is broadly applied to predict bioactivities of small molecules. [1] One pharmacophore model represents chemical features of a compound that are required for a specific biological response, for example a specific arrangement of hydrophobic areas and hydrogen bonds. When a large number of such models is combined, the pharmacological profile of a compound can be predicted, including potential desired and adverse effects. This so-called pharmacophoric profile can guide the identification of biological effects of natural products. [2] The approach especially aims to elucidate the active principle of traditionally used herbal and fungal remedies, thereby accelerating the identification of their molecular mode-of-action. This presentation will introduce the concept of pharmacophoric profiling, describe the setup and data analysis of a profiling run, and show successful application examples to natural products. **Acknowledgements:** This work was supported by the Austian Science Fund (FWF, NFN-project DNTI, No. S 107020-B03, a Young Talents Grant, and the Erika Cremer Habilitation Program, both from the University of Innsbruck). **References:**
1. Langer T., Hofmann R. (2007) Pharmacophores and Pharmacophore Searches. Wiley-VCH, Weinheim, Germany. 2. Rollinger JM. (2009) Phytochem. Lett. 2: 53 – 58.

SL-7 Targeted toxins and saponins – a powerful cooperation

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Saponins from Gypsophila species are known to enhance the cytotoxicity of targeted toxins. In general targeted toxins consist of two components: a toxin moiety with enzymatic activity and a ligand moiety, which mediates specific binding to cancer cells. It was shown that saponins from Gypsophila paniculata L. enhanced the toxicity of a targeted toxin, composed of the N-glycosidase saporin from Saponaria officinalis L. and human epidermal growth factor up to 2,500 000-fold in primary breast cancer cells. Meanwhile the efficiency of the combined application of saponins and targeted toxins was shown in a mouse tumour model. Compared to the single application of the targeted toxin (SA2E), the combination with saponins lead to a tremendous reduction (at least 50-fold) of the SA2E dosage. By this dose reduction side effects were only moderate. This represents an enormous step forward in the targeted anti tumour therapy with recombinant fusion toxins. However, in all these studies a mixture of saponins was used. For the further successful development of a combinatorial anti cancer therapy with targeted toxins and saponins pure saponins are mandatory. This study was intended to isolate pure saponins from Gypsophila paniculata L. in order to scruntinize the toxicity enhancing properties of isolated Gypsophila saponins on targeted toxins. One of the four isolated saponins showed strong, one moderate and two no cytotoxicity enhancing properties. On the basis of these results we determined the structural prerequisites for the saponin-mediated toxicity enhancement of targeted toxins.

SL-8 Discovery of novel neuraminidase inhibiting scaffolds from *Alpinia katsumadai*

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Current antivirals for the treatment of pandemic influenza are mainly directed against neuraminidase (NA) [1]. In the search for new antiviral drug leads from natural sources, the seed extract of the Traditional Chinese Medicinal plant *Alpinia katsumadai* Hayata has been phytochemically investigated and afforded six constituents. Four out of five isolated diarylheptanoids inhibited the NA of human influenza virus A/PR/8/34 of subtype H1N1 in low micromolar ranges. Katsumadain A, which showed an IC_{50} of $1.05 \pm 0.42\,\mu M$, also inhibited the NA of four H1N1 swine influenza viruses with IC_{50} values between 0.9 and $1.64\,\mu M$, and demonstrated antiviral effects in plaque reduction assays [2]. To study the assumed binding interactions in the flexible loop region of the investigated enzyme, extensive molecular dynamics (MD) simulations were performed. The most potent NA-inhibitor from *A. katsumadai* seeds, Katsumadain A, was studied by computational docking and revealed well-established interactions between the protein and the core of this novel NA-inhibiting natural scaffold with excellent surface complementarities. The virtual predictions were in accordance with experimentally-derived SAR data of the investigated diarylheptanoids [2], and have already provided a valuable tool for the identification of further novel natural leads to combat flu. **References:**
1. Krug, R.M. et al. (2009) Trends Pharmacol. Sci. 30:269 – 277. 2. Grienke, U. et al. (2010) J. Med. Chem. 53:778 – 786.

SL-9 Natural products inhibiting the liver stage of the malaria parasite

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Malaria parasite has a complex life cycle involving both human and mosquito as hosts. In human, the sporozoites inoculated by the A*nopheles* mosquito first invade the hepatocytes and develop the liver stage (LS) parasites from which the blood stage (BS) parasites originate. Antimalarial drug discovery is primarily directed against the asexual BS parasites that cause the clinical symptoms of the disease. The LS that has a longer duration, lower parasite loads and causal malaria prophylaxis effect, has not been truly exploited for drug purposes due to technical challenges. Primaquine is the only licensed drug used against LS parasites, but is highly toxic. Only two natural products with low selectivity and unknown targets were reported to kill LS parasites [1]. We investigated an in-house natural product library for LS activity by flow cytometry and immunofluorescence, both based on in vitro infections of *Plasmodium yoelii* in the hepatoma cells (HepG2:CD 81). Recent studies [2,3] indicate that LS parasites exhibit an absolute requirement for *de novo* type II fatty acid biosynthesis (FAS-II) making this pathway an excellent target for LS drug discovery. Hence the compounds were also tested against recombinant FAS-II enzymes. Several lichen metabolites appeared to kill LS parasites, without toxicity on primary mammalian or hepatoma cells, and inhibited multiple crucial plasmodial FAS-II enzymes. Hence, these are the first natural products active against *Plasmodium* LS with a potential target. This presentation will cover our efforts to find natural products targeting the LS malaria parasite. **Acknowledgements:** The School of Pharmacy is gratefully acknowledged for funding. **References:** 1. Carraz, M. et al. (2006) PLoS Med. 3:e513. 2. Vaughan, AM. et al. (2008) Cell. Microbiol. 11:506 – 520. 3. Yu, M. et al. (2008) Cell Host Microbe 4:567 – 578.

SL-10 *In vitro* and *in vivo* activity of cynaropicrin against *Trypanosoma brucei rhodesiense*

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As an outcome of a recent screen for new antiparasitic leads, cynaropicrine was identified as a potent inhibitor of *Trypanosoma brucei rhodesiense* [1]. This guajanolide sesquiterpene lactone found in *Centaurea* and *Cynara* species was more active against *T. b. rhodesiense in vitro* (IC_{50}: 0.3 μM) than against *Plasmodium falciparum* (IC_{50}: 3.0 μM) and *Trypanosoma cruzi* (IC*50*: 4.4 μM). *In vivo* it decreased *T. b. rhodesiense* parasitaemia by 98% after 4 days when 10 mg/kg/d when administered intraperitoneally. The mice had a 100% survival rate after 14 days (control: 0%). Preliminary structure activity studies with natural and semisynthetic derivatives showed the necessity of the 2-(hydroxymethyl), 2-propenoic acid side chain, for the preferential toxicity towards *T. b. rhodesiense*. The interaction of cynaropicrine with trypanothione -a trypanosomatid specific glutathione spermidine conjugate essential for parasite redox metabolism- was studied. Under physiological conditions trypanothione spontaneously forms stable bisadducts with cynaripicrin via a Michael addition of the thiols to the exocyclic double bonds at C-13 and C-3′, as was shown in NMR experiments. This adduct formation was also studied in STIB 900 strain and in NY-at1 (TbMRPA – efflux pump of trypanothione conjugates overexpressing) *T. b. rodesiense* strains. The antitrypanosomal activity of cynaropicrin shown *in vivo* and *in vitro* may be due to interaction with trypanothione metabolism. **References:** 1. Adams, M. et al. (2009) Nat. Prod. Commun. 9: 1377 – 1381.

SL-11 *Eupatorium perfoliatum* L.: Antiinflammatory and antiplasmodial activity of the dichloromethane extract and novel sesquiterpene lactones

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Eupatorium perfoliatum L., Asteraceae, is a medicinal herb from North America with a well documented use for the treatment of fever, flu, and malaria. At present, it is widely used as homeopathic remedy with immunomodulatory activity (e.g. in Contramutan®). In order to elucidate the active compounds and the mechanisms of action, the aerial parts of the plant were extensively studied. From the methanolic-aqueous (7/3 V/V) extract three uncommon glucaric acid derivatives have been isolated [1]. Soxhlet extraction with dichloromethane followed by column chromatography yielded eight sesquiterpene lactones, two germacranolides (euperfolitin [2] and a new heliangolide) and six new guaianolides. Five guaianolides are members of a homologous group with 8-O-tiglic acid, 11-methyl, and 2-oxo-3,4-en functionality. They mainly differ in the degree of oxidation of C-14, ranging from a methyl group up to a free carboxylic acid function. Furthermore, an unusual dimeric guaianolide with a novel mode of linkage between C-14 and C-4 was identified. *In vitro* tests with the macrophage cell line RAW 264.7 indicated that potential immunomodulatory effects of the dichloromethane extract are not due to enhanced phagocytosis but are rather mediated by antiinflammatory activity. This was shown by concentration dependent (1 μg/mL up to 100 μg/mL) inhibition of NO-production from LPS-stimulated macrophages. This effect correlated with a significantly reduced activation of iNOS (Western Blot). Microarray investigation revealed new insights into potential modes of actions. Fractions of the dichloromethane extract also exhibited a moderate inhibitory activity against *Plasmodium falciparum*, which is in accordance with the reported traditional antimalarial use. **References:** 1. Maas, M. et al. (2009) Molecules 14:36 – 45. 2. Herz, W. et al. (1977) J. Org. Chem. 42:2264 – 2271.

SL-12 Antimicrobial activity of *Kielmeyera variabilis*

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Kielmeyera variabilis Mart. (Clusiaceae), a tree commonly known in Brazil as "malva-do-campo", is used in Brazilian folk medicine to treat several tropical diseases such as schistosomiasis, leishmaniasis, malaria, fungal and bacterial infections [1]. Multidrug-resistant *Staphylococcus aureus* (MRSA) have been a major threat to public health in hospitals and the community in the past decade [2]. As part of our on-going project to isolated bioactive compounds from Brazilian species, the anti-staphylococcal properties of the *K. variabilis* was investigated. The MICs were obtained using the methodology described by Gibbons and Udo [3]. The EtOH extracts from leaves (EFK) and stems (EGK) were tested against *S. aureus* strain (SA 1199B) with the MIC values of 128 and 32 μg/mL, respectively. The EGK was partitioned between hexane (EHGK), EtOAc (EAGK) and *n*-BuOH (EBGK). The EHGK showed strong activity against SA 1199B (MIC = 16 μg/mL, compared to the control Norfloxacin MIC = 32 μg/mL). The EHGK was bioassay-guided fractionated using chromatographic techniques (Sephadex® LH-20, silica gel column, SPE and PTLC) leading to the isolation of the bioactive compound **1**. The compound **1** (a phloroglucinol derivate) was tested against six *S. aureus* strains and showed a strong activity with MIC values of 0.25 – 2 μg/mL (Table 1).

Table 1: MICs of compound 1 and standard antibiotic in μg/mL

	SA 1199B	RN 4220	EMRSA 15	EMRSA 16	Xu 212	ATCC 25943
Compound 1	2	0.25	1	0.5	0.25	2
Norfloxacin	32	0.5	0.5	128	8	0.5

Acknowledgements: 1 FAPESP 2 School of Pharmacy, University of London, London, UK. **References:** 1. Alves, T. M. A. et al. (2000) Men. Inst. Oswaldo Cruz. 95:367 – 373. 2. White, C. (2004) BMJ 329:131. 3. Gibbons, S., Udo, E. E. (2000) Phytother. Res. 14:139 – 140.

SL-13 Dual antibiofilm and antimetalloproteinase-9 activities of panduratin A in experimental periodontal disease models: A potent natural periodontotherapeutic

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We examined the efficacy of panduratin A, a chalcone compound isolated from *Kaempferia pandurata* Roxb., on inhibition of oral biofilms and MMP expression by conducting experimental periodontal disease models *in vitro* and *in vivo*. Oral biofilms were formed by multi-species oral colonizers, including *Streptococcus mutans*, *S. sanguis*, and *Actinomyces viscosus*. Human gingival fibroblasts and oral epithelial cells induced by *Porphyromonas gingivalis* were employed as the *in vitro* oral culture models for the expression of MMPs and their signaling pathways. The effect of panduratin A on attenuating the expression of MMP-dependent gingival inflammation was also evaluated by conducting *in vivo* test using lipopolysaccharide (LPS)-induced rat gingiva. Our findings suggest that panduratin A possesses anti-biofilm activity by eliminating oral bacterial colonization during early dental plaque formation. Panduratin A may control periodontal inflammation involving MMP-2 and MMP-9 induction in human gingival fibroblasts and oral epithelial cells *in vitro*. In oral epithelial cells, panduratin A decreases MMP-9 gene expression by suppressing *P. gingivalis* supernatant-stimulated AP-1 activation which may be mediated by blocking ERK1/2 and JNK phosphorylation. Meanwhile, in gingival fibroblasts, panduratin A inhibits *P. gingivalis* supernatant-stimulated MMP-2 gene expression through down-regulating CREB signaling that may be facilitated by attenuation of p38 phosphorylation. Panduratin A also attenuates the expression of MMP-9 secretion, protein, and mRNA in LPS-induced rat gingival inflammation *in vivo*, thus subsequently resulting in the improvement of the gingival tissue morphology. With dual potent activities as antibiofilm and anti-MMP-9 agent, panduratin A could be applied as a promising candidate in periodontotherapy. **References:** 1. Yanti et al. (2009). J Oral Sci 51(1), 87 – 95. 2. Yanti et al. (2009). Biol Pharm Bull 31(1), 110 – 115. 3. Yanti et al. (2009). Biol Pharm Bull 31(10), 1770 – 1775.

SL-14 *In silico* access for the discovery of 11β-HSD1 inhibiting triterpenes from *Eriobotrya japonica*

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An elevated activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) has been associated with metabolic disorders including obesity and type 2 diabetes, with recent findings providing evidence for beneficial effects of 11β-HSD1 inhibitors, making this enzyme a promising therapeutic target [1]. Recently we investigated different extracts of traditionally used anti-diabetic medicinal plants for potential anti-glucocorticoid effects. Among them, the extracts of E. japonica (Thunb.) Lindl. showed a dose-dependent inhibition of 11β-HSD1, with a preferential inhibition of 11β-HSD1 versus 11β-HSD2 measured in cell lysates. These results were confirmed in intact stably transfected HEK-293 cells [2]. In this study the loquat leaves were phytochemically investigated following predictions of a pharmacophore-based virtual screening. Determination of the 11β-HSD1 and 11β-HSD2 inhibitory activities in cell lysates of virtually predicted hits in combination with a bioactivity-guided approach revealed triterpenes from the ursane type as selective, low μmolar inhibitors of 11β-HSD1: corosolic acid (IC50 0.81 μM), 3-epicorosolic acid methyl ester (IC50 5.2 μM), 2-α hydroxy-3-oxo urs-12-en-28-oic acid (IC50 17 μM), tormentic acid methyl ester (IC50 9.4 μM), and ursolic acid (IC50 1.9 μM). Intriguingly, a mixture of loquat constituents with moderate activities displayed a pronounced additive effect. By means of molecular modeling studies and the identification of the 11β-HSD1 inhibiting 11-keto-ursolic acid (IC50 2.1 μM) and 3-acetyl-11-keto-ursolic acid (IC50 1.3 μM) a structure-activity relationship was deduced for this group of pentacyclic triterpenes [3]. The mechanism elucidated in this study together with previously determined pharmacological activities provides this class of compounds from loquat with an astonishing multi-targeted anti-diabetic profile. **Acknowledgements:** This work was granted by the Austrian Federal Ministry of Science and Research and the Austrian Federal Ministry of Health within the Project "Treatment of Metabolic Syndrome by Traditional Chinese Medicine", and the Swiss National Science Foundation, N°31003A-124912. **References:** 1. Odermatt A; Nashev LG (2010) J Steroid Biochem Mol Biol 119: 1 – 13. 2. Gumy C; et al. (2009) Fitoterapia 80: 200 – 5. 3. Rollinger JM; et al. (2010) Bioorg Med Chem 18: 1507 – 15.

SL-15 Lupeolic acid derivatives from *Boswellia* species as inhibitors of the cytosolic phospholipase $A_{2\alpha}$

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Resins derived from *Boswellia* species are traditionally used in chronic inflammatory diseases like rheumatoid arthritis, inflammatory bowel diseases or psoriasis. Recent data disclose a higher risk for patients suffering from these diseases to be affected by occlusive vascular events which partly can be attributed to an elevated activation state of blood platelets [1, 2, 3]. Here, we analyzed the impact of different triterpenic acids isolated from *Boswellia carterii* on the activity of cytosolic phospholipase (cPL)$A_{2\alpha}$. This enzyme plays a central role in inflammation by catalyzing the release of arachidonic acid (AA) from the sn-2 position of phospholipids in cellular membranes [4]. Using a cell-free assay of (cPL)$A_{2\alpha}$ activity we found that two thus far unidentified lupeolic acid derivatives, that is 3α,28-dihydroxylup-20(29)-en-4β-oic acid (OH-LA) and 3α-acetoxy-28-hydroxylup-20(29)-en-4β-oic acid (Ac-OH-LA) act as potent (cPL)$A_{2\alpha}$ inhibitors (IC_{50}= 5 and 12 μM, respectively). Their inhibitory potential on AA release could also be observed in cell-based assays using A23187-stimulated blood cells. In stimulated human platelets the lupeolic acid derivatives also inhibited the formation of metabolites of AA which was restored by supplementation of exogenous AA, supporting selective impact of the compounds on PLA_2 activity. Moreover, OH-LA and Ac-OH-LA inhibited platelet aggregation induced by collagen but not after stimulation with U46619, a thromboxane receptor agonist. Together, we identified two novel hydroxy-derivatives of lupeolic acid from *Boswellia carterii* which act as inhibitors of (cPL)$A_{2\alpha}$. These data suggest a potential of *Boswellia* extracts as anti-inflammatory remedy associated with possible cardiovascular protective functions in chronic inflammatory diseases. **References:** 1. von Hundelshausen P. et al. (2007) Circ Res. 100(1):27 – 40. 2. Collins CE. (1997) Aliment Pharmacol Ther. 11(2):237 – 47. 3. Mallbris L. et al (2004) Eur J Epidemiol. 19(3):225 – 30. 4. Diez E. Et al. (1992) J Biol Chem. 267(26):18342 – 8.

SL-16 Detection of trypsin inhibition and antioxidant effects on TLC

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Activities of plant extracts in cosmetic preparations include trypsin inhibition and antioxidation. Trypsin inhibition detection was adapted from a HPLC procedure [4]. TLC plates sprayed with 4 mg/mL trypsin in TRIS buffer pH 8.2, left at 25ô¤ for 10 min, then sprayed with 1 mg/mL NA-benzoyl-L-arginine 4 nitroanilide (L-BAPA) in TRIS buffer pH 8.2 gave white zones against yellow background for inhibitors after 30 min at 25ô¤; minimum detecta⁄le amount (MLA) of positive control hexamidine diisethionate 1 μg. In situ TLC detection of oxidation of unsaturated lipids through malondialdehyde formation using thiobarbituric acid (TBA) was used [2]. Plates were sprayed with 2.5% v/v linseed oil in dichloromethane, dried for 5 min, then sprayed with 15% aq. perchloric acid, heated at 37ô¤ for 10 min and sprayed with 1% TBA in 50mM aq. NaOH. Antioxidants appear as pale zones against a pink background after 10 min. MDA of positive control propyl gallate 1 μg. A modification of the Griess reaction was used to detect NO scavengers [3]. The developed plate was sprayed with 20mM aq. sodium nitroprusside, left in full daylight for 10 min at 25ô¤ then sprayed with 1% w/v sulfanilimide in 2% aq. phosphoric acid, followed immediately by 0.1% N-(1-naphthyl)ethylene diamine in 2% aq. phosphoric acid. NO scavengers appear after 20 min as pale zones against a purple background; MLA of positive control carboxy-PTIO potassium salt 100 ng. **References:** 1. Dickson, R. et al. (2006) Phytother. Res. 20:41 – 45. 2. Ozgen, U. et al. (2006) Pharm. Biol. 44: 107 – 112. 3. Fox, J.B (1979) Analyt. Chem. 51:1493 – 1502. 4. Schlüter, H. et al. (2008) Anal. Bioanal. Chem. 392: 783 – 789.

SL-17 Search for α-glucosidase and α-amylase inhibitors from Indonesian medicinal plants

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One effective way to treat diabetes is to suppress carbohydrate digestion due to utilization of α-amylase and/or α-glucosidase inhibitors. The objective of our research was to explore the potential of Indonesian medicinal herbs for putative antidiabetic activity via inhibition of α-amylase and α-glucosidase. In vitro activity was determined via inhibition of sucrose and/or maltose hydrolysis by rat intestinal α-glucosidase, and starch azure hydrolysis by porcine pancreatic α-amylase. Among 57 samples that were found to have strong inhibitory activity against α-amylase and/or α-glucosidase, 17 species are here reported for the first time to be potential anti-diabetic herbs. Among these, *Macaranga tanarius* leaves inhibited both α-glucosidase and α-amylase. Five ellagitannins showing inhibitory activity against rat intestinal maltase were successfully isolated and identified. Of the isolated compounds, two were new and were named macatannins A (IC 50 = 0.80 mM) and B (IC 50 = 0.55 mM), while the other compounds were identified as mallotinic acid (IC 50 > 5.00 mM), corilagin (IC 50 = 2.63 mM), and chebulagic acid (IC 50 = 1.00 mM).

SL-18 Quinone reductase inducing polyacetylenes of the Araliaceae: Effects of structure and configuration on activity

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Consumption of "indirect antioxidants" – compounds that increase the production of antioxidant proteins in vivo – is a promising means to prevent or delay diseases in which oxidative stress plays an underlying role. Indeed, indirect antioxidants show potent protective action in animal models of cancer, cardiovascular disease, Parkinson's disease and ischemia. Indirect antioxidants act by increasing the activity of Nrf2, a transcription factor responsible for the constitutive and inducible expression of antioxidant and detoxifying proteins such as quinone reductase (QR), heme oxygenase-1 and superoxide dismutase. In order to identify novel activators of Nrf2, we used the activity of the representative enzyme, QR, as a guide to isolate the active components from the methanolic extracts of two members of the Araliaceae from Canada, *Oplopanax horridus* and *Aralia nudicaulis*. Several related polyacetylenes with potent activity were isolated and a number of structure activity-relationships were observed. Importantly, the stereochemical configuration of these compounds directly impacted their relative potencies as QR inducing agents.

SL-19 Relationship between chemical structure and antioxidant activity of luteolin and its glycosides isolated from *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus*

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The genus *Thymus* (Lamiaceae) is represented by about 200 species worldwide [1]. There are 39 (64 taxa) *Thymus* species in Turkey, 27 taxa of which are endemic [2 – 4]. *Thymus sipyleus* Boiss. subsp. *sipyleus* var. *sipyleus* is an endemic species which grows widely in Turkey [2] and is used as a spice in Turkey [5]. One triterpenic acid (ursolic acid), one phenolic acid (rosmarinic acid), and four flavonoids (luteolin, luteolin 7-O-(6″-feruloyl)-β-glucopyranoside, luteolin 5-*O*-β-glucopyranoside, and luteolin 7-*O*-β-glucuronide) were isolated from the aerial parts of *T. sipyleus* subsp. *sipyleus* var. *sipyleus* and identified. Afterwards, in vitro lipid peroxidation inhibition effects of the compounds were determined using TBA test methods in a bovine brain liposome system. All compounds inhibited lipid peroxidation in various degrees except for ursolic acid. The order of the lipid peroxidation activities of luteolin and its glycosides were: Luteolin 7-*O*-β-glucuronide > luteolin 5-*O*-β-glucopyranoside > luteolin 7-*O*-(6″-feruloyl)-β-glucopyranoside > rosmarinic acid > luteolin. However, the activity order of the compounds was completely different in DPPH radical-scavenging activity. None of the compounds show Fe chelating activity. **References:** 1. Evans W.C. (1989). Trease and Evans' Pharmacognosy, 13th ed. Bailliere Tindall: London, Great Britain. 2. Jalas J. (1982). *Thymus* L., in Flora of Turkey and the East Aegean Islands, Vol. 7, pp. 349 – 382, edited by P.H. Davis, University Press, Edinburgh, UK. 3. Davis P.H., Mill R.R., Kit T. (1988). Flora of Turkey and the East Aegean Islands, Vol. 10, pp. 209 – 504, University Press, Edinburgh, UK. 4. Güner A., Özhatay N, Ekim T., Baser K.H.C. (2000). Flora of Turkey and the East Aegean Islands, Vol. 11, p. 209, University Press, Edinburgh, UK. 5. Özgen U. et al. (2004). Econ. Bot. 58:691 – 696.

SL-20 Anti-inflammatory potential of flavonoid fraction of *Tamarindus indica* Linn (seeds)

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The present study was designed to investigate anti-inflammatory potential of flavonoid fraction of *Tamarindus indica* Linn (seeds) in rodents. The flavonoid fraction was prepared from methanol extract (successive) of *T. indica* seeds (Family: Caesalpiniaceae) by the method of Shinoda [1]. Anti-inflammatory activity was studied by carrageenan, histamine and serotonin induced rat paw edema [2], prostaglandin inhibitory activity [3], acetic acid induced capillary permeability [4], sodium CMC induced leukocytes emigration [5] and cotton pellets induced granuloma test [6] at the dose levels of 12.5, 25 and 50 mg/kg. Indomethacin, aspirin, prednisolone and dexamethasone were used as standard drugs for the respective model. The results were analyzed by One-way analysis of variance (ANOVA) followed by Dunnet's t-test. Oral administration of flavonoid fraction of *T. indica* showed, dose dependent inhibition of edema in acute phase of inflammation. It also significantly ($p < 0.01$) inhibited castor oil induced diarrhoea in mice. Acetic acid induced capillary permeability and total leukocyte count was significantly ($p < 0.01$) inhibited by the flavonoid fraction in a dose dependent manner. The weight of the granuloma formation was significantly ($p < 0.01$) decreased, indicating its effectiveness in the proliferative phase of inflammation. The results obtained suggest the anti-inflammatory effect of flavonoid fraction of *T. indica* seeds in acute and chronic phase of inflammation. **References:** 1. Shinoda, J. (1928) Pharma Soc Jpn. 48 (3): 214 – 220. 2. Winter, C et al. (1962) Proceedings of the Society for Expt. Biol. Med., (111): 544 – 547. 3. Awouters, F. et al. (1978) J Pharm Pharmacol. 30 (1): 41 – 45. 4. Whittle, B. (1964) British J Pharmacol. 22: 246 – 253. 5. Qin C et al. (2001) Chinese Pharmacol. Bull. 17: 715 – 716. 6. Winter, A. (1957) J Ameri. Pharmace. Asso. Sci. Ecol. 46: 515 – 519.

SL-21 Potent immunostimulatory and antioxidant activities of three – (−) catechin-O-rhamnosides from Eastern Nigeria mistletoe

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Mistletoes of Eastern Nigeria origin, Loranthus micranthus, harvested from our local Kola tree, Kola acuminata, was recently reported to be

the most potent specie in terms of antidiabetic [1], immunostimulatory activities [2, 3]. Furthermore, an unpublished data showed that this mistletoe possesses pronounced in vitro antioxidant activities when compared to ascorbic acid. In bid to study the chemical composition of this specie of mistletoe, elaborate bioassay-guided fractionation and purification led to the isolation of three rhamnoside based glycosides namely; – (−) catechin-7-O-rhamnoside, – (−) catechin-3-O- rhamnoside and a 4′-methoxy derivative of the 7-O-rhamnoside. Characterization and unequivocal structural assignments of these compounds were achieved by a combination of UV/visible, IR, NMR, MS, COSY experiments including, HMBC and HQSC. These flavonoid glycosides exhibited very potent immunostimulatory activity on cell line (C57Bl/6 splenocytes) against Lipopolysaccharide and Concanavalin A standards. Their measured antioxidant potentials in terms of effective concentration at 50% reduction in diphenyl picrazyl hydrazyl radical activity (EC50) were remarkably high (< or = to 55. 42 ± 0.99 mg/ml) when compared to ascorbic acid (17.6 ± 1.78 mg/ml) We are herein reporting, for the first time, the presence and some biological activities of three catechin-based rhamnoside glycoside in mistletoe harvested from Eastern Nigeria **Acknowledgements:** The authors specially acknowledge Mr. Alfred Ozioko of BDCP, Nsukka for collection and identification of the plant material. **References:** 1. Osadebe PO, et al (2004) J Ethnopharmacology, 95: 133 – 138. 2. Osadebe PO, et al (2009) J Ethnopharmacology, 125:287 – 293. 3. Osadebe et al (2008), RPMP Vol 27: 473 – 485.

SL-22 Cytotoxic abietane diterpenes from *Peltodon longipes* and their mode of action

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Peltodon longipes (Labiatae), also known as "baicuru amarelo" in south of Brazil, has been used in folk medicine as an anti-inflammatory and antiseptic remedy (1). The effective compounds responsible for these effects are still unknown. Here we report the isolation and structure elucidation of 12 diterpenes from the abietane type. 12,14-dihydroxy-11-methoxyabieta-8,11,13-trien-7-one was isolated for the first time, whereas the other diterpenes have already been known. Structure elucidation was based on 1D-(^{1}H and ^{13}C) and 2D NMR experiments (COSY, HMQC, HMBC and NOESY) as well as EIMS. Cytotoxic activity of these compounds was evaluated against two different cancer cell lines, MIA PaCa-2 (Human Pancreatic Carcinoma Cell Line) and MV-3 (Human Melanoma Cell Line). 7α acetoxyroyleanone, the major compound, showed the highest cytotoxic activity with an IC_{50} value of 4.7 and 7.5 μM, respectively. Interestingly, some diterpenes preferentially inhibited topoisomerase-I in the relaxation assay and gave lower IC_{50} values (2.8 and 4.7 μM) than the known classical topoisomerase-I inhibitor camptothecin (IC_{50} 28 μM). Docking experiments revealed that these diterpenes may target topoisomerase-I differenzially to camptothecin. While camptothecin stabilizes type I topoisomerase-DNA complexes, there is evidence that the diterpenes compete with DNA for topoisomerase binding through direct interaction. A new binding side is suggested which allows correlation with the experimental data. Additionally studies are in progress to give further insights in the mechanism of action. **Acknowledgements:** Financial support from the government Baden-Württemberg (Zukunftsoffensive IV) is gratefully acknowledged. **References:** 1. Mentz, L.A. et al. (1997) Caderno de Farmácia, 13: 25 – 48.

SL-23 Treatment of equine sarcoid with the mistletoe extract ISCADOR® P (*viscum album austriacus*) – a double-blind placebo controlled study

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Equine Sarcoid (ES) is the most common skin tumour in horses with high recurrence rate. No single universal treatment is effective. Viscum album extracts (VAE) are used as adjunct in the treatment of human cancer. We hypothesized that therapy with VAE (Iscador® P) is also effective in Equine Sarcoids. Of 53 horses with ES, 42 were treated solely with VAE or placebo as monotherapy and 11 were treated after selective excision of ES. Horses were randomly assigned to the VAE (n = 32) or placebo group (n = 21). Horses received increasing concentrations of VAE from 0.1 mg/ml to 20 mg/ml or physiological NaCl solution 3 times a week over 105 days s.c. Number, localization, size and type of the ES were documented over 12 months. Cure or improvement rate (decrease of tumour size by 50% or more in at least half of the tumours) were assessed after one year. Sarcoid level assessment included analysis of 1 to 7 clinically relevant sarcoids per horse. In the VAE group, 13 horses (41%) showed improvement. Of these, 9 patients showed complete remission (28%). In the control group only 3 cases (14%), all showing complete remission, were classified as improved. In the VAE group 27 ES (38%) showed complete remission and 48 (67%) improvement after one year compared to 9 ES with complete remission (13%; n. s.) and 17 ES (40%; $p < 0.01$) with improvement in the placebo group. VAE (Iscador® P) represent a safe and effective treatment for ES, particularly in cases with multiple sarcoids.

SL-24 Natural phenolics and nuclear receptors (ER & PPAR): Isolation, structure determination and biological evaluation

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Plants originated mainly from Mediterranean region as well as from other higly deverse hot-spots all over the world have been phytochemically explored in the context of our continuing study towards the discovery of novel, active natural products presenting high affinity to nuclear reseptors. In particular estrogen receptor (ER) and peroxisome proliferator-activated receptor (PPAR) were the target-reseptors under investigation. Our study is mainly focused to the identification of secondary metabolites which present the capability to activate both isoforms of estrogen receptor (ERα & ERβ) [1] as well as the two isoforms of the peroxisome proliferator-activated receptor (PPARγ & PPARδ) [2]. Numerous of secondary metabolites belonging mostly to simple phenolics, to flavonoids and specifically to flavones, flavonols and methylether derivatives thereof and isoflavonoids mainly isoflavones, prenylated isoflavones and 2-arylbenzofurans have been identified using a bioguided approach or LC-MS-based methodologies. The structure elucidation of the isolated compounds was performed via HRMSn and 1 & 2D NMR [3], [4], [5], [6]. The biological evaluation was focused on the determination of the relative binding activity (RBA values) of the purified compounds to ERα and ERβ and/or PPARγ and PPARδ as well as on the exploration of the biological michanism of action using e-screen, reporter gene and analysis of gene expression assays. Determination of their proliferative and/or cytotoxic properties was also performed. A series of novel natural compounds belonging mostly to 2-arylbenzofurans and prenylated isoflavones were identified whereas a numerous thereof found to present significant estrogenic, antiestrogenic, SERM and/or antidiabetic activity [7], [8], [9]. **References:** 1. Miksicek, RJ, (1993) Mol. Pharmac. 44: 37 – 43. 2. Knouff, C. and Auwerx, J. (2004) Endoc. Rev. 25: 899 – 918. 3. Halabalaki, M et al., (2000) J. Nat. Prod. 63 (8): 1672 – 1674. 4. Halabalaki, M et al., (2006) Planta Medica 72, (6) 488 – 493. 5. Halabalaki, M et al., (2008) J. Nat. Prod. 71 (11): 1934 – 1937. 6.Djiogue, S. et al. (2009) J. Nat. Prod. 72 (9): 1603 – 1607. 7. Katsanou, SE (2007) J. Ster. Biochem.

Mol. Biol. 104 (3 – 5): 228 – 36. 8. Dontas I, (2006) Maturitas 53 (2): 234 – 242. 9. Arapogiani N-E (2007), 45th Int. Meeting & Ann. Meeting of GA, Graz, Austria.

SL-25 High-throughput natural products chemistry: Is it possible?

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Sequoia Sciences identifies novel chemistry from its library of structurally diverse small molecules isolated from plants. The proprietary design of this library allows for the screening of these compounds at optimal HTS concentrations without non-drug-like interferences. Sequoia built this analytical process such that rapid isolation and structure elucidation of active compounds could be accomplished. Using the extremely sensitive CapNMR probe, structure elucidation of active compounds is completed on samples of limited mass. Relative to synthetic library HTS, the question remains, is it really high-throughput natural products chemistry? Can the rate limiting step, structure elucidation process, be a higher throughput process? The scientific strategy that Sequoia employs in order to rapidly uncover the chemical diversity contained in plant natural products will be outlined. This presentation will expand upon the ground breaking CapNMR probe by describing the MultiSampleTM CapNMR probe. This advanced capillary NMR probe acquires complete NMR data sets on two samples simultaneously. The Protasis Dual SampleTM Probe (DSP) has now extended the high-throughput process to include NMR data acquisition. This talk outlines sample loading and data acquisition ease of the DSP probe, presenting data on biologically active compounds isolated from preparative HPLC fractions from Sequoia's library. By essentially achieving the same performance for each sample as achieved using a single sample CapNMR probe, the DSP probe provides a doubling of throughput in 1D proton as well as all gradient 2D experiments. Sequoia's inclusion of the DSP probe compliments its current platform technologies for high-throughput natural products research.

SL-26 Challenges in the discovery and development of novel antibiotics – will there be a new drug derived from natural sources?

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Bacterial infections are an ongoing threat to the individual human health status as well as for the societies. The main challenge is resistance to multiple antibiotics which is spreading throughout the world induced through intensive use of existing antibiotics, seriously challenging our ability to treat bacterial infections successfully. Consequently, there is an urgent need for novel (class) antibacterial (AB) compounds without cross-resistance to antibiotics already in use. Global sales of antibiotics are substantial at $33,919 m with significant growth (2005) of 8%. However, in the last two decades only very few new antibiotics reached the market; the market growth is thus dominated by the only two new AB compounds classes launched within the last two decades, Linezolide (Pfizer), and the natural product Daptomycin (Cubist). This lack in success as well as market potential resulted in a decline in R&D efforts – many big Pharma companies abandoned their AB internal programs. What are the technical obstacles for AB discovery? What are the Market and regulatory obstacles? This presentation attempts to summarise challenges in the identification and development of new AB compounds, incl. those derived from natural sources as well as to reflect currently applied strategies to overcome them, incl. successful examples. **References:** 1. J.A. Leeds, E.K. Schmitt, P. Krastel (2006), Expert Opin. Investig. Drugs 15(3). 2. K.M. Overbye, J.F. Barrett (2005), DDT 10: 45. 3. D.J. Payne, M.N. Gwynn, D.J. Holmes, D.L. Pompliano (2007), Nature Rev. Drug. Disc. 6: 29.

SL-27 Silexan, an orally administered Lavandula oil preparation, is effective in the treatment of anxiety disorders and related conditions

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Four clinical trials investigating the efficacy of Silexan in anxiety and related conditions have been completed: (1) In a 10 week, double-blind, randomized study with 221 adults suffering from subsyndromal anxiety 80 mg/d silexan was superior to placebo in reducing the total scores of the Hamilton Anxiety Scale and the Pittsburgh Sleep Quality Index (HAMA, PSQI; $p < 0.01$), and significantly more patients receiving silexan were responders or in remission; ($p < 0.01$) [1]. (2) In 77 patients with generalized anxiety disorder (GAD) 80 mg/d silexan and 0.5 mg/d lorazepam administered double-blind for 6 weeks were comparably efficacious in reducing the HAMA total score and in improving various other measures of anxiety, global impairment and sleep [2]. (3) Another randomized, double-blind, 10-week trial compared 80 mg/d silexan and placebo in 170 patients with anxiety accompanied by restlessness and disturbed sleep. Silexan was superior regarding HAMA total score reduction ($p < 0.05$) and in improving disturbed sleep (PSQI total score: $p = 0.09$), and the patients treated with the herbal drug showed higher responder ($p < 0.05$) and remission rates ($p = 0.10$). (4) In an open-label study the effect of 80 mg/d silexan on neurasthenia and post-traumatic stress disorders (PTSD) was investigated ($n = 50$). During 10 weeks of treatment the patients showed comparable, significant improvements for anxiety, depressed mood, and for waking up frequency and duration ($p < 0.01$) [3]. In all trials silexan was very well tolerated and showed no sedative effects. The results support the efficacy of silexan in anxiety, and show perspectives for further investigations in related indications. **References:** 1. Kasper S., et al. (submitted for publication). 2. Woelk H., Schläfke S. (2010) Phytomedicine 17: 94 – 99. 3. Stange R., et al. (2007) Focus on Alternative and Complementary Therapies 12 (Suppl. 1): 46.

SL-28 Sinupret® – an updated pharmacological view on a success story

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Rhinosinusitis is a common disease in countries with a western lifestyle; its incidence has increased considerably in the past few years. The pathophysiology of rhinosinusitis is dominated by inflammatory processes in the upper airways induced by viral or bacterial infection resulting in obstruction of drainage and ventilation in the nasal cavity and paranasal sinuses. Based on these pathological challenges, the aim of an effective therapy is the restoration of drainage and ventilation. Sinupret®, developed by Bionorica SE, is a uniquely composed herbal medicinal product containing five different herbal components (Gentianae radix, Primulae flos cum calycibus, Rumicis herba, Sambuci flos and Verbenae herba) as active principle. For Sinupret® multiple pharmacodynamic properties were evaluated and will be summarized related to the main indication – acute and chronic rhinosinusitis. Strong antimicrobial activities were determined by in vitro bioassays using microdilution-methods. The minimal bactericidal concentration (MBC) was calculated for gram-positive and -negative bacterial strains, relevant for upper airway infections. A substantial antiviral activity of non-toxic concentrations of Sinupret® against a broad panel of human pathogenic viruses causing infections of the upper respiratory tract was shown after treatment of the infected cells using plaque-reduction assays, analyses of cytopathogenic effects and ELISAs for viral proteins. Sinupret® inhibits expression and activity of pro-inflammatory cytokines in vitro and mediates significant anti-inflammatory therapeutic activity in in vivo inflammation models. Secretolytic effects of Sinupret® lead to activation of the secrete-producing cells and to increased bronchial secretion which was demonstrated in different in vivo models.

SL-29 Safety and tolerability of EPs® 7630 (Umckaloabo®)

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Herbal medicines play an increasingly important role in the perception of physicians and patients looking for equally effective, albeit safer approaches to conventional management of respiratory tract infections (RTIs). This review systematically presents the available evidence on the safety and tolerability profile of EPs® 7630. EPs® 7630 is a herbal drug preparation from the roots of Pelargonium sidoides (1:8 – 10), extraction solvent: ethanol 11 % (w/w), and is widely used in several countries for RTI treatment. Based upon the study reports of all 25 clinical trials as well as post-marketing surveillance studies with EPs® 7630 completed by 2006, this review includes data from 9218 patients with acute or chronic RTIs such as tonsillopharyngitis, sinusitis or bronchitis and from 31 healthy subjects. EPs® 7630 was well tolerated and its safety is underlined by the fact that no serious adverse drug reactions were reported in this large patient population. Comparing EPs® 7630 and placebo groups, adverse events were similar with regard to quality and quantity throughout almost all organ systems and symptoms. The only differences were a slightly higher incidence of gastrointestinal disorders, namely epigastric pain, nausea and diarrhoea, and of hypersensitivity reactions (mostly skin reactions), as well as gingival bleeding and epistaxis associated with EPs® 7630 treatment compared to placebo. Data on treatment-emergent changes in liver enzymes from placebo-controlled trials gave no indication of an unfavourable influence of EPs® 7630. EPs® 7630 therefore is a well-tolerated herbal medicine in the management of RTIs in children from 1 year of age and adults alike.

SL-30 Silibinin in the treatment of chronic hepatitis C – A review of the latest preclinical and clinical findings

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Aims: Silymarin, the extract from milk thistle fruits (Silybum marianum) has been used as a hepatoprotectant since ancient times. The potential benefit of silymarin in the treatment of viral induced liver diseases continues to be discussed controversially. Silibinin, a major component of silymarin, is a well known potent scavenger of several oxidizing species. It has been shown in the past that oxidative stress might mediate HCV-induced activation of STAT-3 and at the same time to impair IFN-alpha signalling, causing resistance to the antiviral action of IFN-alpha in chronically HCV infected patients. Consequently, the hypothesis has been developed that antioxidative properties of silymarin or its constituent silibinin may theoretically improve the response in nonresponder hepatitis C patients to the standard of care (SOC) antiviral therapy with pegylated interferon and ribavirin (PegIFN/RBV). **Clinical findings:** An investigator intiated a multicenter, open-label clinical trial (Prof. Ferenci, University Hospital of Vienna) titled "Nonresponder Study: Multicenter Study to Evaluate the Efficacy of Silymarin in Addition to Combination Therapy with Pegylated Interferon Alfa 2A and Ribavirin in Patients with Chronic Hepatitis C". The objective of the trial was initially to use silibinin as an antioxidant in hepatitis C patients not responding to SOC. Unexpectedly, silibinin administered parenterally, showed a powerful antiviral activity. A recently done interims analysis showed at week 25 that 57 % of the patients (still under PegIFN and RBV combination therapy) were HCV RNA negative. Another therapeutic approach of intravenous administration of silibinin in patients non responding to SOC is the so called "rescue treatment". After completing at least 24 weeks of SOC therapy non-responding patients received additionally 20 mg/kg/d silibinin intravenously for 15 days. Thereafter PegIFN/RBV was continued. It could be shown that silibinin is effective on treatment of nonresponders to full dose of PegIFN/RBV combination therapy. A slightly different approach has been followed by Biermer & Berg in Germany who used silibinin for the successful treatment of a severe CHC-patient in the context of ribavirin. **Pre-clinical findings:** Meanwhile, the ability of silibinin to inhibit HCV enzymatic functions and replication has been tested in HCV RNA-dependent RNA polymerase (RdRp) and NS3/4A protease enzyme assays. Its ability to inhibit replication of an HCV genotype 1b replicon model and the JFH1 infectious HCV model in cell culture was also studied. It could be shown that silibinin inhibited RdRp function and also inhibited both HCV genotype 1b replicon replication and HCV genotype 2a strain JFH1 replication in cell culture. **Summary:** Intravenous silibinin has been shown to be a potent antiviral agent in patients with chronic HCV not responding to standard therapy with pegylated interferon plus ribavirin. Moreover, silibinin has been found to be a direct inhibitor of hepatitis C virus RNA-dependent RNA polymerase in vitro.

SL-31 Approaches for the integration of phytochemicals into classical cancer chemotherapy by modulation of multi-factorial drug resistance

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Drug resistance has dogged oncology since decades. Overcoming drug resistance is urgently required to improve chemotherapy. The multiplicity of drug resistance implies that gene expression profiles predict drug resistance with higher accuracy than single gene expression studies. We analyzed different tumor types by microarray-based mRNA and immunohistochemistry-based protein analyses. Data were subjected to hierarchical cluster analyses. Expression profiles predicted drug response and survival times of patients. This may facilitate the development of individualized therapy options in the future. Strategies are to use either non-cross-resistant phytochemicals or natural products modulating resistance to standard anticancer drugs. We present three examples how natural products and their derivatives modulate resistance to established cancer drugs. (1) The anti-malarial artemisinin from Artemisia annua L. and its semi-synthetic derivatives inhibit the ATP-binding cassette (ABC) transporters P-glycoprotein/MDR1 and MRP1, which contribute to multidrug resistance phenotype of tumors and to the blood brain barrier. (2) Artesunate exerts synergistic activity in combination with Erlotinib, a small molecule tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR) in transfected human glioblastoma multiforme cells. The inhibition of specific kinases in the EGFR downstream signaling pathways is responsible for this synergism. (3) Artesunate synergistically interacts with Rituximab, a monoclonal antibody against CD20 to treat Non-Hodgkin lymphoma. Rituximab affects the transcription factors NF-kappa B, Sp1, and YY1, which leads to altered expression profiles of anti-oxidant stress response or apoptosis-regulating genes, thereby increasing the activity of artesunate towards Non-Hodgkin lymphoma cells.

SL-32 Deoxyelephantopin suppresses lung metastasis of B16 melanoma in mice

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Elephantopus scaber L. (Asteraceae) is a traditional herbal medicine claimed for anti-cancer effects. We evaluated the in vitro and in vivo efficacy of a major sesquiterpene lactone constituent of E. scaber, deoxyelephantopin (DET), for its anti-B16 melanoma cell activity and the underlying molecular mechanism. Our data show that DET could induce cell cycle arrest at G2/M phase at 5 mM, with the decrease of cyclins A, B1, and D1 protein expression. When increased DET concentration to 8 mM, apoptotic hallmarks PARP and caspase 3 were activated in a time-dependent manner. DET also inhibited B16 cell migration accompanied with inhibiting of MMP-9 activity. A non-invasive real-time in vivo imaging system to monitor the melanoma cell growth and metastasis in mice is created in this study. The stable B16 melanoma cell clone carrying COX-2 promoter driven-luciferase gene was established and used to comparative study of the efficacy of DET and a chemotherapeutic drug cisplatin in C57BL/6J mice. We observed that Pre-DET10 (10 mg/kg BW), and cisplatin (CP, 2 mg/kg BW) have similar profound effect on inhibiting lung metastasis of B16 melanoma and increase of median survival rate in tested mice (tumor control: 33 days, pre-DET10: 43 days, CP2: 43 days). Notably, Pre-DET10 treatment has little or no side-effects compared to cisplatin treatment in mice. This report pre-

sents the novel pharmacological effect of DET which may be worthy for further development into an agent against skin melanoma.

SL-33 Experimental evaluation of antiulcer and gastrointestinal effects of *Bridelia ferruginea* stem bark

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The methanol extract (ME) of dried powdered stem bark of *B. ferruginea* obtained by 48 hr cold maceration was subjected to solvent guided fractionation in a silica gel column using petroleum ether, dichloromethane and methanol successively to yield the petroleum ether (PF), dichloromethane (DCMF) and methanol (MF) fractions. The extract (ME) and fractions (DCMF and MF) were assessed for antiulcer activity using indomethacin and ethanol induced ulcers in rats. The effects of the extract and fractions on small intestinal transit was studied using charcoal meal test, also the effect of extracts and fractions on isolated guinea pig ileum and acetylcholine induced contractions of the isolated guinea pig ileum were studied. The extracts and fractions were assessed for acute toxicity and lethality (LD 50)1 and phytochemical constituents (2,3). Collected data were analyzed using one way ANOVA and further subjected to LSD Post Hoc tests. The extract (200 and 400 mg/kg) and fractions (200 and 400 mg/kg) significantly ($P < 0.05$) protected the rats against ethanol and indomethacin-induced ulcers, and inhibited small intestinal propulsion in the order DCMF >MF>ME. In addition, MF (0.03 – 4.27 mg/ml) elicited no inhibition, while DCMF(3.3 – 27 µg) antagonized acetylcholine-induced contractions of the guinea pig ileum; whereas ME (0.03 – 4.27 mg/ml) contracted the guinea pig ileum. Oral LD 50 was estimated to be 2,154 mg/kg. The results suggest that the stem bark of *Bridelia ferruginea* contains constituents with antiulcer potency, in addition to non-polar spasmolytic and polar spasmogenic constituents. **References:** 1. Lorke D. (1983) Arch. Toxicol. 53: 275 – 289. 2. Harborne JBC. (1973) Phytochemical methods. London: Chapman and Hall;. p. 279. 3. Trease GE, Evans WC. (1983) Drugs of biological origin. In: Pharmacognosy 12th ed. United Kingdom: Balliere Tindall. p. 309 – 540.

SL-34 Effects of 20-OH ecdysone, a spinach (*Spinacia oleracea*) derived steroid, on metabolic and temperature regulatory parameters

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Ecdysteroids have protein anabolic effects and spinach (spinacia oleracea) derived β-ecdysone prevents osteoporosis and deterioration of joint cartilage tissue in ovariectomized (ovx) rats [1,2]. It is devoid of estrogens or androgenic properties. In the present study we investigated the effects of Ecd on lipids and oral glucose tolerance test. Ecd augments effects of gamma butyric acid (GABA) in neurons and since GABA-ergic drugs are effective to ameliorate hot flashes in climacteric women, we also investigated the effects of β-ecdysone on hot flashes utilizing a newly developed technique to measure skin temperature in freely moving ovx animals. Rats were either fed with soy free food or with Ecd containing food (18 mg/animal/day for 3 months). An oral glucose tolerance test was performed 4 weeks after ovx. Ecd significantly reduced serum triglycerides, total cholesterol and serum LDL but not HDL concentrations. Basal serum glucose concentrations were lower in Ecd treated animals than in the ovx controls and remained significantly lower following intragastric glucose application. Skin temperature in ovx animals showed pulses (hot flashes) every 20 – 40 minutes which were not seen in intact animals and Ecd treated ovx animals. We conclude that the Ecd treatment has beneficial effects of serum lipids which may be used in the human to prevent arteriosclerosis. Furthermore, Ecd prevents hot flashes which may be useful in the treatment of climacteric complaints of women. **References:** 1. Kapur, P et al. (2010) Phytomedicine;17:350 – 355. 2. Seidlova-Wuttke, D, et al.(2010) J Steroid Biochem Mol Biol 119, (3 – 5):121 – 126.

SL-35 Chemical composition and biological activity of the essential oils from native Australian *Callitris* species

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The chemical composition of the essential oil of the wood of the Australian native cypress *Callitris endlicheri*, *C. intratropica* and *C. columellaris* were determined by GCMS. The colour of the oils from *C. endlicheri* were yellow, yellow green and light green, whilst the distinct blue colour of the oil from *C. intratropica* was in keeping with its generic name 'blue cypress oil'. The sesquiterpene alcohol guaiol (**1**) comprised > 50% of the *C. endlicheri* oil. Significant differences were observed in components between the three accessions. In contrast, the oils of *C. intratropica* showed more subtle variations between collection sites with both oils being high in guaiol (17 – 21%) together with the sesquiterpene lactones dihydrocolumellarin (**2**), 12 – 20%, and columellarin (**3**), 2 – 6%. The green oil from *C. columellaris* was dominantly dihydrocolumellarin.

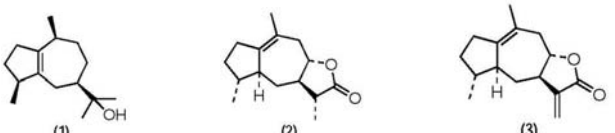

Fig. 1

In vitro cytotoxicity against the mouse lymphoblast P388D 1 cell line of the oils of *C. endlicheri* gave IC_{50} values in the range of 1.25 to 2.72 µg/mL while *C. intratropica* and *C. columellaris* gave IC_{50} values of 9.10 and 5.43 µg/mL, respectively. Guaiol (**1**), dihydrocolumellarin (**2**) and columellarin (**3**) gave IC_{50} values of 2.31, 1.92 and 8.62 µg/mL, respectively. The oil of *C. intratropica* and compounds **2** and **3** were subjected to phagocytosis assay and although the oil (10 µg/mL) showed granulocyte and monocyte activity enhancement of 5.4% and 22.5%, respectively, only columellarin (**3**) showed an enhancement of granulocytes activity (5.2%). Preliminary investigation into the antioxidant activity of the oils showed some activity of the oils, which varied between accessions within the species.

SL-36 The standard is set – can it be implemented? The African Herbal Pharmacopeia

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Africa contributes 25% of the global pool of plant genetic resources currently being traded. While over 5.000 African plant species are known to be used medicinally, few have been described and studied. This enormous potential for African countries can only be utilized with internationally recognized quality standards in place, since their absence constitutes a major barrier to regional and international trade. The preparation and publication of the African Herbal Pharmacopeia (AfrHP) by the Association of African Medicinal Plant Standards (AAMPS, www.aamps.org) has been completed to help address this issue. Fifty important African medicinal plant species have been described comprising relevant safety, efficacy and quality data in order to promote the cultivation and trade of these important medicinal plants. This presentation sheds some light on the issues which arose over compiling and editing the monograph drafts with regards to areas where further research and investigation is warranted. It also discusses strategies for making the pharmacopoeia available and accessible to the African, as well as international business and scientific communities. Finally, it calls upon African regulators to consider adopting these pharmacopoeial standards, since only when turned into legislation can they make the desired impact.

SL-37 African tree of life, Baobab – *Adansonia digitata* L and its development from a traditional herbal to an evidence based modern superfruit

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Natural products in Southern Africa have been used for centuries by local populations for their nutritional and medicinal properties. With increasing commercial interest it has become necessary to investigate the quality and composition of the raw materials which have long histories of use by local populations. Baobab is an indigenous tree from Southern Africa that produces a fruit with a wide range of important nutritional and medicinal properties. The fruit has been traditionally consumed in Africa and more recently has been the focus of several scientific studies investigating its important properties. The Baobab fruit pulp contains high levels of several minerals including calcium and magnesium. The antioxidant capacity of Baobab fruit pulp is significant and the soluble dietary fibres in the pulp are said to have pre-biotic effects. Traditional use of this species provides reasoning for further scientific research to confirm the nutritional and medicinal properties, an approach that has proved to be effective. Traditional knowledge has led to the discovery of Baobab fruit pulp. Due to this knowledge and subsequent scientific evidence of its nutritional properties, this natural product has demonstrated that it is safe for human consumption as a food. It received Novel Food approval in Europe and FDA GRAS (Generally recognized as safe) notification in USA. Baobab fruit pulp is the new superfruit for use in health foods and beverages. **References:** 1. Saka, J. D. K., et al., (1994). Nutritional value of edible fruits of indigenous wild trees in Malawi. Forest Ecology of Indigenous Wild trees in Malawi 64: 245 – 248. 2. Chadare, F. J., A. R. Linnemann, et al. (2009). Baobab Food Products: A review on their composition and nutritional value. Critical Reviews in Food Science and Nutrition 49: 254 – 274.

SL-38 The pharmacophore of thapsigargin

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A derivative of thapsigargin (1) is currently in clinical phase 1 trials for treatment of prostate, bladder, and breast cancer. Thapsigargin has been targeted towards solid tumours by coupling the natural product to a peptide, which selectively is cleaved by enzymes present in excess in the cell walls of the blood vessels nourishing solid tumours. Human kallikrein 2 is an example of such a proteolytic enzyme [1]. The cytoxicity of thapsigargin relates to the ability to inhibit the sarco- or endoplasmic Ca2+ ATPase (SERCA). A pharmacophore model suggest that lipophilic interactions between the acyl groups at O-3, O-8, O-10 and CH3 – 15 are of major importance for the affinity to the binding cavity. Analysis of the X-ray structure of the complex of thapsigargin and SERCA [2] reveals that the segment F(834)FRY(837) andthe segment A(306)IPEGL(311) form the sides of the cavity.

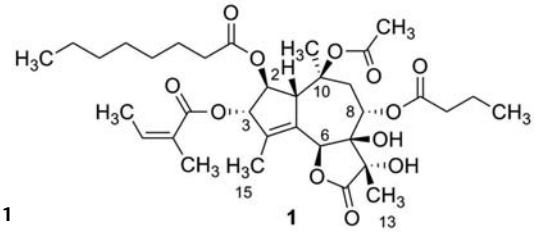

Fig. 1

Even though the angeloyl and acetyl groups are in very short distances from the transmembrane segments replacement of these groups with voluminous but flexible acyl groups only to a minor extent diminish the affinities of the analogues for SERCA. In contrast replacement of these acyl groups with voluminous and stiff groups like 4-benzylbenzoate severely reduces the binding affinity of the analogues. **Acknowledgements:** This work is supported by the Danish Research Council for Strategic Research and the Danish Cancer Society. **References:** 1. Christensen, SB. et al. (2009) Anti-cancer Agents Med Chem 9:276 – 294. 2. Toyoshima, C, Nomura, H. (2002) Nature 418: 605 – 611.

SL-39 Antihyperalgesic activity of salvianolic acid B and its formulations in the chronic constriction injury of the sciatic nerve (CCI) model of neuropatic pain

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Salvianolic acid B (Sal B) is the most abundant and the major active antioxidant from *S. miltiorrhiza* preventing Aβ25 – 35-induced reduction in BPRP in PC12 cells [1], having neuronprotective effect against hydrogen peroxide (H_2O_2)-induced rat pheochromocytoma line PC12 injury [2] and inhibiting HIV-1 replication [3]. In the framework of a large screening of natural constituents, the antihyperalgesic activity of Sal B was tested by the Paw-pressure test, in a model of neuropathic pain produced by a chronic constriction injury of the sciatic nerve. Size exclusion chromatography (Sephadex LH-20) was used for a rapid separation and refinement of Sal B from the crude extract of the roots of *S. miltiorrizha* Bunge (danshen). Hydroalchoholic solutions were used as eluents for a safe and convenient separation obtaining Sal B almost pure by NMR (>96%). Liposomial formulations were prepared using egg phosphatydil choline, cholesterol and 18:0/18:0 PEG 2000 in the case of long circulationg liposome. Encapsulation efficacy of Sal B was evaluated by HPLC-DAD, while size, polydispersion index and zeta potential by dynamic light scattering. Sal B and two formulations, liposomes and long circulating liposomes, administered intraperitoneally at the dose of 100 mg/kg, reverted the mechanical hyperalgesia in CCI treated rats, evaluated in the Paw-pressure test. The antihyperalgesic effect started 15 min. after administration and in the case of long circulating liposomes the antihyperalgesic effect was still significant at 45 min. The prolonged, delayed and increased therapeutic effect of the long circulating liposomes was also supported by preliminary pharmacokinetic studies. **References:** 1. Lin, YH. et al. (2006) Biochem. Biophys. Res. Commun. 348:593. 2. Liu, CS. et al. (2006) Phytomedicine 14:492. 3. Abd-Elazem IS. et al. (2002) Antivir. Res. 55:91.

SL-40 Propolis, a natural agent with therapeutic potential for HTLV-1 adult T-cell leukemia

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HTLV-1 is the etiological agent of an aggressive malignancy of the CD4+ T-cells, called adult T-cell leukemia (ATL) and of certain other severe clinical disorders (1). The viral Tax protein is a key factor in HTLV-1 pathogenicity. A major part of Tax oncogenic potential is accounted for by its capacity of inducing the transcriptional activity of the NF-kB factors, which regulate the expression of numerous cellular genes (2). Persistent activity of NF-?? factors has been proved to play a central role in the pathophysiology of ATL and other clinical disorders. Propolis is a natural product produced by honeybees. An ethanolic extract of propolis (PE) has been used for long time in folk medicine. One of its active components, caffeic acid phenethyl ester (CAPE), was found to be a potent inhibitor of NF-kB activation. The main aim of this project is to pursue the possibility of blocking all Tax oncogenic effects in the cytoplasm and the nucleus, by treatment with these products. The cells were transected with a plasmid expressing Tax protein and plasmids containing the examined promoters. Our results showed that both PE and CAPE substantially inhibited the activation of NF-kB-dependent promoter by Tax. However, only PE could efficiently inhibit also the activation of SRF- and CREB- dependent promoters by Tax. Also, both tested materials strongly inhibited Tax binding to IkBa and b and prevented their induced phosphorylation and degradation by Tax. However, they were not able to prevent Tax or of NF-kB transport to the nucleus. **References:** 1. Yoshida, M. (2005) Discovery of HTLV-1, the first human retrovirus, its unique regulatory mechanisms, and insights into pathogenesis, Oncogene, 24: 5931 – 5937. 2. Pahl, H.L. (1999) Activators and target genes of Rel/NF-kB transcription factors, oncogene 18, 6853 – 6866.

SL-41 Comparative study of *Mentha arvensis* Linn whole plant extracts for antioxidant and antidepressant activity

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Mental disorders contribute significantly to the global burden of diseases. Projections towards the year 2020 show that the neuropsychiatric illness will increase their share from about 10.5% of the total burden of disease in 1990 to 15% in 2020 [1]. *Mentha arvensis* is an indigenous plant of the Lamiaceae family commonly known as '*Pudina*' and '*Mint*' in English. It is reported to exhibit central nervous system (CNS) modulating effects [2]. Hence, the present study was carried out to investigate aqueous and methanol extracts of *Mentha arvensis* Linn extracts for antioxidant and antidepressant activity. Aqueous and methanol extracts of *Mentha arvensis* were preliminary studied for *in vitro* antioxidant activity using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Nitric oxide and Hydroxyl radical scavenging activity methods. Then the extracts were investigated for antidepressant activity by Tail suspension and Forced swim test at dose level of 125, 250 and 500 mg/kg in Swiss albino mice. Fluoxetin was used as a positive control at the dose of 10 mg/kg. Methanol extract showed significant antioxidant and antidepressant activity as compared to aqueous extract. However, there is need for further studies to evaluate its mode of action. **References:** 1. Kastrup M, et al (2007) Dan. Med. Bull 54: 42 – 432. 2. Pia M. Vuorela et al.(2006) Fitoterapia 77:429 – 434.

SL-42 Cardiotonic glycoside determination in in vitro and ex vitro samples of *Digitalis lamarckii* Ivan, an endemic species to Anatolia

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Digitalis lamarckii Ivan is an endemic species to Turkey. *Digitalis* species are medicinally and economically important as they contain cardiac glycosides that strengthen diffusion and regulate the heart rhythm [1]. Moreover, preliminary studies have revealed that digoxin and digitoxin are also effective agents in several cancer treatments [2]. In this study cardenolide patterns in in vitro and ex vitro (from natural populations) samples of *D. lamarckii* were studied. In in vitro samples, digoxin was predominantly found in different tissues (lamina, petiole or whole shoot) of 12 or 18-week old regenerants. Digoxin content was, in general, lower in the 18-week old regenerants than 12-week old ones (Fig. 1).

Fig. 1: *In vitro* regenerant (12 weeks old) produced from 2.0 mg/l BA+0.5 mg/l NAA

Highest digoxin was, however, observed in lamina excised from 12-week old regenerants (130 mg/kg). When varying concentrations of BA alone or in combination with NAA, BA at 1.0 mg/l was found most productive (6.9 mg digoxin/kg). For ex vitro, natural populations were studied with reference to different vegetation periods (June to September). Accumulation of digoxin, gitoxigenin, lanatoside C and digitoxin were estimated in leaf samples, and the higher amounts of digoxin were found in June samples extracted from mid-leaves (1010 mg/kg) and reddish leaves collected in September (1062 mg/kg). On the other hand, gitoxigenin, lanatoside C or digitoxin were found in trace amount. **Acknowledgements:** The authors deeply appreciate the financial support of The Scientific and Technological Research Council of Turkey (TUBITAK) for the project TOVAG-106O470. **References:** 1. Baytop T (1999). Therapy with medicinal plants in Turkey: Past and present. (2nd ed). Nobel kitabevi, Istanbul. 2. Newman et al. (2008). Cardiac glycosides as novel cancer therapeutic agents. Mol. Intv. 8(1): 36 – 49.

SL-43 HPTLC method development and validation for estimation of isovitexin from methanolic extract of *Enicostemma littorale* Blume

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Enicostemma littorale Blume (Chota-Kirayat or Chota-Chirayata) is a glabrous perennial herb belongs to family Gentianaceae [1]. It is used as anti-diabetic, anti-inflammatory, anticancer and antioxidant [2, 3]. In the present study, a new, simple, sensitive, precise and robust high-performance thin layer chromatographic (HPTLC) method was developed and validated for the estimation of isovitexin from methanolic extract of *E. littorale*. Analysis of isovitexin was performed on TLC aluminium plates pre-coated with silica gel 60 RP-18 F-254 as stationary phase. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase consisting of acetonitrile-water (3:7, v/v). Camag TLC scanner III was used for spectrodensitometric scanning and analysis of the plate in absorbance mode at 235 nm. The system was found to give compact spots for isovitexin (Rf value of 0.40). The data for calibration plots showed good linear relationship with r2 = 0.99853 in the concentration range of 50 – 400 spot-1 with respect to peak area. The present method was validated for precision and recovery. The total isovitexin content was found to be 0.4% (w/w) in methanolic extract of *E. littorale*. Statistical analysis proves that the method is reproducible and selective for the analysis of isovitexin. **References:** 1. Murali, B. et al. (2002) J. Ethnopharmacol. 81:199 – 204. 2. Maroo, J. et al. (2003) Phytomed. 10:196 – 199. 3. Sadique, J. et al. (1987) Biochem Med Metabol Biol. 37:167 – 176.

SL-44 Dereplication of Brazilian plants from Cerrado and Atlantic Forest using NMR virtual design and hyphenated techniques

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The need for new and innovative analytical methods that may shed information towards the composition of complex natural mixtures is a keystone on bioprospection programs. Dereplication methodologies associated to state-of-the-art spectroscopic techniques, have been successful in the selection of biologically active extracts. Our research group "NuBBE" has incorporated the use of those methods as well as molecular virtual design using Nuclear Magnetic Resonance (NMR) aiming to increase the understanding of molecular relationships on dynamic natural matrixes and synergism effects of bioactive crude extracts. Highly active crude extracts, from *Abarema lusoria* and *Calea pinnatifida*, previously *in vitro* screened using human cell lineages such as HL-60 (Leukemia), MDA-MB435 (Melanoma), HCT-8 (Colon) and SF-295 (Glioblastoma), were analyzed using HPLC/DAD-HRMS and compared with *in silico* databases aiming to detect known plant metabolites. NMR data from the crude extracts was processed and compared with an NMR molecular virtual designed environment containing all known compounds for each species. Flavonoids, such as 8-methyl-naringenine, 4′,5,7-trihydroxy-3′,6-dimethoxy-8-methylflavanone, 3′,4′,5,6,7-pentahydroxy-8-methyldihydroflavonol and the benzophenone bis (2-hydroxy-4,6-dimethoxy-3-methylphenyl) metanone, were detected in *A. lusoria*. From *C. pinnatifida* we were able to detect flavonoids, such as 4′,5,7-trihydroxy-3-methoxy-6,8-dimethylflavanone, 4′,7-dihydroxy-5-methoxy-6-methyldihydroflavonol, 5,6,7-trihydroxy-3′,4′-dimethoxy-8-methylflavanone and, 3′,4′-dimethoxy-5,7-dihydroxy-6,8-dimethyldihydroflavonol. The occurrence of flavonoids may be associated to the original activity re-

vealed in the *in vitro* assays. However, further studies must be performed in order to establish molecular synergism effects since there is no significant antineoplasic activity reported for those metabolites once isolated.

SL-45 HPLC-LC/MS/MS of secoiridoid glycoside and flavonoids in *Enicostemma littorale*

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Enicostemma littorale Blume. a small herb of family Gentianaceae is commonly used as an antidiabetic agent. Its anti-inflammatory, antioxidant, hypoglycemic and anticancer activities were reported [2, 3]. Swertiamarin, a bitter secoiridoid glycoside, alkaloids, flavonoids, phenolic acids and xanthones were isolated [3]. In the present study, new HPLC-LC/MS/MS method was developed for the analysis of Secoiridoid Glycoside and Flavonoids in the ethyl acetate fraction of the Enicostemma Littorale. HPLC-UV analysis was carried out on a Perkin-Elmer HPLC system with diode array detector using a RP-18 Column (250 x 4.6 mm; 5 µM Kromasil). Mobile phases used included 0.2% formic acid and pH 3.5 with Ammonia in water (solvent A), and Acetonitrile (solvent B). A gradient was used as 0 – 10 min, 25% B; 10 – 20 min, 80% B; 20 – 33 min, 80% B; 33 – 34 min, 10% B; and 34 – 40 min, 10% B. HPLC-LC/MS/MS analysis was performed with an Acquity UPLC and Xevo Quards MS system. The operational conditions used were: capillary voltage at 3.5 V, cone 35 V, dissolveation temperature at 600ô¤C, MS 2 scan from 100 to 900 amu. Mass spectra of Swertiamarin, Secoiridoid Glycoside was m/z 374 and Swertisin m/z 446, prenylated flavanoids m/z 356 and m/z 358.This method is suitable for the routine analysis, as well as for the separation and identification of known and novel Secoiridoid Glycoside and Flavonoids. **References:** 1. Vasu, V. et al. (2005) J. Ethnopharmacol. 101: 227 – 282. 2. Kavimani, S. et al. (2000) J. Ethnopharmacol. 71: 349 – 352. 3. Vishwakarma, S. et al. (2004). Pharm. Biol. 42: 400 – 403.

SL-46 Assessment of the phenolic content and free radical scavenging capacity of extracts obtained from the pericarp of *Garcinia mangostana L.*

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The pericarp of *Garcinia mangostana* L. is popularly used as a food supplement due to its high amount of phenolic compounds i. e., xanthones, flavonoids and tannins which promote high free radical scavenging activity [1]. This work was designed to examine the amount of phenolic constituents and the free radical scavenging capacity of extracts obtained from the pericarp of *G. mangostana* collected from 15 different locations in Thailand. The 95% ethanolic extracts were prepared by Soxhlet extraction. The average content of total phenolic compounds, total tannins and total flavonoids per 100 grams of extract in all samples were found to be 22.33 ± 3.25 g of gallic acid equivalent, 36.38 ± 8.46 g of tannic acid equivalent, and 4.13 ± 1.10 g of quercetin equivalents evaluated using Folin-Ciocalteu procedure, protein precipitation method, and aluminium colorimetric method, respectively. The average EC_{50} of all extracts was found to be 17.59 ± 5.69 µg/mL determined by the DPPH scavenging method. The regression analysis between the amount of total phenolics and total tannins in the extracts exhibited a high positive relationship (r = 0.9302), while the correlation coefficient between antiradical activity ($1/EC_{50}$) and the content of total phenolics and total tannins of all ethanolic extracts were both higher than 0.8, indicating a significant positive relationship between these parameters. Therefore, phenolic compounds and tannins seem to play an important role as free radical scavengers in pericarp extracts of *G. mangostana*. **Acknowledgements:** This study was granted by Mahidol University Research Fund. **References:** 1. Pothitirat, W. et al. (2009) Fitoterapia 80: 442 – 447.

SL-47 Chemopreventive potential of rhamnosyl depsides from *Inga laurina*

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Fabaceae family is composed of 650 genera with ca. 18.000 species and is found in tropical and subtropical regions as shrubs and trees1, including species as Inga laurina, I. edulis and I. marginata, which are used as popular medicines in Brazil to treat stomach and inflammatory disorders. Few species of Inga genus have been investigated although some present important biological properties. Recent studies have shown the presence of flavonoids and other phenolic compounds from Inga species, which might be associated to their reported uses. 1,2 Preliminary analysis of Inga laurina leaves crude EtOH extract by TLC test nebulized with beta-carotene solution and in a liposome model using Fe2+ as radicalar reactions initiator indicated the presence of antioxidant compounds. Its phytochemical study led to the isolation of four new gallic acid derivatives from the EtOAc fraction from liquid-liquid partition of the EtOH extract, followed by chromatographic procedures including RP-HPLC. Their structures were determined through extensive 1D and 2D NMR and MS analysis as: 3-(2-rhamnopyranosyl-1,5-dihydroxyphenyl) gallate (1), 2-(1-rhamnopyranosyl-3,5-dihydroxyphenyl) gallate (2), 2-[1-(4′-galloyl)rhamnopyranosyl-3,5-dihydroxyphenyl] gallate (3) and 5-[1-rhamnopyranosyl-2-galloyl-3-hydroxyphenyl) gallate (4). As observed in the literature, Inga genus presents compounds with antioxidant activity, which was confirmed by the results obtained from the TLC analysis with beta-carotene as spray reagent. The free radical scavenging activities of compounds 1 – 4 were confirmed by the DPPH and ABTS assays, and their chemopreventive index (CI), evaluated through the induction of quinone-reductase enzyme, was comparable to the positive control BNF3, suggesting the chemopreventive potential of Inga laurina. **Acknowledgements:** FAPESP for research grant 04/07932 – 7 awarded to DHSS (PI), and scholarships to MCCM and LAS; CAPES, for scholarship to SRM; and CNPq, for research fellowships to DHSS, MCMY and CPS. **References:** 1. Lokvam, J et al. (2005) J. Chemical Ecol. 31, 11. 2. Lokvam, J et al. (2007) J. Nat. Prod. 70, 134. 3. Cuendet, M. et al. (2006) J. Nat. Prod. 69, 460.

SL-48 Adaptogenic-related activity and phenolic content of selected ginseng-like herbs in Thailand

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Eleven plants traditionally as adaptogens were collected from the north and northeastern parts of Thailand and evaluated for adaptogenic-related properties as well as total phenolic and total flavonoid contents. Plant samples were extracted by various methods then were tested for in vitro antioxidant activity and were investigated for phenolic and flavonoid contents by spectrophotometric techniques. Tested extracts showed antioxidant activity with EC50 ranged from 14.50 ± 1.04 to 783.68 ± 19.94 and 11.18 ± 2.60 to 745.24 ± 24.54 µg/mL using DPPH scavenging assay and thiobarbituric acid reactive substances (TBARS) method, respectively. Their total phenolic and total flavonoid contents are in the range of 1.93 ± 0.04 to 31.74 ± 1.08 g% chlorogenic acid equivalent (g% CAE) and 0.38 ± 0.01 to 12.39 ± 1.40 g% rutin equivalent (g% RE), respectively. The leaf decoction of Acanthopanax trifoliatus (ATD) exhibited strong antioxidant activity with high amount of phenolic and flavonoid contents. ATD was further tested for in vivo anti-anxiety activity using light-dark task [1] and hole-board test [2] with 30 mg/kg Phenobarbital as a positive control. Animals orally receiving ATD at the concentrations of 500 to 1000 mg/kg significantly ($P < 0.05$) increased the number of entries (80%) and time spent (90%) in light chamber in light-dark task. For the hole-board test, animal group receiving 1000 mg/kg

ATD significantly increased the number of head-dip (37%). The results indicated that plant samples, especially the leaves of Acanthopanax trifoliatus possess antioxidant and anti-anxiety activities supports their ethnomedical uses as adaptogenic agents. **Acknowledgements:** The Graduate Program Development under the Collaboration between Thailand Institute of Scientific and Technological Research and Universities. **References:** 1. Crawley, J. et al. (1980) Pharmacol. Biochem. Behav. 13:167 – 170. 2. Treit, D. et al. (1981) Pharmacol. Biochem. Behav. 15(4):619 – 626.

SL-49 The effectiveness of a standardised *Echinacea* preparation in preventing colds, flus and other respiratory disorders for air-travellers

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Air travel, especially on intercontinental flights can be stressful. Studies have shown that passengers' well-being is influenced by the cabin environment such as quality of cabin air, oxygen pressure in the cabin, motion or vibration and oil additives used in aircraft engines [1, 2]. Various studies have reported and investigated the occurrence of nasal dryness [3] and the increased risk of developing upper respiratory disorders such as allergic rhinitis [4] and attracting virus or bacteria induced respiratory infections [5, 6]. Studies have shown that Echinacea, one of the most widely used herbal medicines worldwide, can decrease the severity of cold symptoms and improve quality of life if used as early treatment [7 – 9]. Some studies also suggest positive effects for the prevention of the common cold when using Echinacea [8]. Here we report on a randomised, double-blind placebo controlled clinical trial with 183 participants who were travelling return from Australia to America, Europe or Africa for a period of 1 – 5 weeks on non-stop commercial flights via economy class. Participants were using coated Echinacea (standardised to 4.42 mg alkylamides) or placebo tablets, and trial dosing consisted of three protocols (priming, travel and sick), depending on the phase of travel of the participants and their health status. Outcomes were assessed using a survey which included questions about upper respiratory symptoms (based on WURSS-44), quality of life (based on SF-36) and the occurrence of additional air travel related symptoms. Each participant completed this survey before travel (baseline), after travel (return) and at 4 weeks after return from travel (follow-up). Preliminary results so far indicate that at follow-up a significantly lower number of participants reported respiratory illness in the Echinacea treated group compared to placebo ($P = 0.02$) and a significantly higher number of participants treated with Echinacea reported a "good" quality of life compared to placebo ($P = 0.005$). Moreover, a significantly higher number of participants in the placebo group compared to the Echinacea group reported respiratory illness at follow-up compared to baseline ($P = 0.044$) suggesting that the placebo group had not returned to the pre-travel level in terms of respiratory illness. **References:** 1. Nicholson, A.N., et al. Travel Med Infect Dis, 2003. 1(2): p. 94 – 102. 2. Hinninghofen, H. et al., Auton Neurosci, 2006. 129(1 – 2): p. 80 – 5. 3. Norback, D., et al., Scand J Work Environ Health, 2006. 32(2): p. 138 – 44. 4. Ohrui, N., et al., Ann Allergy Asthma Immunol, 2005. 95(4): p. 350 – 3. 5. Vogt, T.M., et al., J Travel Med, 2006. 13(5): p. 268 – 72. 6. Evans, A., et al., Aviat Space Environ Med, 2006. 77(9): p. 974 – 6. 7. Gillespie, E.L. et al., Conn Med, 2006. 70(2): p. 93 – 7. 8. Woelkart, K. et al. Planta Med, 2008. 74(6): p. 633 – 7. 9. Linde, K., et al., Cochrane Database Syst Rev, 2006(1): p. CD000530.

SL-50 Mechanisms of the anti-proliferative and pro-apoptotic effect of the herbal fixed combination STW 5 on colon adenocarcinoma cells in vitro

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The herbal preparation STW 5 is widely used in the treatment of functional dyspepsia and other motility-related gastrointestinal disorders. Our objective was to determine the molecular mechanisms of the anti-proliferative and pro-apoptotic effects on colon adenocarcinoma cells (HT-29). Cells were treated with diclofenac (Diclo), aspirin (ASA), STW 5 or its components STW 6 (*Iberis amara* totalis), STW 5-K II (peppermint), STW 5-K VII (milk thistle) or STW 5-K VIII (lemon balm). Anti-proliferative effects were measured with sulforhodamine. Apoptosis was identified by YO-PRO-1® staining. Treatment with Diclo (0.1 mM), ASA (2.5 mM), STW 5 (100 µ g/ml) or its components STW 6 (12.5 µ g/ml), STW 5-K II (50 µ g/ml), STW K VII (100 µ g/ml) or STW 5-K VIII (25 µ g/ml) inhibited proliferation by ca. 50 – 60% (ASA or Diclofenac 45 – 50%). STW 5 (as well as ASA or Diclo) induced apoptosis 3 to 4- fold. 100 µg/ml STW 5 showed a 20% or 30% induction of Caspase-3 or BAX expression, whereas ASA or Diclo revealed inhibitory effects. 100 µg/ml STW 5 inhibited the Bcl2 mRNA expression. STW 25 – 100 µg/ml up-regulated the expression of NFkB p65 subunit. Our data suggest that STW 5 and some of its components show antiproliferative and pro-apoptotic effects on HT-29 cells in vitro, possibly due to an activation of the caspase cascade and the NFkB pathway. Active concentrations of STW 5 are, in relation to therapeutic doses, comparable to those of ASA and Diclo, suggesting a similar favourable effect on colon carcinoma risk.

SL-51 Does willow bark extract have a sildenafil-like action? Effects of different fractions on the synthesis of cGMP and NO in human umbilical vein endothelial cells (HUVEC)

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Objectives: In the past years several methods have been implemented for testing substances which may exhibit sildenafil like properties. Willow bark extract, used in therapy of painful musculoskeletal diseases, has been supposed also to exhibit effects on endothelial cells and thus induce NO and might then indirectly increase cGMP and act slidenafil-like. We therefore investigated different fractions with a HUVEC cell model. **Method:** We measured intracellular concentrations of cGMP and the release of NO after treatment of HUVEC cells with 5 different fractions (A-E) of the standardized willow bark extract STW 33-I (extraction medium water, DER 16 – 23:1), covering the whole spectrum of components from lipophilic to hydrophilic compounds. **Results:** Fraction E showed a significant cGMP enhancement in concentrations from 10 – 100 µg/ml. In investigating the validity of the assay this effect was comparable to a positive control (NO donor and PDE5 inhibitor (TO156)). Fractions A and E increased NO release whereas fraction C led to inhibition of NO release. **Conclusions:** Fractions of willow bark extract increasing NO release and cGMP level, such as fraction E, might be interesting candidates for natural aphrodisiac products with a sildenafil-like mode of action. Fractions exhibiting the property to reduce NO release like fraction C might be further tested for their anti-inflammatory response as previous research findings from the study of inflammatory diseases showed the importance of free radicals like NO in enhancing the disease outcome. Taken together, this study shows that willow bark extract has several exciting modes of action possibly interesting for further indications.

SL-52 Effects of ethanolic extract of *Pedalium murex* Linn. on sexual behavior and reproductive parameter of male rats

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Throughout the entire ages, men and women have incessantly pursued all means to enhance, maintain and bring back their sexual ability, or to stimulate the sexual desire of the opposite sex [1].The vajikaran rasayana is special class of Ayurvedia that deals with herbs associated with an improvement of male sexual potency and thereby ensure a supraja, or better progeny. The main aim of Vajikaran was achievement of successful copulation for healthy reproduction, along with an improvement in sexual pleasure as an additional benefit [2]. Dried fruit of *Pedalium murex* known as bada gokhru is one amongst the traditionally used herbs to enhance sexual dynamics. Administration of 50, 100, 150 mg/kg b.w. ethanolic extract of *P. murex* improved overall sexual behavior

and reproductive parameter in male rats. We propose a possible mechanism of action of *P. murex* and experimental evidence in support of the hypothesis. Administration of extract had pronounced anabolic and spermatogenic effect in treated animals as evidenced by gains in the body or reproductive organs weight and as reflected in histology of testis. The treatment also markedly affected sexual behavior of animals, sperm count and sperm motility. Improvement in sexual behavior of rats was characterized by increased mount and intromission frequency and reduced mount and intromission latency. Treatment with *P. murex* improved FSH, LH and testosterone levels[3]. These studies suggest the role of *Pedalium murex* in improving sexual function by its action on HPG (hypothalamus pituitary gonadal) axis. The experiments justify the role of bara gokhru as Vajikaran Rasayana. **Acknowledgements:** AICTE, New Delhi for providing National Doctoral Fellowship **References:** 1. Thakur, M. et al. (2007) Sex Disabil. 25 (4): 203 – 207. 2. Chauhan, NS. et al. (2008) Nat Prod Res. DOI: 10.1080/14786410802588493. 3. Chauhan, NS. et al.(2009) Int J Impot Res. DOI:10.1038/ijir.2009.62.

SL-53 The hawthorn special extract WS® 1442 protects against endothelial barrier dysfunction – elucidation of the underlying molecular mechanisms

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Due to its profound cardiac effects, the hawthorn (Crataegus spp.) extract WS® 1442 (Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany) is an approved drug against heart failure stage NYHA II. Heart failure is accompanied by inflammatory processes leading to endothelial hyperpermeability and edema formation. However, data about an effect of Crataegus on endothelial barrier dysfunction and the underlying molecular mechanisms are lacking. In vivo, WS® 1442 abrogated the histamine-induced extravasation of FITC-dextran from venules of the mouse cremaster muscle. In cultured human endothelial cells, Crataegus inhibited thrombin-induced macromolecular permeability. By applying biochemical and microscopic techniques, we revealed that WS® 1442 abrogates the detrimental effects of thrombin on crucial subcellular regulator systems of endothelial barrier stability: adhesion junctions, the F-actin cytoskeleton, and the contractile apparatus. Mechanistically, we found that Crataegus inhibits the thrombin-induced rise of intracellular calcium, which is accompanied by an inactivation of PKC, and reduces RhoA activation. Moreover, Crataegus activated barrier-protecting mechanisms: it increased endothelial cAMP levels, which consequently activated Rap1 and PKA. However, PKA was not crucially involved in barrier protection. Rac1 and cortactin were activated by WS® 1442 leading to enhanced cortical F-actin bundles. By using Epac1-targeting siRNA, we found that the Crataegus-induced Epac/Rap1 activity is important for the activation of cortactin. Taken together, our study provides evidence that Crataegus effectively inhibits endothelial hyperpermeability in vitro and in vivo. Mechanistically, we elucidated a dual role of action of WS® 1442, since it inhibited the barrier-stabilizing Ca2+/PKC/RhoA pathway and activated the barrier-protecting cAMP/Rap1/Rac1 signaling network.

SL-54 Combination of ferulic acid and antibiotics as effective antibacterial agents

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Ferulic acid (FA) is a monophenolic phenyl propanoid occurring in plant materials including rice, green tea and coffee beans. In this study we have shown that FA interacts in a synergistic way with antibiotics such as amikacin, ampicillin, ciprofloxacin, erythromycin and vancomycin against Gram negative (*Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*) and positive (*Staphylococcus aureus*) bacteria. The interaction is quantified by checkerboard and time-kill curve assays. The Fractional Inhibitory Concentration (FIC) index shows that FA is synergistic with amikacin against all the organisms, except *P. aerugionsa* (see Table below). *S.aureus* was found to be highly sensitive to the combinations and *P.aeruginosa* was the least. Time kill curve studies was done to study the parameters such as killing rate (K_{max}) and area under killing curve (AURKC) for the combinations, namely phytochemical and antibiotics and were compared with the values from individual compounds. The K_{max} and AURKC for FA and amikacin combination against *E.coli* was −2.7 h-1 and −15.6 respectively and was statistically significant than the individual treatments. FA possessed bacterial membrane damaging activity. Our study showed that FA was synergistic with few of the antibiotics tested; therefore food rich in this natural compound could positively interact with antibiotics under *in vivo* conditions too.

Table 1: FIC index indicating the interaction of FA with antibiotics against various bacteria. (Synergy is indicated by underline)

	Microorganisms									
Phyto-chemical	E. coli					E. aerogenes				
	Ami	Amp	Cip	Ery	Van	Ami	Amp	Cip	Ery	Van
FA	0.38	0.62	0.47	0.48	0.52	0.18	0.76	0.52	0.51	0.68

	Microorganisms									
Phyto-chemical	P. aeruginosa					S. aureus				
	Ami	Amp	Cip	Ery	Van	Ami	Amp	Cip	Ery	Van
FA	0.54	0.68	0.49	0.73	0.66	0.16	0.65	0.36	0.44	0.23

SL-55 Investigation of Iranian *Artemisia annua* sedative effects in mice

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Artemisia annua (Asteraceae) commonly known as sweet wormwood or Qinghao is an annual herb/shrub native of Asia [1]. The plant grows broadly in Caspian Sea shores in North of Iran [2]. In China, the aerial parts of this plant are a source of artemisinin, which is an antimalarial compound [3]. This study was aimed to establish the scientific basis of reported ethno-medicinal use of *Artemisia annua* as sedative agent. The plants were gathered from Gilan Province in Iran. Plant aerial parts were extracted with methanol and concentrated in vacuum. Methanol extract was partitioned into chloroform, petroleum ether and ethyl acetate soluble fractions. Each fraction was administered intraperitonealy (i.p.) in male mice with different concentrations (50, 100 and 200 mg/kg) and for evaluation of sedative activity, immobility time was determined using open-field test. Flumazenil (3 mg/kg, i.p.) as a benzodiazepine receptor antagonist was injected 15 min before chloroform fraction (200 mg/kg, i.p) to clarify the mechanism of action. Compared to control group (saline-treated mice), the chloroform fraction significantly (***$p < 0.001$) increased immobility time in a dose-dependent manner. Flumazenil decreased immobility time induced by chloroform fraction significantly (*$p < 0.05$).

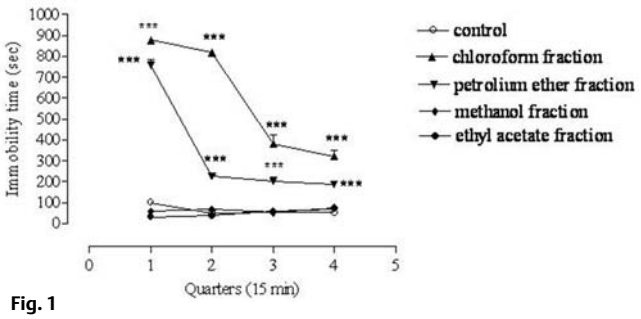

Fig. 1

The results of the present study suggest that *A. annua* growing in Iran has sedative effects which are probably mediated via benzodiazepine receptors pathways **Acknowledgements:** The authors would like to thank the authorities of Pharmacy Faculty, Tehran University of Medicinal sciences for its financial support. **References:** 1. Wright, C.W. (2002) Artemisia, Taylor and Francis, London, 107 – 117. 2. Zargari A. (1997) Medicinal Plants, Tehran University Publications, Tehran, Iran, 3:65 – 66. 3. Gupta, S. et al. (2002) Antimicrob Agents Chemother. 46(5): 1510 – 1515.

SL-56 Cyclobutane dimer iridoids from *Tabebuia argentea* Britt. (Bignoniaceae)

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Tabebuia argentea Britt. (Bignoniaceae) is a tree native of South America and it is used in folk medicine as anti-inflammatory and against influenza [1]. Previous studies of *T. argentea* apolar extracts reported the isolation of beta-sitosterol, methyl cinnamate, lapachol, ethyl *p*-hydroxycinnamate, betulinic acid, 3,4′,5-trihydroxy-7-methoxyflavone, veratric acid, and *p*-anisic acid [2] while phytochemical studies of the genus *Tabebuia* led to the characterization of iridoids [3], and flavonoids [4]. As a part of an investigation on plants acclimatized at the El Zoharia Research Garden of Cairo, we carried out a phytochemical study of polar extract of the plant. Two new cyclobutane dimer iridoids, one new pinoresinol type lignan, together with many known iridoids and flavonoids, were isolated and characterized from the aerial parts methanol extract. Their structures were elucidated by 1D and 2D NMR spectroscopy, as well as ESI mass spectrometry. In the framework of a screening to search for natural compounds as HSP90 inhibitors, all *T. argentea* isolated compounds were tested through Surface Plasmon Resonance, a technique that allowed to study the protein/ligand interaction [5]. Some iridoids bound the protein with a good affinity constant. **References:** 1. Agra, M.F. (1996) Plantas de Medicina popular dos Cariris Velhos, Paraiba Brasil: espécies mais comuns. Editora União, João Pessoa, Brasil. 2. Barbosa-Filho, J.M. et al (2004) Phyton: 221 – 228. 3. von Poser, G.L. et al (2000) Biochem. Syst. Ecol. 28: 351 – 366. 4. Prakash E.O., Rao J.T. (1999) Fitoterapia 70: 287 – 289. 5. Dal Piaz, F. et al. (2009) J. Med. Chem. 52: 3814 – 3828.

SL-57 Effects of kaerophyllin against hepatic fibrosis in rats

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Engulfment of apoptotic bodies (ABs) of hepatocytes is associated with activation of hepatic stellate cells (HSCs) and liver fibrosis in vivo. The present study is to investigate the in vitro and in vivo antifibrotic effects of kaerophyllin [α-(*trans*-3,4-dimethoxybenzylidene)-β-(3,4-methylenedioxybenzyl)-γ-butyrolactone] from *Bupleurum scorzonerifolium*. Liver fibrosis was induced by thioacetamide (TAA; 200 mg/kg, i.p.) injection twice weekly for 6 weeks. Two groups of rats received either high or low doses (15 mg/kg and 30 mg/kg, twice daily) of kaerophyllin by gavage in rats. LX 2, a human hepatic stellate cell line, incubated in the presence of UV-induced HepG2 ABs was used to investigate the engulfment of ABs by HSCs and the role of kaerophyllin in the inhibition of HSCs activation. Phagocytosis of ABs by LX 2 induced activation, with production of collagen I and asmooth muscle actin (aSMA), increased motility and NF kB activity. Kaerophyllin inhibited LX2 phagocytosis of ABs and activation. TAA administration induced liver fibrosis, which was attenuated by kaerophyllin treatment, with reduction of GOT and GPT levels and aSMA protein expression. Kaerophyllin reduced TAA-induced liver fibrosis and inhibited HSC activation and engulfment of ABs.

SL-58 Novel phenalenone derivatives

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Marine-derived fungi are an important source of pharmacologically active natural products. Investigation of the crude extract of the fungus Coniothyrium cereale provided a new series of phenalenone derivatives, obtained by HPLC separations. The structures of the compounds were established on the basis of extensive spectroscopic studies (1 H; 13C; HSQC, COSY, NOESY and HMBC), mass spectral analysis (LC/MS and HRESIMS), UV and IR. The absolute stereochemistry is based on the specific optical rotation and CD spectra which were compared with published data. Structurally most unusual and unprecedented is the lactame-containing compound 7. The compounds are subjected to cytotoxicity and antimicrobial evaluation and also towards the the enzymes human leukocyte elastase (HLE), trypsin, chymotrypsin, acetylcholinesterase, papain, cholesterolesterase and thrombin. Compounds 6 and 7 showed good in vitro cytotoxicity of IC50 = 41 and 27 μM respectively. Compounds 6 and 8 showed antimicrobial activity of MIC = 7.8 and 15.6 μg/mL respectively. Compounds 1, 2 and 7 showed potent inhibition of HLE with IC50 values of 7.16, 10.9 and 13.3 μM, respectively.

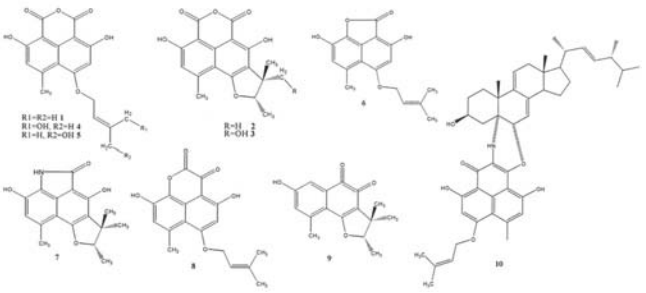

Fig. 1: Structures of metabolites isolated from *Coniothyrium cereale*

Acknowledgements: Financial support from the Egyptian Government and from BMBF (project No. 03F0415A)

SL-59 Hepatoprotective effect of *Averrhoa bilimbi* Linn. methanol extract on carbon tetrachloride induced liver damage in albino rats

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Averrhoa bilimbi is an indigenous plant of the Averrhoaceae family, which was used traditionally for liver disorders [1]. However, there were no scientific reports available on its hepatoprotective activity. Hence, the present study was carried out to investigate the hepatoprotective effect of *Averrhoa bilimbi* Linn. methanol extract on carbon tetrachloride (CCl_4) induced liver damage in albino rats. Liver toxicity was induced by intraperitoneal injection of CCl_4 at the dose of 1 ml/kg with a gap of 72 hrs for 10 days. Blood serum marker enzymes like glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), total protein and bilirubin were estimated [2]. Administrations of *Averrhoa bilimbi* methanol extract at the doses of 250 and 500 mg/kg, *p.o.* showed significant ($p < 0.01$) decrease in serum GPT, GOT, ALP, and bilirubin levels as compared to negative control. The total protein level was decreased due to hepatic damage induced by CCl_4 and it was found to be increased in methanol extract of *Averrhoa bilimbi* treated group. Treatment of rats with CCl_4 led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA), which was associated with a significant reduction of the hepatic antioxidant system like reduced glutathione (GSH) [3]. These biochemical alterations resulting from CCl_4 administration were significantly ($p < 0.01$) inhibited by treatment with *Averrhoa bilimbi* methanol extract. The results were comparable with silymarin at the dose of 100 mg/kg, *p.o.* The data suggest that *Averrhoa bilimbi* methanol extract may act as a hepatoprotective and antioxidant agent. **References:** 1. Kirtikar K, Basu B (1981) Indian medicinal plants. 2nd edition, International Book Distributors, 443. 2. Shahjahan, M. et al. (2004) Indian J Med Res 120: 194 – 198. 3. Shenoy, A. et al (2002) Ind J Pharmacol.46 (2): 167 – 174.

SL-60 Inhibition of histamine-induced inflammatory reactions in intestinal mucosa by STW 5

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Herbal medicine is a therapeutic option in the therapy of irritable bowel syndrome (IBS), a disease, for which an inflammatory etiology is discussed. In such inflammatory reactions in the gastrointestinal tract, histamine seems to be an important trigger. So the question raised, whether STW 5, a herbal medicine with clinically proven efficacy in the therapy of IBS (2), and consisting of a fixed combination of 9 herbal extracts, has anti-inflammatory and antioxidant effects (3). For the measurements of these effects a pharmacological model involving mucosa preparations from mouse ileum was developed. As a marker of inflammatory reactions, free radical production was measured via luminol-enhanced chemiluminescence. Histamine (5 to 100 µmol/L) strongly increased this parameter. Similar to the antioxidant trolox, STW 5 had a significant inhibitory effect even in dilutions down to 0.1 µl/ml). From the extracts contained in STW 5, those from peppermint and chamomile showed highest effects, that of greater celandine herb was least active. It can be concluded, that ileal mucosa preparations stimulated by histamine are a model with significant relevance in studies on diseases involving intestinal inflammation. The inhibitory effects exerted by STW 5 and its constituents in that reaction might be involved in its therapeutic effects. **References:** 1. Breunig et al. 2007, J Physiol 583:731. 2. Madisch et al. 2004, Aliment Pharmacol Ther 19, 271 – 279; Germann et al. 2006, Phytomedicine, 13, SV, 45 – 50.

Posters

Aphrodisiaca from plants

P001 Plants used for aphrodisiacs purposes in central South America: southwestern Mato Grosso state, Brazil

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We studied plants used as aphrodisiacs in central South America, region dividing the watershed of the La Plata River (Pantanal-North) and Amazon (High-Graporé Juruena), Mato Grosso-Brazil. Informants (63) of the collected data (2004 – 2005), were healers (people reference-popular herbal medicine) residents in the cities studied. The species most used for aphrodisiacs are: I-Catuaba [*Anemopaegma arvense* (Vell.) Stellfeld & J.F. Souza, (Bignoniaceae)]; II-Nó-de-Cachorro [*Heteropterys aphrodisiaca* Machado, (Malpighiaceae)]; III-Tiririca [*Cyperus rotundus* L., (Cyperaceae)]. Besides these, less frequently, are also mentioned as an aphrodisiac and/or sexual problems to solve: IV-Ginseng-brasileiro [*Pfaffia glomerata* (Spreng.) Pedersen, (*Amaranthaceae*]; V- Melancia [*Citrullus lanatus* (Thunb.) Matsum. & Nakai, (Cucurbitaceae)]; VI-Carobinha [*Jacaranda semiserrata* Cham., (Bignoniaceae)]; VII-Salsaparrilha [Herreria salsaparilha Mart., (Asparagaceae)] e VIII-Velame [*Macrosiphonia velame* (A. St.-Hil.) Müll. Arg., (Apocynaceae)]. The "prepared aphrodisiacs" most used are obtained from the roots of I and II extracted in wine or rum. The species I, II and VIII have occurred in the native grassland and savanna land of central Brazil, and the species VI and VII are also native place where there is or was sparse forest, but the species III, IV and V are exotic occurrence of spontaneous or cultivated. There is increased demand for these plants in the region studied. The people believe in the effective aphrodisiac activity of some species, especially for I and II. Phytochemicals studies using extracts of the roots of the species "I" and "II" showed a significant effect on the seminiferous tubele of adult rats Wistar[1], which may be promising. **Acknowledgements:** Institutional support- UNEMAT, FAPEMAT and EMPAER-MT; Suport provided for all PLAMED team-project: Bonila MGO, Carniello MA et al. **References:** 1. Chieregatto, L.C. (2005). Effect of the extracts of Heteropterys aphrodisiaca O. Mach. and Anemopaegma arvense (Vell.) Stellf. on Seminiferous Tubule of Adul Rats Wistar. UFV, Viçosa (BR).

P002 Damiana (*Turnera diffusa* Willd.) – a traditionally used aphrodisiac as modern PDE-5 inhibitor

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Selective phosphodiesterase-5 (PDE-5) inhibitors like Sildenafil, Tardanafil or Vardenafil are commonly used for the treatment of Erectile Dysfunction (ED). PDE-5 inhibitors are generally not considered as aphrodisiacs because they do not have any direct effect on the libido. However, increased ability to attain an erection may be interpreted as increased sexual arousal by users of these drugs. An aphrodisiac is a substance that increases sexual desire. One link between PDE-5 inhibitors and aphrodisiacs might be the herbal drug Folia Damiana. The leaves of Damiana (Turnera aphrodisiaca) have a long history of traditional use as aphrodisiac in Mexico [1]. Nowadays Damiana products claimed to increase libido and sexual performance are widely marketed, whereas scientific data are scarce: Damiana extracts were shown to stimulate sexual behaviour in rats [2,3]. Our previous investigations showed for the first time, that Damiana leaf preparations influenced PDE-5 activity in vitro [4]. In the present study a crude 60% w/w ethanolic Damiana extract was investigated for its inhibitory activity on PDE-5 in vitro and was further optimised using different purification techniques. In conclusion, PDE-5 inhibition might be a possible mechanism of action of Damiana. An human study on the effects of FB9389 on mild Erectile Dysfunction in male subjects is currently in progress. **References:** 1. Mills S., Bone K (2005) The Essential Guide to Herbal Safety, 358 – 359, Elsevier. 2. Arletti, R. et al. (1999) Psychopharmacology 143:15 – 19. 3. Estrada-Reyes, R. et al. (2009) J Ethnopharmacol 123:423 – 429. 4. Patent Application WO2008071684.

Authentication of plants and drugs/DNA-Barcoding/PCR profiling

P003 Metabolotaxonomy of Tibetan medicinal plant *Halenia elliptica* with HPLC

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Halenia elliptica (Gentianaceae) has an extensive distribution and was a traditional medicines use in China [1]. It possess the ability to reduce fever, detoxify and act as choleretic and liver tonics, it has been mainly used for the treatment of hepatic and choleric and inflammatory diseases, such as hepatitis, cholecystitis. Some other Gentianaceae species, in particular, *Swertia erythrosticta*, *S. franchetiana* and *S. tetraptera* are often marketed as *H. elliptica,* and therapeutic effects of *H. elliptica* are not achieved. In this study, a simple, reliable and reproducible method, base on high performance liquid chromatography (HPLC), for developing chromatographic fingerprints to discriminate among these species is described. The data of fingerprints of *H. elliptica* and its adulterants established by HPLC were all processed with two kinds of mathematic methods including correlation coefficient and cosine value of vectorial angle to validate their similarities. The chromatographic profiles including retention time and peak area are different between *H. elliptica* and *Swertia* species. The similarity coefficients of *H. elliptica* and three *Swertia* species (*S. erythrosticta*, *S. franchetiana* and *S. tetraptera*) were of 0.325, 0.436 and 0.774, respectively. *H. elliptica* was closely related to *S. tetraptera*, with similarity coefficients of 0.774. This conclusion agrees fully with results from molecular and morphology studies *S. tetraptera* is the closest living relative of *H. elliptica*. This method provides effective and accurate identification of *H. elliptica*. **Acknowledgements:** This research was supported by the Natural Science Foundation of China (NSFC 30770153 to CY Xue). **References:** 1. Yang YC. Tibetan Medicines. Qinghai People Press, Qinghai. (in Chinese); 1991. p. 111.

P004 Authentication and identification of Chinese plant materials *Periploca sepium* Bunge, *Acanthopanax gracilistylus* W. W. Smith and *Acanthopanax senticosus* (Rupr. & Maxim.) Harms.

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The increasing number of plant species used in herbal medicinal products leads to misidentification, incorrect plant substitutions and adulterations. Nevertheless authenticated raw material is the basic starting point for the development of a botanical product. One example is the challenging differentiation between Periplocae Cortex (*Periploca sepium* Bunge, family Asclepiadaceae), Acanthopanacis Cortex (*Acanthopanax gracilistylus* W. W. Smith, family Araliaceae) and Acanthopanacis senticosi Radix et Caulis (*Acanthopanax senticosus* (Rupr. & Maxim.) Harms. or *Eleutherococcus senticosus* Rupr. & Maxim.) Maxim., family Araliaceae). In fact, some Acanthopanacis Cortex plant material used in herbal products has been identified as its common substitute Periplocae Cortex. This is possibly due to confusion because of the Chinese names of the plants: "Ciwujia" refers to Acanthopanacis senticosi Radix et Caulis and "Wujiapi" to Acanthopanacis Cortex. The pinyin name of Periplocae Cortex is "Xiangjiapi" or northern "Wujiapi" to be distinguishable from southern "Wujiapi" (Acanthopanacis Cortex). Nevertheless 80 percent of Acanthopanacis Cortex in the Chinese domestic market is estimated to be Periplocae Cortex [1,2]. For the bioassay-guided fractionation of anticancer activities of both plants authentication of plant material and plant extracts from different origin was conducted by microscopic and chromatographic methods such as TLC, GC and HPLC. The odour compounds 2-hydroxy-4-methoxybenzaldehyde, vanillin, borneol, thymol and linalool and the eleutherosides E, B and E1 were used as chemical markers. As the results were not distinct enough, they were ensured by DNA sequence analysis of the nuclear ribosomal internal transcribed spacer (ITS) of the raw materials. ITS sequence analysis finally allowed a clear discrimination of the two genera *Periploca* and *Acanthopanax* [3].
References: 1. Bensky, D., Clavey, S., Stöger, E. (2004) Chinese Herbal Medicine – Materia Medica. 3 rd edition. Eastland press Inc. Seattle, USA. 2. Chinese Pharmacopoeia Commission (2005) Pharmacopoeia of the People's Republic of China. Volume I. English version. People's Medical Publishing House. Beijing, China. 3. Maruyama, T., et al. (2008) Authentication of the Traditional Medicinal Plant *Eleutherococcus senticosus* by DNA and Chemical Analyses. Planta Medica 74. 787 – 789.

P005 Authentication of the traditional Tibetan medicinal plant *Lygodium japonicum* using MALDI-TOF spectrometry

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Botanical supplements for health enhancement are being increasing used all over the world [1]. The integrity of herbal supplements is of great importance because adulteration or contamination of a botanical product can have a direct effect on the efficacy or even safety of the product by introducing unknown phytochemicals [2]. Matrix associated laser desorption ionization time of flight (MALDI TOF) MS has been shown to be a rapid and sensitive method for characterization of organisms [3]. Here we provide an innovative, based on MALDI TOF mass spectrometry method for the quick molecular identification of *Lygodium japonicum*. This species is a perennial fern belonging to the family Lygodiaceae that has a long history of widespread use in traditional Tibetan folk medicine. Highly specific mass spectrometric profiles from 8 different *Lygodium* species were obtained. Signals generated from proteins with molecular weights of about 11 kDa have been selected as specific biomarkers for unambiguous discrimination. Several biomarkers for *Lygodium japonicum* (m/z 11522.47, 1129.65, 11676.32) were defined. Structural characterization by mass spectrometry of these proteins generating biomarker signals allowed us to identify them as *Lygodium japonicum*. **Acknowledgements:** This research was supported by the Natural Science Foundation of China (NSFC 30770153). **References:** 1. Zhao, Z et al.(2006) Planta Med 72:865 – 874. 2. Lum, MR et al. (2005) Planta Med 71:841 – 846. 3. Alispahic, M et al (2010) J Medical Microbiology 59: 295 – 301.

P006 Application of HPLC-Tandem-MS for authentication of the traditional Tibetan medicinal plant *Rhodiola rosea*

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Rhodiola rosea, a high-altitude plant, is extensively being used in traditional folk medicine in Tibet to treat fatigue, asthma, haemorrhage, depression, anaemia, impotence, gastrointenstinal ailments, infections, and nervous system disorders [1]. Some other *Rhodiola* species, in particular, *R. alterna*, *R. brevipetiolata*, *R. crenulata*, *R. kirilowii*, and *R. sachalinensis* are often marketed as *R. rosea*, and thus, the therapeutic effects of *R. rosea* are not achieved. In an attempt to develop a mass spectrometry method for discriminating among these species, peptides from the six *Rhodiola* species were analyzed and isolated by high-performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-Tandem-MS). On the basis of the mass spectral fingerprints, 27 *Rhodiola* samples were successfully differentiated by principal component analysis (PCA) of the mass spectral raw data. The PCA results were also validated with cluster analysis and supervised PCA analysis. Using these fingerprints, some *R. rosea*-specific peptides were detected. This method provides effective and accurate identification of *Rhodiola rosea*. **Acknowledgements:** This research was supported by the Natural Science Foundation of China (NSFC 30770153). **References:** 1. Brown RP et al. (2010) Herbal Gram 56: 40 – 52.

P007 Molecular survey of the Thai herbal drug "Reo noi" based on internal transcribed spacer (ITS1) nucleotide sequence analysis

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"Reo noi" has been commonly used as Thai folk medicine to treat dyspepsia and gastric diseases. It is derived from the ripe fruits of some *Amomum* plants (Zingiberaceae), such as *A. uliginosum*, *A. villosum* and *A. xanthioides* [1]. The identification of "Reo noi" commercial herbal drugs is generally based on their morphological characteristic. In this study, we collected ten "Reo noi" herbal drugs from the main traditional herbal drug supplier throughout Thailand. Their macroscopic characters were observed and internal transcribed space1 (ITS1) nucleotide sequences of nuclear DNA were analysed. Our results revealed that "Reo noi" herbal drugs all had similar morphological characters whereas the molecular data revealed three different nucleotide sequences. Six samples of "Reo noi" herbal drugs had the same nucleotide sequence as authentic *A. uliginosum*. This analysis revealed that the majority of "Reo noi" herbal drugs were obtained from *A. uliginosum* and the other species should be considered as either substitutes or adulterants. **Acknowledgements:** This work was financial supported from Faculty of Pharmacy, Maha SaraKham University and Department of Medical Sciences, Ministry of Health, Thailand. **References:** 1. Farnsworth, N. R., Bunyapraphatsara, N. (1992) Thai medicinal plants. Prachachon. Bangkok.

P008 Medicinal plant identification: molecular identification of different *Taxus* species by DNA fingerprinting (TAXUS-DNA-ID)

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Taxus represents an important genus since the remote antiquity. Over the last fifty years specific attention was devoted by the pharmaceutical research to the characterization and purification of Taxus-derived chemical constituents as tricyclic diterpenes with a taxane nucleus as potent anticancer agents. In particular, *Taxus x media* Rehder is of interest for the taxol content and *Taxus baccata* L. for the accumulation of 10-desacetylbaccatin III [1]. Species identification and classification of the commercial specimens is very important but at the same time controversial, because morphological characteristics for species diagnosis are few. The objective of the present study was to develop molecular markers that can be used for the identification and differentiation of the aerial part of these species of high relevance for the pharma sector. In the present work, the innovative technology of SNP (Single Nucleotide Polymorphism) genotyping was used to identify species-specific DNA markers for unequivocal identification of the following species and cultivars: *T. baccata, T. brevifolia, T. canadensis, T. cuspidata, T. globosa, T. fuana, T. floridana, T. x media* and cultivars (*Browni, Densiformis, DGS, Hicksii, Wardi, Runyan, Tautoni), T. wallichiana* and cultivars. A molecular DNA fingerprint method for the authentication of Taxus species was developed and validated for its applicability to processed specimens. The DNA fingerprint method TAXUS-DNA-ID enables the rapid and reliable identification of species and cultivars of Taxus. The use of this method opens the route to precise and timely quality controls for origin and purity of Taxus-derived raw materials. **References:** 1. Bruneton J.,1999 Pharmacognosy. Lavoisier publishing. Condé-sur-Noireau (France).

P009 Phytoequivalence in the global marketplace for botanical products (III): using yeast functional genomics to characterize *Equisetum arvense* extracts from America, Asia and Europe

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Using phytochemical profiling by HPTLC, HPLC-PDA-MS/MS, we previously reported that extracts of *E. arvense* originating from America, Asia and Europe exhibited qualitative and quantitative differences in flavonoid glycosides and phenyl carboxylic acid content [1]. Extracts are considered equivalent and interchangeable by manufacturers of herbal medicines if certified to have originated from the same species and prepared using similar extraction solvents and procedures. The assumption underlying current industry practice is that phytochemical variability between extracts does not impact on their pharmacological activity. Unfortunately, this assumption cannot be easily tested, as human clinical studies would be prohibitively expensive and take years to complete. Therefore, there is a need for simpler, laboratory-based model systems for the characterization of the pharmacological activity of complex herbal extracts. We explored the use of *Saccharomyces cerevisiae*, the best-studied eukaryotic organism, for this purpose. Specifically, we investigated the effect of *E. arvense* extracts on *S. cerevisiae* gene expression using Affymetrix Yeast 2.0 genechip microarrays. Linear modelling and principal component analysis was used to identify differenzial gene expression elicited by the extracts. Changes were found in expression of genes involved in mRNA translation, drug transport and phospholipids metabolic pathways. Comparison of the observed patterns of gene expression with the phytochemical composition of the extracts revealed that the phytochemical variation was reflected in their effect on yeast gene expression. Together, the data show that functional genomics in *S. cerevisiae* may be developed as a sensitive bioassay for the quality control of herbal extracts. **References:** 1. Lee, S. et al. (2008) Planta Med 74(9): 921 – 922.

P010 Using 6-gingerol content and gene mapping to identify two types of Gingers used in Thai traditional medicine

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Two species of ginger are used in Thai traditional medicine. *Zingiber officinale* Roscoe are commonly known as Khing or Khing Haeng which is widely used as food, carminative, stimulant and mixing in polyherbs remedies for balancing patient conditions. Another ginger is *Zingiber ligulatum* Roxb. [1], sometimes called Khing Klang or Khing Haeng which is only used as carmiative. The later ginger has less punget smell but often misunderstood and misused as Khing Haeng. Fresh rhizomes of gingers from 12 sources in 4 parts of Thailand were collected and studied for microscopic characters and 6-gingerol content. Also, the fresh leaves were studied for DNA profiles using amplified fragment length polymorphism (AFLP) methodology [2]. HPLC analyes revealed that 6-gingerol was found only in *Z. officinale*, not in *Z. ligulatum*. In addition, the DNA mapping was different in the two ginger species investigated, whereas the microscopic examination of dried rhizomes showed no differences. These results lead to the question whether commercial dried gingers from various sources were *Z. officinale* or not. Dried ginger rhizomes were purchased from 14 sources, represented 4 parts of Thailand, and was studied for standardization according to Thai Herbal Pharmacopoeia. The 6-gingerol was found in 12 samples in range of 0.72 – 8.24% w/w. The other 2 gingers were 6-gingerol-free were recollected as fresh specimens for growing, and then confirmed by AFLP as *Z. ligulatum*. **Acknowledgements:** Thammasart University Research Fund for financial support. **References:** 1. Hooker JD (1894) Flora of British India vol.1: 245. 2. Thai Herbal Pharmacopoeia (2000) vol. 2. 3. Vos P. et al (1995). Nucleic Acids Research 23: 4407 – 4414. 4. Yildirim F, Akkaya MS (2006). Genetic Resources and Crop Evolution 55: 1033 – 1042.

P011 Rapid molecular authentication of the medicinal plant *Taraxacum mongolium* from its adulterants by ribosomal DNA internal transcribed spacer (ITS)-primed polymerase chain reaction

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Taraxacum mongolium (TM) is a traditional Chinese medicine (TCM) that used as an important component in healthy drink in Taiwan. Due to its similarity on morphological features between TM and *Taraxacum officinale*, *Ixeridium laevigatum*, *Youngia japonica*, *Ixeris chinensis*, *Emilia sonchifolia* var. *javanica*, is very common misused and becomes its adulterant of *Taracum mongolium*. In this study, the internal transcribed spacer 1 (ITS1) nuclear ribosomal DNA (nrDNA) served as DNA barcode and allele-specific sequence-primed polymerase chain reaction were exploited for their application in the differentiation of TM from its related adulterants. Using extracted genomic DNA from TM and others *Taraxacum* plants leaves as template, the PCR reaction was performed with a set of specifically designed primers. The results showed that highly specific 250 bp PCR product of TM was successfully amplified; however, not any fragment was amplified from other *Taraxacum* plants and species. This indicated that our specific primers can be used to discriminate TM form other *Taraxacum* species. Applying this method to detect DNA barcode, it might be an alternative way to rapidly authenticate plants used in TCM and to develop a specific TM "identification kit" in the future. **References:** 1. Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. (2008) Rev Med Virol, 18: 407 – 421.

P012 *Diospyros villosa* root botanical identification

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Diospyros villosa L. (de Winter) var. *villosa* (Ebenaceae) is an African shrub, which naturally occurs in Mozambique. Roots of *D. villosa* are used in the local traditional medicine as cathartic remedy for stomach and intestinal complaints [1]. The present work aims to establish botanical criteria to determine most relevant morphological roots features. Methodology includes microscopic and macroscopic analysis of transversal and long-cross section of entire, fragmentized and powdered plant material by light and scanning electron microscopy techniques. Quantitative microscopy studies were also performed. The most useful microscopic characters for roots identification purposes as herbal drug are: periderm composed of cork with 5 to 7 layers of tangentially elongated rectangular cells and few layers of phellogen and phelloderm; cortex with 6 to 8 layers of parenchymatous cells with frequent groups of 8 to 10 sclereids, oval to elongate in shape, crystalliferous brachysclereids with a short roughly isodiametric form and prismatic calcium oxalate crystals to go around; tanniferous cells (detected by histochemical reaction with potassium dichromate) and some cells with irregular cavities filled with quinones (detected by histochemical reaction with Börntraeger reactive) present in the cortical parenchyma; phloem crossed by uniseriate medullar rays and prominent xylem with bordered pitted vessels; numerous single and composed starch grains occur on the cortical parenchyma, inside the medullar ray cells and into the medullar parenchyma. Fragments of the above mentioned structures have also been observed on the powdered material. Obtained morphological characters allowed the *D. villosa* root identification and should be included in a future pharmacopeia monograph. **References:** 1. Hutchings A et al. (1996) Zulu Medicianl Plants. University of Natal Press. Pietermaritzburg.

P013 The application of micrographic parameters in the quality control of *Moringa oleifera* leaf

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Moringa oleifera Lam. (Moringaceae), commonly known as "drumstick tree" or "horse radish tree" is native to north India but is now found throughout the tropics. Leaves of this high medicinal value plant have been reported to have antihypercholesterolemic action [1] and those with other risk factor, such as hypertension [2] or diabetes mellitus [3]. Reports have also described the plant to be highly potent anti-inflammatory agent, antitumour activity and hepatoprotective against antitubercular drugs such as isoniazid and rifampicin [4]. Many uses for leaves include: biogas, green manure, domestic cleaning agent, biopesticide and it is particularly useful as a human food [5]. Our work aims to set *M. oleifera* leaf botanical identification parameters to the whole, fragmentized and powdered plant material by means of light and scanning electron microscopy. The most useful micrographic parameters observed on the leaf are: A bilateral organization with 1 – 2 cell layers of palisade tissue on the upper epidermis and spongy parenchyma with a small intercellular space volume on the lower epidermis; a surface showing a slightly sinuous cuticle in both epidermises; anomocytic stomata with an irregular distribution, surrounded by 4 to 6 subsidiary cells; calcium oxalate cluster crystals on the palisade parenchyma; gland canals in the parenchyma central veins; unicellular non-glandular trichomes scarce. The powdered material is characterized by the presence of fragments containing the above-named structures. Results of this study will be very useful in the identification of this medicinal and edible plant as raw material for use by the pharmaceutical and alimentary industry. **References:** 1. Ghasi, S. et al. (2000) J. Ethnopharmacol. 69:21 – 25. 2. Faizi, S., et al. (1998) Planta Med. 64 (3):225 – 228. 3. Kar, A., et al. (2003) J. Ethnopharmacol. 84 (1):105 – 108. 4. Fakurazi, S. et al. (2008) Food Chem. Toxicol. 46:2611 – 2615. 5. Fahey, JW (2005) T. F. L. Journal 1: 5.

P014 Morphological characters for the identification of *Lonicera caerulea* var. *altaica* dried fruits

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Dried fruits of *Lonicera caerulea* var. *altaica* (Caprifoliaceae) are used in traditional Mongolian medicine to treat liver, stomach and cardiac diseases [1]. Although some previous comparative studies were reported on the *Lonicera* genus [2], there aren't any studies on the morphoanatomical characterization of the fruits of *L. caerulea* var. *altaica* (syn. *L. altaica*). The aim of this work was to provide botanical markers useful for the identification of this edible dried fruit, as an eventual raw material for the pharmaceutical and alimentary industry. Used methodology includes the macroscopic and microscopic analysis of the whole, sliced and powdered dried fruits by light and scanning electron microscopy. Quantitative microscopy studies were also performed. Macroscopically, the dried fruit is a dark blue berry that presents a conspicuously wrinkled outer surface. The micro-morphological characters that have diagnostic value to characterize the fruit, are as follows: pericarp composed of epicarp with one layer of cells with superficial polygonal contour with anomocytic stomata, red coloured parenchymatic mesocarp and endocarp with sclereids and calcium oxalate crystals druses; a collenchymatic sheath on the peduncle pericycle and vascular bundles; seeds with a spindle-shaped naked without wing; browning exotesta and endotesta layers and the embryo is surrounded by several layers of endosperm cells with droplets of oil and few calcium oxalate crystals druses type on the cotyledons. The powder fruit is characterized by the presence of fragments of the above-named structures. Obtained morphological characters can be included in a future quality control monograph of *L. caerulea* var. *altaica* fruit. **References:** 1. Ariunaa Z. & Khaidav T. (2008) Book of Abstracts. Medical University of Lublin, Faculty of Pharmacy. Lublin, pps 68. 2. Jacobs B. et al (2009) Annals of Botany 104: 253 – 276.

P015 Modern approaches to characterise the quality of Propolis

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Propolis is a natural resinous material. It is used in folk medicine, in cosmetology and in food industry for health foods and nutrition supplements and claimed to improve human health and prevent diseases. In Germany there are also authorized traditional medicinal products. For many parts of Europe *Populus* species are the main sources of propolis. Our research project addresses characterisation of the quality of propolis and products derived thereof by recent analytical and molecular biological methods in order to analyse and compare samples of different origin. Characterisation of quality is based on HPLC, GC and MS techniques [1] as well as metabolomic approaches by NMR-fingerprint in combination with Principal Component Analysis. An alternative characterisation is performed by sequencing of ITS-regions of DNA isolated from propolis samples to identify the plant species from which samples are originating. PCR-related techniques are also a promising tool to retrace the origin of propolis products. In selected samples from Germany DNA from *Populus* and *Betula* species were identified whereas in a commercial sample of green Brazilian propolis DNA from *Baccharis dracunculifolia* was identified [2]. Chemical profiling and NMR-fingerprinting of studied samples of different origins displayed significant differences, e.g. quality and quantity of aromatic and aliphatic acids and esters, flavonoids etc. The application of such modern analytical techniques is necessary to characterise complex products like propolis and to guarantee a reproducible quality for a safe and adequate use of propolis products. The antimicrobial activities of all studied samples will be evaluated as well. **References:** 1. Popova et al.(2009) Phytochem. 70 (10), 1262 – 1271. 2. De Moura SA et al. (2009) eCAM 10: 1 – 9.

P016 Identification of herbal substances in finished herbal medicinal products

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Because of the diversity of botanical products in the market it is necessary to look for complementary methods that may also be useful to retrace herbal substances. We are evaluating the potential of PCR-related techniques and NMR-fingerprinting focussing on herbal substances from therapeutic systems of non-European origin and selected plant families e.g. Lamiaceae and Ranunculaceae. A challenging topic is the authentification of the herbal substance in products containing rhizomes of Black Cohosh (*Cimicifuga racemosa*). This perennial plant is native to North America and is of major importance in the US dietary supplements market [1]. In Europe, a monograph on Cimicifugae rhizoma is under development by the HMPC at the EMA [2]. Reports have been published on the occurrence of rare toxic liver disease after application of products containing *Cimicifugae rhizoma*. One hypothesis to explain this toxicity is based on possible contaminations with other species. Therefore, we analysed commercial samples using a PCR-based method targeting the ITS-region. Contaminations with *Acer rubrum*, *Morus murrayana* and *Collinsonia canadensis* were detected in few samples. These plants are also native to North America. In a second approach, the samples were extracted with dichlorine methanol and a metabolomic fingerprinting via H-NMR-measurements in combination with PCA was accomplished to further prove the identity of the respective samples. The data derived from NMR-profiling demonstrate that the contaminations are only due to minor traces of contaminating material. **References:** 1. Kan He et al. (2006) J Chromatogr A. 2006 April 21; 1112(1 – 2): 241 – 254. 2. www.ema.europa.eu, HMPC, European community monographs, draft monograph Cimicifugae rhizoma, 2010.

P017 Authentication of *Euclea natalensis* leaf by botanical identification

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Microscopical characters are well established criteria in the identification and authentication of herbal drugs. Leaves of *Euclea natalensis* A. DC. (Ebenaceae) are commonly used in the treatment of headache and toothache, as well as for abdominal complaints and as purgative [1]. The purpose of this study was to determine the most relevant diagnostic characters to identify leaves of *E. natalensis* by light microscopy (LM) and scanning electronic microscopy (SEM). Used methodology includes the microscopic analysis of the whole, fragmentised and powdered dried leaves (minimum of 30 adult leaves). Results showed that among the most useful micro characters for leaf identification are the following: a thick cuticle on both epidermises; the dorsiventral organization of the mesophyll, with one to two cell layers of palisade parenchyma on the upper epidermis and spongy parenchyma with rounded cells on the lower epidermis; the midrib vascular bundle surrounded by a sclerenchymatous tissue; the xylem with numerous small vessels, radially arranged; the presence of calcium oxalate prism crystals; numerous paracytic and diacytic stomata frequent in lower epidermis with an irregular distribution and conical unicellular non-glandular trichomes, with thick walls, sometimes curved near the base and are also fairly abundant (Image 1).

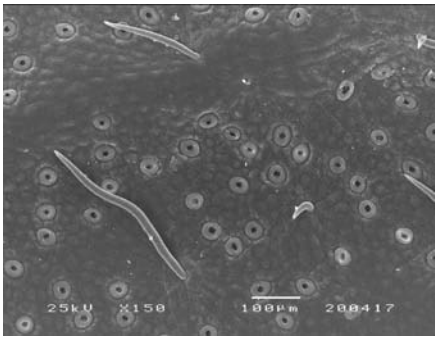

Fig. 1: Non-glandular trichomes and stoma random distribution on lower epidermis, by SEM (scale bar: 100 µm)

A developed collenchyma is present under the lower epidermis of the midrib area and near the upper epidermis. The powdered material is characterized by the presence of fragments containing the above named structures. Obtained results can be useful on quality control protocols involving this medicinal plant. **References:** 1. McGaw LJ. et al. (1997) Phytother Res 11:113 – 117.

P018 Cloning of gene encoding chalcone isomerase (*CHI*) from *P. candollei* and expression of genes involved isoflavonoid biosynthesis pathway in seedling plants

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Pueraria candollei (Fabaceae), a Thai medicinal plant used for rejuvenation in elderly, is major source of isoflavonoids [1]. Like other flavonoids, isoflavones are synthesized from phenylpropanoid pathway [2]. The aims of this investigation were to clone the gene encoding chalcone isomerase (*CHI*) from *P. candollei* using PCR-base cloning and to study the expression of genes involved isoflavonoid biosynthesis pathway. Total RNA from leaf, stem and root of twenty-day-old seedling plants were extracted then the first-strand cDNA was synthesized to be DNA templates. For cloning of *CHI*, forward and reverse primers were designed according to chalcone-flavanone isomerase cDNA from *P. lobata* (GenBank no. Q43056). The plasmids contain 672 bp of open reading frame *CHI*s, code for 223 deduced amino acids, were sequenced and then were aligned to compare the similarity. Multiple alignment of *CHI* from *P. candollei* with other leguminous plants showed highly identity (> 80%). In addition, the *CHI* encoding genes showed the active sites residues including conserve regions as same as *CHI* from other plants [3]. For expression study, semi-quantitative RT-PCR analyses were employed using primers designed based on existing sequences of 4 genes from *P. lobata* and 2 genes from *Glycine max*. The optimal PCR conditions for amplification of each target gene are summarized. The highest expressions of chalcone reductase, chalcone synthase, chalcone isomerase, isoflavone synthase and hydroxy-isoflavone dehydratase were found in roots whereas isoflavone-*O*-glucosyltransferase was found highly in leaves. The results are useful for further study in the isoflavonoid biosynthesis pathway in *Pueraria candollei*. **Acknowledgements:** The Thailand Research Fund (DBG4980009), The Royal Golden Jubilee Ph.D. Program (PHD/0143/2548) **References:** 1. Prathanturarug S. et al. (2000) Seminar on herbal development in Thailand, Bangkok, Thailand. 2. Dewick, PM. (1994) The flavonoids. Chapman & Hall. London 3. Jez, JM. et al. (2000) Nature 7:786 – 791.

Biodiversity

P019 Isoflavonoids with insecticide and larvicide activities from *Muellera frutescens* Standl.

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Many plants of the Fabaceae family, are used as fish poisoning and insecticides [1,2]. The genus *Muellera* in this family is represented by only seven species of climbers and trees and is distributed over south and central America. In 1984, Geesink proposed to consider *Muellera* as *Lonchocarpus* synonymous [3]. The Lonchocarpus genera is well known in French Guiana because of its traditional utilization as a fish poisoning [4]. However, one study only reported ethnobotanical use of *M. frutescens* as an ichtyotoxic plant. Furthermore, no ethnopharmacological use or biological activity data was ever reported in the literature for this plant. Many chemical studies dealing with *Lonchocarpus* genera have been published [5,6,7]. Phytochemical investigation of barks, stems and rarely leaves described isolation of active isoflavonoids named rotenoids. This study aims at conducting a phytochemical survey of *Muellera frutescens* in order to evaluate whether or not *Muellera* is closely related to *Lonchocarpus* and eventually isolate new bioactive secondary

metabolites. Three different extracts (hexane, ethyl acetate and methanol) of bark, roots and leaves were prepared and tested on various biological assays. We discovered insecticide and larvicide activities for all extracts, and none of them exhibited cytotoxicity on human cells. The bioguided fractionation of the most active extract (bark hexane extract) allowed us to isolate eight isoflavonoids, the structures of which were elucidated by spectroscopic methods. It was found that *Muellera* is indeed closely related to *Lonchocarpus*, therefore corroborating Geesink's proposal. **References:** 1. Thasana, N., et al. (2001.) Heterocycles 6, 1121 – 1125. 2. Kumar, P. el al. (1989) Phytochem 28, 916 – 918. 3. Geesink, R. (1984) Taxon 33, 742 – 743. 4. Moretti, C., Grenand, P. (1982). J. ethnopharmacol. 139 – 160. 5. Blatt, C.T.T et al. (2002). Phytotherapy Res. 16, 320 – 325. 6. Fang, N et al. (1999) J. Agric. Food Chem. 47, 2130 – 2136. 7. Cabizza, M. et al. (2004) J. Agric. Food Chem. 52, 288 – 293.

P020 The FairWild Standard – A fair and sustainable deal for wild-collected ingredients throughout the supply chain

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The increasing demand for natural products in the sectors of food, cosmetics, wellness and medicinal ingredients poses major ecological and social challenges. The high pressure on potentially vulnerable plants can endanger local ecosystems and livelihoods of collectors who often belong to the poorest social groups in the countries of origin. In order to deal with these challenges, a standard and certification system for sustainable wild-collection of plants was developed by different stakeholders and interest groups, leading to the establishment of the FairWild Foundation in 2008. The Foundation is responsible for the maintenance and implementation of the FairWild standard that consists of sound ecological as well as social and fair-trade principles and criteria. FairWild Foundation aims to provide a worldwide framework for implementing a sustainable, fair and value-added management and trading system for wild-collected natural ingredients and products thereof. FairWild Foundation informs, advises and assists customers with projects in sustainable wild-collection and implementation of the standard. In addition, FairWild Foundation advises on the inclusion of sustainable and fair management principles in conservation strategies, trade policy and other regulations. While encouraging sustainable and fair business practices, the FairWild Standard also focuses on influencing consumer choice. The FairWild Standard is based on existing traditional knowledge and appropriate resource management, as well as on Fair Trade principles and ILO Standards. The Standard is geared to a worldwide approach.

P021 The effects of salt stress on yield and some components in Chamomile genotypes during growth stage

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One possible management option for growers, dealing with decreases in chamomile production caused by salinity. Our objectives were to investigate the effects of salinity levels on yield and some yield components in growth stage of two chamomile spices. Eight genotypes of Matricaria recutita and Matricaria aurea were used in this study. The treatments included salt levels of control, 6, 12, and 18 dSm-1 in sand culture with nutrient solutions. The salinity stress exerted at 2 durations: The first from seedlings stage and second duration of stress exertion began at stem elongation to harvest. The traits measured plant height, root length, the number of leaf per plant, node numbers, and stem fresh and dry weight, roots fresh and dry weight. The salt application trials indicated that dry matter yield was decreased with increasing NaCl doses. The dry matter yields were higher in control than that of the 18 dSm-1 NaCl levels. All the criteria investigated suggest therefore that M. aurea were superior to M. recutita genotypes. Positive and highly significant relationships existed between DM yield with PH (0.36**), RL (0.31**), IN (0.14*), LN (0.18**), SFW (0.51**), RFW (0.42**), RDW (0.33**), and RRW (0.23**). Path analysis showed that plant height (36.7%), Root fresh weight (27.0%), and stem fresh weight (16.5%), had strong positive direct effect, in that order internode number (15.2%), stem relative water (23.6%) and root dry weight (14.3%) had strong negative direct effect. There was a significant difference between genotypes studied for all traits except for root relative water content.

P022 Chemodiversity of Polynesian liverworts

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Liverworts are known as rich sources of secondary metabolites, which volatile constituents such as terpenoids belong to a wide variety of carbon skeletons. These metabolites can be used as chemosystematics markers and are very helpful for taxonomic significance because liverworts are very small plants which morphological classification is extremely difficult. Aiming to assess the diversity of Polynesian liverworts, chemical analysis (GC-MS, 1D and 2D ^{1}H and ^{13}C NMR) of volatile constituents of six species (*Trichocolea pluma*, *Chandonanthus hirtellus*, *Mastigophora diclados, Jungermania* sp., *Plagiochila* sp. and *Cyathodium foetidissimum*) collected in French Polynesia had been performed. All the investigated species are chemically different, each species biosynthesized own peculiar compounds and some of them are biomarkers of the liverworts species. *T. pluma* biosynthesized characteristic isoprenyl phenyl ethers, herbetane-type sesquiterpenoids for M. *diclados*, cembrane-type diterpenoid are peculiar for *C. hirtellus* and fusiccocane-type for *Plagiochila* sp. Interesting chemical constituents had been also in these Polynesian liverworts such as: vanillic acid methyl ester firstly reported to occur from the Marchiantophyta (*T. pluma*), skatol produced by *C. foetidissimum*, (E)-ectocarpene and dictyotene previously found in marine algae and now detected in *C. hirtellus*. These relevant findings express part of the biodiversity specificity of Polynesian bryophytes [1]. **Acknowledgements:** This work was supported in part by Grant-in-aid for Open Research (A.L.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. **References:** 1. Ludwiczuk, A. et al (2009), Nat. Prod. Com.10: 1387 – 1392.

P023 Chemical composition – biological activities of selected samples of propolis from South Greece

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Propolis (bee glue) is a well known natural product with healing properties. The chemical composition of propolis is highly variable and depends mainly on the local flora. Greece is characterized by high biodiversity flora, assuming different propolis composition. In the framework of our continuing work on propolis samples from Greece and all around the world, four propolis samples from Peloponnese and one from the island of Kos were analyzed by GC/MS after silylation. In these samples the diterpenes: totarol, totarolone, abietic acid, imbricataloic acid, isoagatholal, agathadiol, communic acid, junicedric acid, 13-epi-cupressic acid and isocupressic acid have been identified as major constituents while the characteristic propolis flavonoids were either in small amounts or even absent. These findings confirm the presence of conifer trees as propolis plant sources in Greek samples. Diterpenes have been previously found in propolis samples from North-West Greece [2], Crete and Italy (Sicily) [3] while propolis from the temperate zone having Populus spp. as plant sources [1] have large amounts of esters of phenolic acids and flavonoids. All the samples showed significant antibacterial activity against nine Gram-negative and -positive human pathogenic bacteria and three fungi, probably due to the large amounts of diterpenes, while the antioxidative activity of all samples has been assayed as well thorough Rancimat method. **References:** 1. Bankova, V., et al. (2000) Apidologie 31:3 – 15. 2. Melliou, E., Chinou, I. (2004) Planta Medica 70:1 – 5. 3. Popova M. et al. (2010) J. Agric. Food Chem. 58: 3167 – 3176.

P024 Expression of 3β-HSD and P5βR, genes coding for Δ5 – 3β-hydroxysteroid dehydrogenase (3β-HSD) and progesterone 5β-reductase (P5βR), in leaves and cell cultures of *Digitalis lanata* EHRH.

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Plants of the genus *Digitalis* produce cardiac glycosides that are used in the therapy of cardiac insufficiency in humans [1]. 3β-HSD and P5βR are both supposed to be important enzymes in the biosynthesis of these products [2, 3, 4]. Enzyme activity and expression of the respective genes encoding 3β-HSD and P5βR were demonstrated in cardenolide-accumulating leaves of *Digitalis lanat*a but also in cardenolide-free permanent cell suspension cultures (K3OHD) initiated from D. *lanata* leaves. P5βR activity was 3.7 times higher in *D. lanata* leaves (7.8 μkat/kg protein) than in K3OHD cells (2.1 μkat/kg protein) (analysed by GC-MS). 3β-HSD activity in *D. lanata* leaves and K3OHD cells was detected by HPLC and it was found that the activity is about in the same range in leaves (13.9 μkat/kg protein) and in suspension-cultured cells (11.3 μkat/kg protein). Expression of the respective genes, namely AY585867.1 (P5βR gene) and DQ466890.1 (3β-HSD gene), was demonstrated by real-time qPCR analysis. The expression of the 3β-HSD gene in leaves (8.7 mRNA level) is nearly twice as high as in K3OHD cells (4.9 mRNA level). Analyses revealed that P5βR is most strongly expressed in *D. lanata* leaves (42 mRNA level). The relative mRNA level was about 3.2 times higher than in suspension cells (13.3 mRNA level) which nicely fits to the activity data. Since a new, inducible progesterone 5β-reductase was identified recently [5] the expression of the respective gene, P5βR2, in K3OHD cells and *D. lanata* leaves is now under examination and the new results will be presented and discussed. **References:** 1. Hoppe, UC et al. (2005) Z Kardiol 94:488 – 509. 2. Kreis, W. et al. (1998) Planta Med 64:491 – 499. 3. Kreis W, Müller-Uri F (2009) Ann Rev Plant 40:304 – 363. 4. Gärtner, DE. Et al. (1990) FEBS Lett 271:239 – 242. 5. Pedro Peréz-Bermúdez et al. (2010) New Phytol 185:687 – 700.

P025 Evolution and function of *progesterone 5β-reductase* genes in angiosperms

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About 60 genera of the angiosperms have been described to contain 5β-cardenolides [1, 2, 3]. They occur in monocots, basal eudicots, rosids and asterids. The unpredictable occurrence of 5β-cardenolides in the angiosperms raises the question whether this trait has evolved only once or several times during evolution. The analysis of genes suggested to be involved in cardenolide biosynthesis may help to address this question. Progesterone 5β-reductases (P5βR, P5βR2) are thought to catalyse a step in the 5β-cardenolide biosynthesis, namely the conversion of progesterone to 5β-pregnane-3,20-dione. Therefore we isolated 11 new P5βRs orthologues from several 5β-cardenolide-free and 5β-cardenolide-producing plant species. All sequences were analysed *in silico* and were shown to be highly conserved. They contain certain motifs that qualify them as members of a class of stereo-selective enone reductases. Phylogenetic analysis shows that in the angiosperms P5βRs form two separate clusters. The cladogramme generated from protein sequences showed that one cluster (Cluster II) of P5βRs correlated nicely with the assumed phylogenetic relationship of the species in the cluster. This implied that these genes have evolved from a common ancestral gene. A second cluster contained *p5βr*-genes of *Populus*, *Vitis* and *D. purpurea*. All three genera also contained paralogues in cluster II. In this group only the *D. purpurea* P5βR2 was recently described to encode a functional P5βR that can be induced by stress [4]. The occurrence of these closely related functional P5βRs in 5β-cardenolide-free and 5β-cardenolide-containig plants let us presume that cluster II P5βRs are involved in more than just 5β-cardenolide biosynthesis. **References:** 1. Singh, B., Rastogi, R.P. (1970), *Phytochem*. 9: 315 – 331. 2. Meleto, C.P., Medarde, M., San Feliciano, A. (2000), *Molecules* 5: 51 – 81. 3. Kreis, W., Müller-Uri, F. (2010), Biochemistry of sterols, cardiac glycosides, brassinosteroids, phytoecdysteroids and steroid saponins. In: Wink M. (Ed.) *Biochemistry of Plant Secondary Metabolism.* Volume 40, 2nd Ed. 304 – 363, CRC Press, Sheffield. 4. Perez-Bermudez, P. et al. (2010), *New Phytol.* 185: 687 – 700.

P026 Variation in the essential oil content and composition of a Finnish oregano collection

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Fifteen oregano samples (*Origanum vulgare* L.) from eleven different locations in southern Finland plus one commercial Greek oregano (*Origanum viride* ssp. *viride*) were cultivated in Mikkeli, south-eastern Finland in 2008 and 2009. The plants were harvested at flowering time in August 2009, dried and stored in paper bags. The plant material was submitted to hydrodistillation in a Karlsruher-Stahl apparatus for two hours in order to isolate the oil and determine the oil content. The oil content varied from 0.2 to 0.8% in the Finnish oreganos and was 3.7% in the Greek oregano. The oils were analysed by GC-MS using an unpolar RTX-1 and a polar Stabilwax column (30 m x 0.20 mm i.d. Restek Corporation, CA, USA). The identification was based on retention indices and comparison with library spectra. Altogether 47 components were identified and the identified constituents represented 64.9 – 96.9% of the total oil. The dominant constituents were sabinene (up to 17%), γ-terpinene (up to 12%), β-caryophyllene (up to 20%), germacrene-D (up to 17%), caryophyllene oxide (up to 23%) and carvacrol (up to 85%). A cluster analysis of the essential oil compositions classsifed the oils into five groups: 1) β-caryophyllene/germacrene-D, 2) caryophyllene oxide, 3) carvacrol, 4) β-caryophyllene and 5) sabinene/germacrene-D type. Only the Greek oregano was a carvacrol type. Great variations in oregano oils of Mediterranean origin have been reported (1, 2), but not in oregano of Finnish origin. **References:** 1. Figueredo, G et al. (2006) Flav Fragr J 21: 134 – 139. 2. De Martino, L. et al. (2009) Molecules 14: 2735 – 2746.

P027 Changes in distribution and structure of wild *Origanum vulgare* L. (Lamiaceae) populations during the last decade in Armenia and implications for conservation

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Armenia has a rich flora of ca. 3600 plant species (ca. 50% of the entire Caucasian flora), distributed across (semi)desert, steppe, forest and alpine landscapes. Native plant biodiversity and conservation status of these species, particularly those with economic value, needs further assessment [1]. Anthropogenic threats to biodiversity (overpopulation, deforestation and urbanization) have simultaneously hindered research and increased the need for it. Of the ca. 500 species with medicinal/economic use records, ca. 50 are used in folk medicine, including both wild-collected (e.g. Crataegus sp., Hypericum perforatum) and cultivated (e.g. Chamomilla recutita, Mentha piperita) species [2]. Only limited information, however, on genetic biodiversity, population structure, and conservation status of these species is available. From 2006 – 2009, field studies were conducted to re-locate wild Origanum vulgare L. populations based on historical records, and discover new populations. The growth, phenological and habitat characteristics, population size and location (GPS mapping), were assessed. Historical records indicated that this species occurred widely in the central/northern regions, but nearly half the populations had vanished. Remaining populations diminished in size, plant number and experienced fragmentation during the study period. 3 new populations were located in the south/south-eastern regions, indicating that the abundance and distributional range is expanding here. Anthropogenic threats included: poor land management, increasing population pressure, and excessive collection of plants. This research provided baseline data for the development of ex situ and in vitro strategies to conserve unique genotypes, and assess the sustainability of wild populations according to IUCN Red Book Criteria, of this important species in Armenia. **References:** 1. IUCN, WHO, WWF (1993). Guidelines on the Conservation of Medicinal Plants, IUCN, Gland, Switzerland, 50 p. 2. Fayvush, G., Danielyan T., Nalbandyan A. (2004) Armenia as a producer of medicinal plants: possibilities and perspectives. Available online (accessed 12 April 2010): http://www.nature ic.am/NCSA/Publication/Medical_Plants_eng.pdf.

P028 The essential oil composition of some *Lavandula* species grown in Romania

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The genus Lavandula belongs to the family of Lamiaceae and consists of 30 species [1]. They are aromatic evergreen shrubs widely distributed. Parts used are the flowering tops from which the essential oil is obtained [2,3]. The most well known is L. angustifolia (true lavender) which preparations are traditionally used to treat symptoms of neurotonic disorders [3]. Lavender oil has been used empirically since ancient times for its antiseptic properties and today is used in pharmaceuticals and cosmetic products [1,2]. Five different Lavandula species, subspecies, cultivars or hybrids harvested from the Botanical Gardens from Galati, Romania, were investigated for their essential oil composition. The species were: Lavandula angustifolia Mill., L. angustifolia subsp. angustifolia, L. angustifolia subsp. pyrenaica (DC.) Guinea, L. angustifolia cv. Munstead, L. hybrida Rev. (L. angustifolia Mill. x L. latifolia Medik.). Essential oils were obtained from dried inflorescences by hydrodistillation and analysed by means of GC/FID and GC/MS. The identification of the volatile compounds was based on comparison of their retention indices (RI), and mass spectra with those obtained from authentic samples and/or NIST/NBS, Wiley libraries and literature. Linalool (20.0 – 36.0%) and linalyl acetate (12.9 – 22.5%) were the main components in all analysed samples. **References:** 1. Mabberley, D.J. 1997. The Plant-Book. Cambridge University Press, Cambridge. 2. Leung A.Y., Foster S. 1996. Encyclopaedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics, 2nd ed., John Wiley & Sons Inc, New York. 3. Blumenthal M. 2000. Herbal Medicine. Expanded Commission E Monographs. American Botanical Council, Austin.

P029 In vitro shoot cultures of endemic *Cyclopia genistoides* (Honeybush) as a source of valuable polyphenolic compounds

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The plants from Cyclopia genus (Honeybush) are endemic, South-African shrubs characteristic for the fynbos plant formation of the Cape Floristic Region. Aerial parts of several Cyclopia species, including C. genistoides, are used to manufacture the honeybush herbal tea, recognized by its dinstictive sweet, honey-like aroma, low level of tannins and lack of caffeine [1,2]. Extracts obtained from Cyclopia plants are rich in polyphenols, including xanthones (mangiferin and isomangiferin), flavanones (hesperidin), flavones and isoflavones, and as such exhibit substantial antioxidative and antimutagenic activity [1,2,3]. In vitro propagation protocol for C. genistoides has been established. Solid Schenk-Hildebrandt (SH) medium supplemented with 2.0 mg l-1 6-(γ,γ-dimethylallylamino)purine (2iP) and 0.22 mg l-1 thidiazuron (TDZ) have been shown to be the best for shoot culture initiation, as well as for the development of high number of microshoots. Shoot elongation procedure involved explant cultivation on the full strength SH medium supplemented with 1.0 mg l-1 indole-3-butyric acid (IBA), followed by 30-day long growth on SH medium with reduced sucrose level, supplemented with the same amount of IBA. As a result, elongated shoots representing intact plant morphology were obtained. Root induction was achieved on solid SH medium with reduced sucrose and nitrate concentrations, supplemented with 5.0 mg l-1 indole-3-acetic acid (IAA) and 50.0 mg l-1 citric acid. The regenerated plants were acclimatized in the glasshouse, with the use of peat/gravel/perlite subtrate (1:1:1). Both regenerated plants and in vitro microshoots of C. genistoides were evaluated for the production of selected polyphenolic compounds (LC-ESI-MS and LC-DAD analysis). **Acknowledgements:** the study was supported by grant no N N302 041936 from Polish Ministry of Education and Science. **References:** 1. Joubert E. et al. (2008) J. Ethnopharmacol. 19:376 – 412. 2. Kokotkiewicz A., Luczkiewicz M. (2009) Fitoterapia 80:3 – 11. 3. Joubert E. et al. (2008) J. Agric. Food Chem. 56:954 – 963.

P030 Genetic analyses of the endangered medicinal plant *Podophyllum emodi*

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The medicinal use of Podophyllum emodi Wall (Himalayan Mayapple; family: Berberideceae), a high-altitude plant species native to the alpine and sub alpine areas of Himalayas, dates back to ancient times [1]. The plant has been used in traditional Chinese System of Medicine for treatment of a number of ailments. To determine the population structure and outcrossing rate across the range of the species, we conducted AFLP analysis using four primer combinations for 31 populations. The genetic diversity of this species was high based on the level of polymorphic loci (155 of 197 loci; 92.31%) and Nei's gene diversity (ranging from 0.14532 to 0.2435; overall 0.2446). There was significant population genetic differentiation (G(ST)= 0.287; circle minus (II)= 0.221 from the Bayesian f = 0 model). Results from the AMOVA analysis suggest that a majority of the genetic variance is attributed to variation within populations (69.43%), which is also evident from the PCoA. An estimate of the outcrossing rate based on genotypes of progenies from seven of the 31 populations using the multilocus method from the program MLTR ranged from 0.695 to 0.903, suggesting that the species is predominantly outcrossing. These results are encouraging for conservation, signifying that populations may persist due to continued genetic exchange sustained by the outcrossing mating system of the species. **Acknowledgements:** This research was supported by the Natural Science Foundation of China (NSFC 30770153). **References:** 1. Chawla R et al. (2005) Mol Cell Biochem 273: 193 – 208.

P031 Searching for plant-derived natural products by *in vitro* cultivation

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Modern cancer therapy uses more and more natural plant compounds for the development of new therapeutics during the last years. Some of them are rather approachable by the use of bioreactor and/or cell culture techniques than by green house cultivation or wild collection. More than 200 different species of herbs and rare medicinal plants with traditional use were taken into *in vitro* culture. In bioreactors with fully automated nutrient and gas exchange root, shoot and cell cultures were cultivated under different growth conditions. About 500 extracts were analysed by Analyticon's LC/MS-based Chemodiversity Profiling Platform. Combining bioreactor technology with high throughput screening methods high productive plants were selected according to their biomass production and content of active compounds. First results have been shown that *in vitro* cell and organ cultures can produce quantitatively more active compounds than plants of wild collection or green house culture. Compared to this, 20% of the bioreactor material showed a new chemical pattern of natural compounds. In addition, spectrum of secondary metabolites varies at different *in vitro* culture types. The most promising *in vitro* cultures were selected for scale-up in 5 to 10 l bioreactors to optimise the production of preferable compounds. Fractionation of selected extracts and structure elucidation demonstrate that *in vitro* cultures are able to produce secondary metabolites with the same structural diversity as traditionally grown plants. Therefore, *in vitro* cultivated plants combined with the screening platform technology are an attractive source for identification of novel drugs and providing natural plant compounds as pharmaceutical products.

P032 A comparative study on phenolic profiles and antioxidant activities of *Aloe* Species

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Plants contain a diverse group of phenolic compounds with the structural requirements of free radical scavengers. Therefore, crude extracts of fruits, herbs, vegetables and other plants materials are increasingly of interest in the food and pharmaceutical industries [1]. The effects of different extracting solvents have been tested for the extraction of phenolic compounds from plant material [2]. In the regions of Canary Islands (African Northwestern Coast), it is prevailing all the year a high level of solar radiation. This force plants to develop defence mechanisms against excessive production of free radicals through the accumulation of antioxidant substances. Chemical studies of several species of plants from Canary Island reported quantity differences in the chemical composition, as compared to the rest of plants found in other regions [3]. This prompted us to evaluate total phenolic content, antioxidant activities and the differences in the phenolic profiles of the crude extracts derived Aloe vera plants. Aloe species have been used for a long time in folk medicine for the treatment of constipation, burns and dermatitis. On the present study we compare the total phenolic contents (TPC) and antioxidant activities of several extracts derived from Aloe vera plants. Extracting solvents significantly affected TPC determined using the Folin-Ciocalteu method. Antioxidant activity of the crude extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The phenolic compounds present in the extracts were identified and quantified by reverse phase high performance liquid chromatography (RP-HPLC) techniques. Significant differences in the phenolic profiles among extracts were observed. **Acknowledgements:** This research was supported partly by the Caja Insular de Ahorros de Canarias and the Consejería de Vivienda y Arquitectura, Agricultura, Ganadería y Pesca y Agua del Cabildo Insular de Gran Canaria. **References:** 1. a) Prior, R.L. & Cao, G. (2000). Horticulture Science, 35:588 – 592; b) Steinmetz, K.A. & Potter, J. D. (1996). Cancer Causes and Control, 2:325 – 351. 2. Pinelo, M., Rubilar, M., Sineiro, J. & Nunez, M. J. (2004). Food Chemistry, 85:267 – 273. 3. a) Triana, J., López, M., Rico, M., González-Platas, J., Quintana, J., Estévez, F., León, F., González, A., Bermejo, J. (2003). Journal of Natural Products, 66:943 – 948; b) Triana, J., López, M., Pérez, F.J., González-Platas, J., Quintana, J., Estévez, F., León, F., Bermejo, J. (2005). Journal of Natural Products, 68:523 – 531.

P033 Taxane production in hairy roots of *Taxus x media var. Hicksii* carrying taxadiene synthase gene

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Paclitaxel is the active agent of Taxol® (Bristol-Myers Squibb), one of the most effective anticancer plant – derived drug showing activity against different cancers. Moreover paclitaxel has shown great promise as a locally delivered antirestenotic agent and also seems to be an effective agent for the treatment of Alzheimer's disease and other neurodegradive diseases [1,2]. A very promising approach for the production of paclitaxel and related taxanes, without forest harvestation, is provided by plant cell suspension cultures and its production has been recently successfully scaled-up [3]. Two lines of hairy roots were obtained as a result of infection of 10-year old plantlets cultivated in vitro on solid DCR medium [4]. Two bacterial strains were used: *Agrobacterium rhizogenes* and modified *A. tumefaciens* with Ri plasmid. These both bacterial strains carried taxadiene synthase gene. Taxadiene synthase leads cyclization of GGPP to taxa 4(5), 11(12)-diene. This is first committed step of paclitaxel biosynthetic pathway. The hairy roots were cultivated in liquid hormone-free DCR-Medium [5] in dark. Medium supplementation with precursor L-phenylalanie and/or elicitor methyl jasmonate are underway to test the growth of roots and taxane accumulation in roots and post-culture media. **References:** 1. Nims, E. et al. (2006). Metab. Eng. 8: 385 – 394. 2. Li, G. et al. (2003). J. Neurochem. 84: 347 – 362. 3. Frense D. (2007). Appl. Microbiol. Biot. 73: 1233 – 1240. 4. Gupta, P.K., Durzan, D.J. (1985). Plant Cell. Rep. 4: 177 – 179. 5. Syklowska-Baranek, K. et al. (2009). J. Plant. Physiol. 166: 1950 – 1954.

P034 The first report of microsatellite primer pairs for genetic studies in jojoba [*Simmondsia chinensis* (Link) Schneider

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Jojoba [*Simmondsia chinensis* (Link) Schneider] is an important industrial plant, native to Arizona, southern California and northern Mexico. The product of primary interest in jojoba is the seed oil, which consists of esters formed from acids and alcohols with chain lengths of 20 or 22 carbon atoms. As many as 300 products containing jojoba have appeared in markets in recent years and the use of jojoba products is expected to increase in future [1]. The use of DNA markers in jojoba breeding is limited and the best method for jojoba improvement has been the selection of plants with desirable characteristics. DNA markers have been extensively used in plant improvement studies [2,3,4]. In the present study a total of 10 microsatellite markers were identified using a strategy described in [5] and this is the first report on jojoba microsatellite markers. In order to evaluate microsatellite primer pairs (Table 1), genomic DNAs of several jojoba samples were extracted using a DNA extraction protocol described in [6]. Results indicated that these primer pairs are very useful in genetic studies of jojoba.

Table 1: List of microsatellite primer pairs and related information

Locus	Forward Primer (5→ 3')	Reverse Primer (5' → 3')	Motif	Size (bp)
JMA01	ACACCAGATTCCAGAGGCATA	ATTCGTCAAAGGGGATGATG	$[CT]_8$	198
JMA02	AGAGTACGCGGGAAGCAGT	TGCTGGCAAGGGAGGTAATA	$[AG]_8$	600
JMA03	AGTCGTTTCCCCTGCTTTTC	CTTCTGCTTATCCCCCTCATC	$[CT]_7$	320
JMA04	GGACCTCTGCCCTTCTTCTT	TGGCGTCTTCACTGCTACTC	$[GT]_{11}$	500
JMA05	CGGGGATTTATAGTCTTCACTCTC	GTCCAGGCTTCAGACCAGAG	$[TC]_{13}$	214
JMA06	GCATCTGCCATTTTATGTTCAG	AACCCAGTTCCAGCTTCATC	$[AAT]_5$	180
JMA07	GCCAAGTGGGGATGTAGAGA	GGGGACTGAACTCCACCAA	$[GA]_8$	165
JMA08	GGAACCACAATGGCAACG	CGCAGGAAGGTCGTAAACTG	$[TCT]_9$	185
JMA09	GCGGGGAAAGTGTTACGC	GATTAGCAGAGAAACCAAGGGACT	$[AG]_{15}$	190
JMA10	AGTCAGAGTCACAGAGCAATGAA	AAGAGATTAGCAGAGAAACCAAGG	$[TCT]_5$	700

References: 1. Ince, A.G. et al. (2009) Genet. Resour. Crop. Ev. doi: 10.1007/s10722 – 009 – 9516 – 1. 2. Karaca, M., Ince, A.G. (2008) J. Genet. 87:83 – 86. 3. Ince, A.G. et al. (2009) Genet. Resour. Crop. Ev. 56:211 – 221. 4. Ince, A.G., Karaca, M. (2009) J. Sci. Food Agric. 89:168 – 176. 5. Ince, A.G. et al. (2008) Plant Cell Tissue Organ Cult. 94:281 – 290. 6. Karaca, M. et al. (2005) Anal. Biochem. 343:353 – 355.

P035 Phytochemical investigation of *Hypericum xylosteifolium* (Hypericaceae), a Caucasus endemic species

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The small mountainous country of Georgia lies within the Caucasus Biodiversity Hotspot, which has ca. 2750 endemic vascular plant species. High interest in assessing the biodiversity and conservation status of selected native plant species of Georgia, particularly those with potential or existing economic value, exists [1]. As part of a collaborative research project, an IUCN Red List assessment of the endemic species *Hypericum xylosteifolium* (taxonomic section *Inodora*) for Georgia and the northern Caucasus region was conducted and distribution maps based on historical information and recent field studies prepared. Correspondingly, due to the medicinal value and interesting phytochemistry of related *Hypericum* species, a preliminary phytochemical examination of cultivated material from this species was conducted. Dichloromethane extracts of the fruits were analyzed by chromatographic means (TLC, OC, HPLC) and structure elucidation of isolated pure compounds was performed using data from NMR and MS. This research resulted in the isolation of several γ-pyrone derivatives, including hyperenone A (1) and B (2), which have been previously reported from *H. mysurense* (sec-

tion *Ascyreia*) (2). Interesting aspects of this observed chemical convergence are discussed.

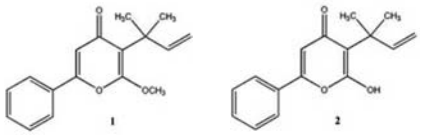

Fig. 1: Gamma-pyrone derivatives from *Hypericum xylosteifolium*

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P036 Development of new set of EST-SSR primer pairs for celery (*Apium graveolens* L.)

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Apium graveolens L. is the only cultivated species in the genus *Apium* and consists of three distinct taxonomic varieties. Root celery (*A. graveolens var. rapaceum*) is characterized by swollen hypocotyls and root tissue that results in a strongly flavored, globelike structure. Leaf celery (*A. graveolens var. secalinum*) has slender, leafy petioles and is used mostly for their leaves for medicinal purposes [1]. Common celery (*A. graveolens var. dulce*) has succulent, solid petioles and is used for fresh vegetable or spice, the extraction of terpenes, furano-coumarins. Interestingly, celery has recently become very popular in molecular biology, as a source of an endonuclease (CEL I) which is used in targeting induced local lesions in genomes (TILLING) studies. The use of molecular markers in celery has been limited to a few studies using isozymes, restriction fragment length polymorphism, random amplified polymorphic DNA, and amplified fragment length polymorphism. Among DNA markers, simple sequence repeat technique (SSRs or microsatellites) has many advantages since they are co-dominant, highly polymorphic, and polymorphism can be detected with a simple polymerase chain reaction (PCR) procedure [2,3]. Microsatellite primer pairs developed in this study (Table 1) will be very helpful in genetic studies and identification of gene containing microsatellite in celery [4].

Table 1: List of microsatellite primer pairs and related information

Locus	Forward Primer (5→3')	Reverse Primer (5'→ 3')	Motif	Size (bp)
AG01	CGATGGTGGCTATGTCGGTA	TGAAGTGGGGGTTGAGTAGAATC	[AAT]18	61
AG02	TGGCAATGAGTGGTGCTCT	CGCAAGTCGTGAAGATAAGTAGAAT	[ATA]12	60
AG03	TGATACTGCCTCTGACCCAAT	GTGCGTGGAAAGTGATGAGA	[ATC]14	59
AG04	AGTGCCAAAACTGGGAACATT	ATGAAGGTGGAGGACGAGTG	[CCTTCA]12	60
AG05	CCACTCATTGCTGTCCTAACAC	GGTCCATTTCTCTGCGTTTG	[CT]10	60
AG06	CCTGAACCTGACAATCATACGG	CCAAAACCTCCAACAAGAATAAGAG	[CT]30	61
AG07	GGCACCAGCAAAGATGGATA	GGCGTCTCTCCGACCAAC	[GA]22	61
AG08	GTTGACTGGTATTCTTGTTCATCTG	GCTCTATCCATTTCTCTCTTGTTTC	[GAT]16	59
AG09	ATCCCCTTTCTCCCCCTTT	ACCTCCTGCTCATCAACCTCT	[GAT]20	60
AG10	CGGGGAACCAAACTCCTAC	CCCTACTTCAACAGCCATCC	[TC]18	59
AG11	TCGCTCATCTCTCTCATCTCA	TGACCTCCGTAAAATCCAAA	[TC]18	58
AG12	CTTCCTCTCCCACCACAAAG	CACCAGCACCCCAGGTAATA	[TTC]8	60

Acknowledgements: This study was supported by the Scientific Research Projects Coordination Unit of Akdeniz University **References:** 1. Muminovic, J. et al. (2004) Plant Genet. Resour. 2:189 – 198. 2. Karaca, M., Ince, A.G. (2008) J. Genet. 87:83 – 86. 3. Ince, A.G. et al. (2009) Genet. Resour. Crop. Ev. 56:211 – 221. 4. Ince, A.G. et al. (2008) Plant Cell Tissue Organ Cult. 94:281 – 290.

P037 Tracing the evolution of benzoic acid-specific type III polyketide synthases (BPS and BIS) in Bonnetiaceae, Guttiferae *sensu lato* and Podostemaceae, evidence from phytochemistry

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Biphenyl synthase (BIS) and benzophenone synthase (BPS) are type III polyketide synthase (PKS) enzymes, responsible for the biosynthesis of a rich diversity of plant secondary metabolites, and share a common ancestor with chalcone synthase (CHS) enzymes, which contribute to the biosynthesis of flavonoid precursor molecules [1, 2]. BIS is the key enzyme in biosynthesis of biphenyl and related dibenzofuran derivatives, commonly found in Rosaceae (order Rosales), while BPS catalyzes the production of benzophenone precursors, which are further modified to form xanthones and benzophenone derivatives, commonly found in Guttiferae *sensu lato* (order Malpighiales). These classes of compounds can serve as defence molecules, elicited in response to microbial infection [2, 3]. As part of a project to examine the distribution of secondary metabolites stemming purely or in part from cyclization of benzoyl-primed polyketides in *Hypericum* (Hypericaceae) and related taxa, the distribution of xanthones and benzophenones in a clade, strongly supported by molecular evidence [4], containing Bonnetiaceae, Calophyllaceae, Clusiaceae, Hypericaceae and Podostemaceae was examined and is reported. Biphenyl derivatives were identified in members of all families except Bonnetiaceae, stimulating the formation of the following questions: (1) are biphenyls present in Bonnetiaceae, but not yet isolated? (2) was BIS silenced in the line giving rise to Bonnetiaceae, following the split with sister taxon, Calophyllaceae? and (3) has BIS been retained in other families of Malpighiales, which along with Rosaceae belong to the broader Eurosid I clade, or has it evolved twice within higher plants?

Acknowledgements: This research was supported in part by a grant from the Austrian Science Foundation (FWF, Project T345). **References:** 1. Schröder (1999) Comprehensive Natural Products Chemistry, vol. 1. Elsevier Science, Amsterdam, pp. 749 – 71. 2. Beerhues and Liu (2009) *Phytochemistry* **70**: 1719 – 27. 3. Franklin et al. (2009) Phytochemistry **70**: 60 – 8. 4. Wurdack and Davis (2009) *American J. Botany* **96**: 1551 – 70.

P038 Screening of nine organelle DNA loci for polymorphism in twenty-two medicinal and aromatic plant species

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Total genomic DNAs along with organelle DNAs from *Cannabis sativa* L., *Cuminum cyminum* L., *Humulus lupulus* L., Pimpinella anisum L., *Trigonella foenum-graecum* L., *Phlomis lycia* D. Don, *Salvia sclarea* L., *S. fruticosa* Mill., *S. tomentosa* Mill., *S. dichroantha* Stapf., *S. virgata* Jacq., *Sideritis erythrantha* Boiss. Et Heldr. Apus Bentham, *S. pisidica* Boiss. & Heldr. Apud Bentham, *S. arguta* Boiss. Et Heldr., *S. perfoliata Linnaeus*, *S. stricta* Boiss. & Heldr. Apud Bentham, *S. libanotica* Labill. subsp. linearis (Bentham) Bornm., *Origanum majorana* L., *O. onites* L., *Melissa officinalis* L., *Rosmarinus officinalis* L. and *Teucrium* L. were extracted according to [1] and analyzed [2]. Polymerase chain reactions of the samples were performed as described in [3,4] using primer pairs listed in Table 1. Amplified products of three chloroplast gene segments were not polymorphic across the plant species under the study. However, these gene segments can be used in Cleaved Amplified Polymorphic Sequence (CAPS) markers [5] to investigate their use in genetic studies. Analyses indicated that among the 9 loci, 6 were polymorphic across the 22 plant species giving PIC values ranging from 0.86 to 0.66 (Table 1). This study indicated that the use of polymorphic 6 organelle loci could be used as diagnostic markers in plant identification and genetic studies of medicinal and aromatic plant species [2,3].

Table 1: List of nine organelle specific primer pairs

Locus	Forward Primer (5'→3')	Reverse Primer (5'→3')	PIC*
trnH-psbA (chloroplast)	TGATCCACTTGGCTACATCCGCC	GCTAACCTTGGTATGGAAGT	0.860
cp-trnK (chloroplast)	GGGTTGCCCGGGACTCGAAC	CAACGGTAGAGTACTCGGCTTTTA	0.000
cp-trnS-psbC (chloroplast)	GGTTCGAATCCCTCTCTCTC	GGTCGTGACCAAGAAACCAC	0.000
psbC (chloroplast)	GGTCGTGACCAAGAAACCAC	GAGCTTGAGAAGCTTCTGGT	0.000
18S rRNA-5 SrRNA (mitochondrion)	GTGTTGCTGAGACATGCGCC	ATATGGCGCAAGACGATTCC	0.814
Nad1B-Nad1C (mitochondrion)	GCATTACGATCTGCAGCTCA	GGAGCTCGATTAGTTTCTGC	0.661
Nad5F-Nad4R (mitochondrion)	CAGTGGGTTGGTCTGGTATG	TCATATGGGCTACTGAGGAG	0.722
Rps14 (mitochondrion)	CACGGGTCGCCCTCGTTCCG	GTGTGGAGGATATAGGTTGT	0.710
trnL (chloroplast)	GGGGATAGAGGGACTTGAAC	CGAAATCGGTAGACGCTACG	0.680

*PIC: polymorphism information content values

References: 1. Karaca, M. et al. (2005) Anal. Biochem. 343:353 – 355. 2. Ince, A.G. et al. (2009) Genet. Resour. Crop. Ev. 56:211 – 221. 3. Karaca, M. et al. (2008) J. Sci. Food Agric. 88:2508 – 2516. 4. Karaca, M., Ince, A.G. (2008) J. Genet. 87:83 – 86. 5. Ince, A.G. et al. (2010) 25:491 – 499.

P039 Changes in distribution and structure of wild *Origanum vulgare* L. populations during the last decade in Armenia and implications for conservation

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Armenia has a rich flora of ca. 3600 plant species (ca. 50% of the entire Caucasian flora), distributed across (semi)desert, steppe, forest and alpine landscapes. Native plant biodiversity and conservation status of these species, particularly those with economic value, needs further assessment [1]. Anthropogenic threats to biodiversity (overpopulation, deforestation and urbanization) have simultaneously hindered research and increased the need for it. Of the ca. 500 species with medicinal/economic use records, ca. 50 are used in folk medicine, including both wild-collected (e.g. *Crataegus* sp., *Hypericum perforatum*) and cultivated (e.g. *Chamomilla recutita*, *Mentha piperita*) species [2]. Only limited information, however, on genetic biodiversity, population structure, and conservation status of these species is available. From 2006 – 2009, field studies were conducted to re-locate wild *Origanum vulgare* L. populations based on historical records, and discover new populations. The growth, phenological and habitat characteristics, population size and location (GPS mapping), were assessed. Historical records indicated that this species occurred widely in the central/northern regions, but nearly half the populations had vanished. Remaining populations diminished in size, plant number and experienced fragmentation during the study period. 3 new populations were located in the south/south-eastern regions, indicating that the abundance and distributional range is expanding here. Anthropogenic threats included: poor land management, increasing population pressure, and excessive collection of plants. This research provided baseline data for the development of *ex situ* and *in vitro* strategies to conserve unique genotypes, and assess the sustainability of wild populations according to IUCN Red Book Criteria, of this important species in Armenia. **References:** 1. IUCN, WHO, WWF (1993). Guidelines on the Conservation of Medicinal Plants, IUCN, Gland, Switzerland, 50 p. 2. Fayvush, G., Danielyan T., Nalbandyan A. (2004) Armenia as a producer of medicinal plants: possibilities and perspectives. Available online (accessed 12 April 2010): http://www.nature-ic.am/NCSA/Publication/Medical_Plants_eng.pdf.

P040 CAPS and DAMD-PCR assays for species identification of *Convolvulus* L. (Convolvulaceae)

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Convolvulus is the second largest genus consisting of approximately 250 species within the family Convolvulacea [1]. Some *Convolvulus* species are important weeds which cause economic losses in the field crops and some other species have valuable medicinal properties. Many species in the genus *Convolvulus* have phenotypic plasticity which is the ability of an organism to change its morphology, physiology or development in response to environmental changes. Due to the phenotypic plasticity and natural hybridization between taxa, identification of some of the species in the genus is problematic or impossible using morphological markers. Among the molecular markers, DNA sequence variations have been extensively used in plant improvement and identification studies [2,3,4]. This study utilized a total of 42 plant samples collected throughout Turkey and containing 12 different species to investigate potential Cleaved Amplified Polymorphic Sequence (CAPS) and Directed Amplification of Minisatellite-region DNA (DAMD-PCR) markers suitable in identification of species. Total DNAs were extracted using a DNA extraction method described in [5]. Results indicated that in comparison to mitochondrial gene segments, chloroplast gene segments were diagnostically useful in plant identification in *Convolvulus*. For instance amplified products of chloroplast trnH-psbA regions produced valuable markers after digestion with *Hinf* I and *Vsp* I restriction enzymes. CAPS assays using the nuclear ribosomal DNA internal transcribed spacers (ITS) and *Hinf* I enzyme digestion produced species specific markers. This study also indicated that DAMD-PCR assays were very useful in plant and species identification in Convolvulus. This study is the first report of DAMD-PCR application on Convolvulus. **Acknowledgements:** This study was supported by the Scientific Research Projects Coordination Unit of Akdeniz University **References:** 1. Gonzalez, A.V., Gianoli, E. (2004) Acta Oecologica 26:185 – 190. 2. Karaca, M., Ince, A.G. (2008) J. Genet. 87:83 – 86. 3. Ince, A.G. et al. (2009) Genet. Resour. Crop. Ev. 56:211 – 221. 4. Ince, A.G., Karaca, M. (2009) J. Sci. Food Agric. 89:168 – 176. 5. Karaca, M. et al. (2005) Anal. Biochem. 343:353 – 355.

P041 CAPS-microsatellites are suitable for Ginger (*Zingiber officinale* Roscoe) genetic studies

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Ginger (*Zingiber officinale* Roscoe) is a perennial plant in the family Zingiberaceae. Rhizomes of plant have been used safely for thousands of years in folk medicine and cooking. Although production areas increase, ginger production is seriously being affected by several diseases. DNA based technologies can be applied for the identification of wild germplasm or commercially important plants with disease resistance genes. However, there exists limited research on the characterization of ginger germplasm. DNA markers are valuable for germplasm identification and plant genetic mapping studies [1,2]. Expressed sequence tags (ESTs) in data bases can be used in development of DNA markers suitable for ginger genetic studies [2,3]. However EST-based microsatellites have low level of polymorphism [4]. Recently it has been reported that monomorphic microsatellite markers can be converted into polymorphic markers using a method called CAPS-microsatellites [4]. Up to date researchers and commercial companies have developed more than one thousand restriction enzymes. This study was undertaken to identify types of restriction enzymes suitable in CAPS-microsatellite analysis. Using Sequencher software we identified restriction enzymes which frequently digest ESTs (Table 1) [5]. Genomic DNAs of two ginger samples were extracted and amplified with several EST-based microsatellite primer pairs [3,4]. Results indicated that CAPS-microsatellite markers are valuable in genetic studies in *Zingiber officinale* Roscoe.

Table 1: List of restriction enzymes suitable in CAPS-Microsatellite analysis

Restriction Enzymes		
Aci I	Hinf I	*Nla* IV
Alw I	Hph I	Rsa I
Apo I	Mae I	*Sau*96 I
Bsl I	*Mae* III	ScrF I
*Bst*7 II	Mbo I	Sec I
*Cac*8 I	*Mbo* II	SfaN I
Dde I	Msp I	Taq I
Fnu4 H I	Mwo I	Tfi I
Hae III	*Nia* III	*Tru*9 I

References: 1. Karaca, M., Ince, A.G. (2008) J. Genet. 87:83 – 86. 2. Ince, A.G. et al. (2008) Plant Cell Tissue Organ Cult. 94:281 – 290. 3. Ince, A.G. et al. (2010) Mol. Breed. 25:645 – 658. 4. Ince, A.G. et al. (2010) 25:491 – 499. 5. Karaca, M. et al. (2005) Anal. Biochem. 343:353 – 355.

P042 Mono- and dinucleotide repeat DNA content differs in dioecious *Asparagus* (*Asparagus officinalis* L.)

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Asparagus (*Asparagus officinalis* L.) is a green vegetable with high antioxidant activity among the commonly consumed vegetables. This plant is naturally dioecious and female plants are homozygous for the recessive alleles (mm) while male plants are either homozygous (MM) or heterozygous (Mm) at the sex locus [1]. In the present study we reported that among the expressed gene/gene segments of asparagus deposited into data bases, mononucleotide and dinucleotide repeat contents were significantly different (Table 1) using a computational approach [2]. Results indicated that genes in male asparagus contained more mononucleotides while female contained fewer amounts of mono-

nucleotide repeats containing genes. On the other hand dinucleotide containing genes in female were much higher than that of the male asparagus. Mono- and di-nucleotide repeat content differences between female and male asparagus indicated that microsatellite instability of mono- and di-nucleotide tandem repeat sequences is much higher than tri-, tetra- penta- and hexa-nucleotide repeat sequences. Although repeat content differences among genes have been previously reported in other plant species [3,4] this study is the first report on repeat content differences between plant sexes. Repeat content differences between the sexes may contribute differenzial expression of genes [5] which may affect the chemical properties of asparagus.

Table 1: Repeat content differences between male and female *Asparagus officinalis* L.

Sex	# Bases	#EST	# EST-SSR		Mono-		Di-		Tri-		Tetra-		Penta-		Hexa-	
			Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
Male	4165918	5188	1003	982.5	660	594.4	114	156.0	170	175.6	5.0	3.5	9	10.5	45	42.5
χ^2			0.43		22.287***		16.109***		0.38		0.0		0.352		0.388	
Female	1812283	3234	407	427.4	193	258.5	110	67.9	82	76.39	0.0	1.5	6	4.6	16	18.5
χ^2			0.977		16.635***		26.095***		0.41		1.516		0.464		0.336	
Total	5978201	8422	1410		853		224		252		5		15		61	

EST: Expressed Sequence Tags, Obs: Observed, Exp: Expected, SSR: Simple Sequence Repeats, ***: statistically significant at $P < 0.0001$.

Acknowledgements: This study was supported by the Scientific Research Projects Coordination Unit of Akdeniz University **References:**
1. Jamsari, A. et al. (2004) Theor. Appl. Genet. 108:1140 – 1146. 2. Ince, A.G. et al. (2008) Plant Cell Tissue Organ Cult. 94:281 – 290. 3. Ince, A.G. et al. (2010) Mol. Breed. 25:645 – 658. 4. Ince, A.G. et al. (2010) 25:491 – 499. 5. Ince, A.G., Karaca, M. (2009) J. Sci. Food Agric. 89:168 – 176.

P043 Immunomodulating effects of extracts from Icelandic marine invertebrates

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Iceland has a unique position in the North Atlantic, with marine biodiversity largely unexplored with respect to chemical constituents. In particular, the confluence of cold-water currents from Atlantic gyres and warm-water geothermal activity accounts for a unique marine biogeography that has never been evaluated for the potential of marine natural product diversity. The aim of this project is to investigate the immunomodulating effects of extracts and fractions obtained from marine invertebrates collected around Iceland. As dendritic cells play an important role as a bridge between the innate and adaptive immune response the effects of extracts from marine organisms were tested by analysing the cytokine secretion and expression of surface molecules on human monocyte-derived dendritic cells. Sixty extracts were prepared by solvent extraction [dichloromethane/methanol (1:1)] and screened for immunomodulating effects. Four extracts at the concentration 100 µg/mL were shown to be cytotoxic and seven reduced both IL-12p40 and IL-10 secretion and CD 86 expression. An active extract of the sponge, *Isodictya palmata*, was further fractionized using modified Kupchan partition and the chloroform and hexane fractions were shown to be active in the dendritic cell model. Further fractionation and characterization is in progress.

P044 The cultivation of medicinal desert plants

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In the medicinal plant industry, the rising demand for medicinal plants, the increasing awareness of extinction of species and the recent technological developments in agriculture enhance the need for and benefit of the cultivation of medicinal plants. The aim of our research is to find new medicinal crops and to get a better understanding of the relationships between secondary metabolites production and environmental conditions. We focus in our research on three wild perennial species that are commonly used in traditional Middle Eastern medicine: Artemisia judaica- the major constituents of the plant's essential oil are camphor and piperitone [1]. Artemisia Sieberi- the plant's essential oil consists mainly of camphor and 1,8-cineole [2]. Origanum Dayi- It's essential oil contains mainly 1,8-cineole, α-terpineol, (E)-sabinene hydrate, (E)-sabinene hydrate acetate, terpinen-4-ol and linalyl acetate [3]. Plants from two different sources (cultivar cuttings and wild seeds) were grown in the field under three water regimes: 100, 70 and 30% of the daily potential evapotranspiration (PET). The following parameters were examined: rate, volatile composition (using GC-MS), relative water contents, osmotic potential, photosynthetic rate, photochemical efficiency and the allelopathic effect on weed growth. The results indicate that the three species are highly productive under controlled field conditions even under relatively low irrigation regimes. Artemisia judaica exhibited high allelopathic effects. Our hope is that this research, which combines plant physiology, chemistry and agriculture, will be useful for both scientists and farmers. **References:** 1. Ravid, U. et al. (1992) j. Flavour and Fragrance 7:69 – 72. 2. Feuerstein, I. et al. (1986) j. Phytochemistry 25:2343. 3. Dudai, N. (2003) j.Flavor and Fragrance 18:334 – 337.

P045 Pyrrolizidine alkaloids in *Senecio* species from the urban area of Vienna (Austria)

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Senecio species (Asteraceae) are poisonous plants due to pyrrolizidine alkaloids present in all plant parts. Here is reported the alkaloid distribution in the different above ground plant organs from Senecio erraticus Bertol., S. inaequidens DC, S. jacobea L., S. vernalis Waldst. & Kit. and S. vulgaris L. collected during summer from various habitats in the urban area of Vienna, Austria to get actual data about the alkaloid contents of these plants. The dried plants were separated in stems, leaves and inflorescences, ground and extracted with methanol/HCl. After reduction with Zn dust and extraction with dichloromethane the alkaloids were analysed by GC/MS [1]. All investigated species contained macrocyclic pyrrolizidine alkaloids. The identified alkaloids were senecivernine, senecionine, seneciphylline, integerrimine, retrorsine, usaramine, erucifoline and acetyl-erucifoline. The highest alkaloid contents were found in the inflorescences where total contents up to 10 mg/g DM could be recorded. Inflorescences representing 35% of the plant dry matter contained about 80% of the total alkaloids of the plant. **Acknowledgements:** We gratefully thank Mrs. H. Michitsch, Mrs. N. Kopf and Mr. Patrick Zwickl for their technical assistance **References:** 1. Witte, L. et al. (1992) Phytochemistry 31, 559 – 565.

P046 Shoot culturesa of some species from the genus *Phyllantus* as a source of biologically active secondary metabolites

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Genus *Phyllanthus* (*Euphorbiaceae*) comprises numerous species commonly used in folk medicine in Asia and South America in the treatment of liver diseases and genitourinary disorders. According to literature data, species from the genus *Phyllanthus* are a rich source of lignans, alkaloids and flavonoids. The aim of the study was to recognize the chemical composition of the shoot cultures of the following *Phyllanthus* species: *P. grandifolius*, *P. juglandifolius, P. multiflorus*, *P. glaucus* and *P. amarus*. Selective production of catechins was revealed in shoot cultures of *P. grandifolius* and *P. juglandifolius* harvested on MS medium supplemented with BAP and TDZ. Catechins possess strong antioxidant activity and play a role in the inhibition of carcino- and mutagenesis. The presence of the following compounds was affirmed in catechin complex: (−)-epicatechin, (+)-catechin, (−)-epigallocatechin and (−)-gallocatechin. Only traces of catechins are present in the shoot culture of *P. multiflorus* and *P. glaucus*. The presence of flavan-3-ols derivatives was confirmed

using HPTLC and UFLC methods. In the shoot cultures of all investigated species β-sitosterol and β-amyrin were determined using HPTLC method. In the shoot culture of *P. multiflorus* harvested on SH_0 the presence of flavonoids was revealed. Among the analyzed plant material only the shoot culture of *P. glaucus* harvested on MS medium supplemented with BAP and IBA contained alkaloids belonging to securinine-type. Securinin and its derivatives are GABA antagonists and inhibit inflammatory process induced by β-amyloid protein. In the shoot cultures of *P. amarus* the presence of complex of lignans, mainly phyllanthin and hypophyllanthin, was revealed using HPTLC and HPLC methods. These compounds are considered to be responsible for the antiviral activity of *Phyllanthus* species, especially in the treatment of *hepatitis B*. **References:** 1. Calixto, J. B. et al. (1998) Med. Res. Rev. 18: 225 – 258.

P047 Bioactive secondary metabolites from Tunisian medicinal plants

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The Tunisian biodiversity has been the subject of several biological and chemical investigations [1 – 3]. In the aim to continue our efforts to develop the Tunisian natural resources, we undertook phytochemical and biological studies of some plants selected according to their wealth essentially of terpenoids. We studied some Apiaceae (*Ferulago, Ferula*), Liliaceae (*Allium*) and Chenopodiaceae (*Atriplex*) representatives. Some of these genus are known for their high content in essential oils and terpenoids (sesquiterpenoids, coumarins, triterpenoids and steroids) [4 – 6], and for their pharmacological properties in particular immunostimulant, cytotoxic, hepatoprotective, antiviral, antimicrobial, anti-mutagenic, hypotensive and hypoglycemic activities. Our analytic and preparative chromatographic studies on some organic extracts (MPLC, VLC, CC, PTLC) led to isolation of 25 pure compounds (coumarins, sesquiterpene-coumarins, terpenes, phenols and saponins). Their structures were elucidated on the basis of chemical and spectroscopic evidence mainly 1D (^{1}H, ^{13}C) and 2D NMR (HSQC, HMBC, COSY, TOCSY, NOESY). Some of the prepared extracts (non volatile and essential oils) and pure compounds were tested for their cytotoxic, antibacterial, antifungal, acetylcholinesterase and antioxidant activities. **Acknowledgements:** The authors are thankful to the IFC-Tunisie (Institut Français de Coopération en Tunisie) for a scholarship to A. J. **References:** 1. Jabrane, A et al. (2009) Chem. Biodiv. 6:881 – 889. 2. Jabrane, A et al. (2010) Chem. Biodiv. 7:392 – 399. 3. Ghouila, H et al. (2009) Tetrahedron Lett. 50:1563 – 1565. 4. Abd El-Razek, M H. et al., (2001), Phytochem. 58:1289. 5. Hostettmann, K., Marston, A. (1995). Saponins. Cambridge: Cambridge University Press. 6. Siddiqui, B. S et al. (1994) Phytochem. 37:1123 – 1125.

P048 Three new medicagenic acid saponins from *Polygala micrantha*

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The cosmopolitan Polygalaceae plant family, consists of approximately 1000 species distributed in 15 genera [1]. The genus includes herbaceous perennial plants, shrubs and small trees, and has a subcosmopolitan distribution. *Polygala micrantha*, a medicinal herbaceous plant, is said to be used as a purge in Cameroon. Polygala species have already been investigated, resulting in the isolation of presenegenin and medicagenic acid glycosides [2 – 3] but no previous phytochemical study has been undertaken on *P. micrantha*. Phytochemical investigation of the roots of *P. micrantha* yielded 9 triterpene saponins, including three new medicagenic acid saponins (**1**-**3**) and six known presenegenin saponins. The structures of the new compounds were elucidated by extensive spectroscopic 1D and 2D NMR analysis and by comparison of their NMR data with those of related compounds. **Acknowledgements:** The authors are grateful to the Conseil Régional de Bourgogne, France for financial support. **References:** 1. Lacaille-Dubois, M.A. et al. (2005) Phytochem. Rev. 4: 139 – 149. 2. Haddad, M. et al. (2003) Helv. Chim. Acta 86: 3055 – 3065. 3. Mitaine-Offer, A.C. et al. (2003) Helv. Chim. Acta 86: 2404 – 2413. 4. Teng, R.-W. et al. (2002) Magn. Reson. Chem. 40: 424 – 429.

P049 Phytochemical variability of populations of *Aloysia citriodora* from Argentina

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Aloysia citriodora Palau (*Verbenaceae*) is one of the most widely used herbs in the Argentine traditional medicine as antispasmodic, sedative and for the treatment of stomachache. Its worldwide use has promoted its cultivation not only in our country but also in several Latin American countries, Europe and Asia. There are official monographs in Argentine (FA) and European Pharmacopoeias (PhEU, as "lemon verbena) for the pharmaceutical quality control. Our working group is devoted to update the respective monograph of the FA, taking in account the detected phytochemical variability of local populations. Parameters for a quality profile of volatile metabolites were previously established [1,2]. At this time we have evaluated the phytochemical variability of several populations using the technique by the European Pharmacopoeia (HPLC), in order to identify the best materials for crops affordable for the pharmaceutical industry. We analyzed 22 populations from 12 regions from Argentina, both in culture and wild materials. Our results show significant differences in the contents of verbascoside: from 0.5 to 4.8%, being 2.5% the minimum accepted value in the PhEu. Other peaks were detected in the HPLC profiles, some of them with similar UV spectra as verbascoside, meanwhile others with polyphenolic UV pattern. Therefore, in vitro assays will be undertaken to determine if the quantity of verbascoside itself affects proportionally the pharmacological activity. **Acknowledgements:** Projects UBACYT BO-14 and PICT-2008 – 1969, Rita Confessore for technical support **References:** 1. Di Leo Lira, P, van Baren, CM, Retta, D, Bandoni, AL, Gil, A, Gattuso, M, Gattuso, S. (2008). J. Essent. Oil Res. 20:350 – 353. 2. Gil, A, van Baren, CM, Di Leo Lira, PM, Bandoni, AL. (2007) J. Agric. Food Chem. 55:8664 – 8669.

P050 Triterpenoidal saponins from *Hydrocotyle bonariensis*

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The genus *Hydrocotyle* is an aquatic or semi-aquatic plant having antioxidant and antitumoral activity [1, 2]. Earlier investigations have reported on a therapeutic action on ulcer, wounds, eczemas, and antitumour activity of compounds from *Hydrocotyle* species [3, 4]. This genus is particularly well represented in temperate areas and higher tropical region [5]. There are very rich in saponins, which have many interesting biological activities such as anti-inflamatory, antimicrobial, molluscicidal, cytotoxic, and antitumor properties [6]. In view of the biological and pharmacological importance of this type of secondary metabolites, we have focused our intention in the study of *H. bonariensis*. It is a hairless and creepy perennial plant. In the present research, we describe the

isolation and structure elucidation of six new oleanane-type triterpenoidal saponins, Bonarienosides A-F (**1** - **6**) from the 70% aqueous MeOH extract of *Hydrocotyle bonariensis* roots. Their structures were established on the basis of NMR spectroscopic data (^{13}C, ^{1}H, COSY, HMBC, TOCSY, and NOESY) and mass spectrometry. **Acknowledgements:** The authors are grateful to the Conseil Régional de Bourgogne, France for financial support. **References:** 1. Yu, F. et al. (2007) Phytomed. 14: 166 – 171. 2. Jayashree, G. et al. (2003) Fitoterapia 74: 431 – 434. 3. Malhotra, C.L. et al. (1961) Ind. J. Pharm. 23: 106. 4. Miyata, S. et al. (1981) Anticancer Crude Drugs and their Prescription 185. 5. Stevens, C. et al. (2000) Missouri Botanical Garden Press. 110 – 115. 6. Lacaille-Dubois, M.A., Mitaine-Offer A.C. (2005) Phytochem. Rev. 4: 139 – 149.

P051 Diversity of medicinal plants used by the population of the state of Mato Grosso, Brazil

Rieder A

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The population of Mato Grosso at the present consists in a greater proportion of families who migrated to this State of Brazil after the 1960 s. These were mixed and coexist well with the native population regionally. This miscegenation this culturally enriched region of central South America where there are at least three biomes (Amazon Rainforest, Pantanal, Cerrado), with great diversity of species. This along ethnobotanical studies reveals the use of expressive diversity of medicinal species. We mention below the results of some studies conducted in the State of Mato Grosso (MT), Brazil. In 1994 a study with 12 healers in the capital of Mato Grosso (Cuiabá) found that they were attending public offering 225 species considered medicinal. In 2001, involving the entire state of MT, 648 teachers listed 67 species as the most important medicinal plants used by them. In 2002, another ethnobotanical study found that 339 residents of Cáceres (MT) listed 86 species of medicinal herbs grown in their backyards for their own use. In 2005, a study involving 21 municipalities and 63 informants Southwestern MT noted that the main medicinal plants used formed a list of 503 species. A more recent study (2009) dedicated to knowing which plants use to control diabetes in MT noted that the available literature provides a list of 133 species is formed. It is believed that the diversity of species listed is mainly related to floristic richness of biodiversity and cultural center in this Brazilian region of South America. **Acknowledgements:** UNEMAT-institutional support, and FAPEMAT EMPAER-MT; support afforded by the whole project team PLAMED: Bonila MGO, Carniello M, Ramos A PR et al.

P052 Screening of *Satureja khuzestanica* Jamzad and *S. rechingeri* Jamzad collections for high yielding genotypes

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Satureja khuzestanica Jamzad and *S. rechingeri* Jamzad are endemic species of Lamiaceae growing wild in Iran. The essential oils of both species are rich in Carvacrol, a phenolic compound widely used in food, feed and pharmaceutical industries. At the beginning of a breeding and domestication program, a collection of *S. khuzestanica* and *S. rechingeri* including 12 populations and 1200 genotypes were investigated for morphological traits, herbal drug yield, oil and carvarcol content. Variation was higher within rather than between populations. Essential oil content varied between 0.3 to 5.4% for *S. khuzestanica* and 1.8 – 9.45% for S. rechingeri. Carvarol was major component of all analyzed samples of *S. khuzestanica* (83.1 – 96.5%) and *S. rechingeri* (85.2 – 97.2%). Studied genotypes were also highly variable based on morphological traits and herbal drug yield. Elite genotypes were selected for future breeding program. **References:** 1. Jamzad, Z. (1996) Ann. Natu. Mus. Wien 98: 75 – 77. 2. Jamzad Z. (1994) Iran J. Bot. 6: 215 – 218. 3. Pank, F. et al. (2004). Zeit. Arz.Gew. 9:72 – 79.

P053 Determination of the best selection criteria for genetic improvement of seed and oil yield in spring safflower cultivars

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Identification of the important seed and oil yield components in safflower as an important medicinal plant in Iran is very efficient in genetic improvement of these traits via indirect selection. For this reason, ten spring safflower cultivars were sown in a randomized complete block design with three replications in research field of Islamic Azad University, Khorasgan branch. Results of correlation, regression and path analysis revealed that traits 1000-seed weight and seed number plant-1 has considerable positive and direct effects on plant seed yield and accounted for the largest amount of variation exist in this trait. On the other hand, traits 1000-seed weight, days to physiological maturity and seed number plant-1 were the most important plant oil yield components and suggest for breeding of this trait. Over all, results revealed that selection for higher amounts of traits 1000-seed weight and seed number plant-1 can improve indirectly plant seed and oil yield in spring safflower cultivars especially in early breeding generations. **Key words:** Spring safflower, indirect selection, correlation analysis, step-wise regression and path analysis. **References:** 1. Abolhasani, Kh. and Saeidi, G. (2006). Evaluation of drought tolerance of safflower lines based on tolerance and sensitivity indices to water stress. J. Sci. Technol. Agric. Natur. Resour. 10 (3): 419 – 422. 2. Arslan, B. (2007). Assessing of heritability and variance components of yield and some agronomic traits of different safflower cultivars. Asian. J. Pl. Sci. 6 (3): 554 – 557. 3. Ashkani, J., Pakniyat, H. and Ghotbi, V. (2007). Genetic evaluation of several physiological traits for screening of suitable spring safflower genotypes under stress and non-stress irrigation regimes. Pak. J. Bio. Sci. 10 (14): 2320 – 2326.

P054 Chemical composition of the essential oil of *Lilium ledebourii* from Iran

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Lilium ledebourii (Liliaceae), locally named "Susan-e-Chelcheragh" is an endemic and rare species growing on the highlands of Damash region in Gilan Province and Fandeghlo forests in Ardabil Province north part of Iran. The habitat of this species is under IUCN protection, Category III (Natural Monument) by the Iranian Department of Environment since 1976. This plant is a unique species of Lilium genus that is native of north Iran [1]. The aim of the present study was the investigation of the chemical composition of the essential oil of *L. ledebourii* (Apiaceae) from Iran by GC/FID and GC/MS methods. The plant material was collected during its flowering stage in June, 2008, on the Ardabil Mountains of the North-West of Iran. The essential oil was obtained by hydrodistillation of the dried herb using a Clevenger type apparatus. The oil yield calculated on moisture free basis was 0.1%. Twenty-six compounds were characterized in the essential oil of Lilium ledebourii, representing 99.1% of the oil, of which linalool (74.1%), E-6-tetradecanyl acetate (11.7%), benzyl salycilate (2.7%) and hexadecanal were found to be the major components. Analysis of oil revealed that oil was rich in linalool. **Acknowledgements:** This work has been supported by Shahid Beheshti University Research Council. **References:** 1. Saeidifard, M., Hosseini, S.M., Padasht Dehkaei, M.N., (2008) Rostaniha 9 (2 (32)):137.

P055 Chemical composition of essential oils from leaves and twigs with leaves of two new varieties of common sage (*Salvia officinalis* L.)

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Common sage (*Salvia officinalis* L.), member of the Lamiaceae family, is one of the oldest and the most important medicinal plant species [1]. Essential oils obtained by hydrodistillation from leaves and twigs with leaves of two new varieties named Miracol and Nikita BG (Botanical Garden) of *Salvia officinalis* were investigated to evaluate their chemical composition. The constituents of the essential oils have been characterized using gas chromatography and mass spectroscopy analyses (GC-MS) [2]. In all volatile oils, monoterpen-ketones (mainly, α-thujone and camphor) were most abundant compounds: 49.49% – 53.73% for Miracol variety and 50.73% – 53.03% for BG Nikita variety. Therefore, the essential oils contained significant amounts of monoterpen-hydrocarbons (Miracol variety: 17.32% – 20.34%; 13.96% -14.02% for BG Nikita variety) and monoterpenoxides (1,8-cineol): 12.85% -13.92% in Miracol variety; 13.94% – 15.18% in BG Nikita variety. β-thujone and manool diterpene were present only in the BG Nikita sage variety. **References:** 1. Lu Y, et al. (2002) Phytochemistry. 59:117 – 140. 2. Bernotiene G, et al. (2007) Chemija. 18 (4): 38 – 43.

P056 A new tetralone from *Diospyros cauliflora*

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Diospyros is a large genus of the mainly tropical trees within the Ebenaceae family, many of which possess considerable economic importance. Although many species of this genus have been intensively investigated [1], Diospyros cauliflora Blume has never been previously investigated for its chemical constituents. Chemical investigation of the chloroform soluble fraction of the methanol extract of its roots has led to isolation of lupeol, betulinic acid, nicotinamide, 7-hydroxy-4′-methoxyflavone (1), 2,5-dimethyl-7-hydroxychromone (2) and a new compound 3,4-dihydro-4β,6-dihydroxy-5-methoxy-2α-methyl-1(2 H)-naphthalenone (3). The new compound 3 appears to have been formed by reduction of 2-methyl-5-methoxy-6-hydroxy-1,4-naphthoquinone (4), a representative of the characteristic naphthaquinones of the Diospyros species [2]. However, the occurrence of compound 3 within this thoroughly investigated genus has no precedence.

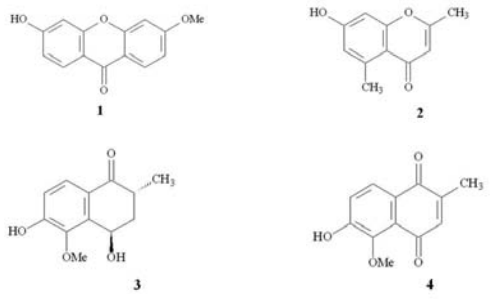

Fig. 1

Acknowledgements: This work is supported by CIIMAR pluriannual. W. Auamcharoen thanks Thailand Research Fund (TRF) for the Royal Golden Jubilee Scholarship PHD/0163/2546. **References:** 1. Mallavadhani, U et al. (1998) Phytochemistry 49: 901. 2. Okuyama, E et al. (1999). Chem. Pharm. Bull. 47, 1473.

P057 Phytochemical evaluation of some natural populations of *Achillea*, *Hypericum* and *Thymus* from Romanian Eastern Carpathians used in traditional medicine

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The phytochemical composition from the analyzed species was investigated in order to be used in phytopreparations for promoting ethnobotanical traditions from the Eastern Carpathians mountain range. The main phenolic compounds from *Achillea* samples identified by RP-HPLC-UV are apigenin, luteolin, apigenin-7-glucoside, luteolin-7-glucoside, caffeic acid and chlorogenic acid; the highest concentration was found in *A. millefolium* and *A. distans* methanolic extracts and the lowest concentration was observed for *A. pannonica*. For *Hypericum samples* we identified the major phenolic compounds as hyperoside and quercetin. A raised content for hyperoside (721.80 mg/100 g d.w.) and quercetin (1017.62 mg/100 g d.w.) was found in *H. perforatum* samples. Important amounts were observed also for *H. maculatum* samples (altitude: 1000 m). By the same method we identified and determined some of the major phenolic compounds for *Thymus* wild populations: chlorogenic acid (37.51 mg/100 g d.w. for *T. glabrescens*), rosmarinic acid (1083.8 – 1545.39 mg/100 g d.w. for T. *pulegioides*), apigenin (20.46 mg/100 g d.w for T. pulegioides ssp. montanus) and luteolin (10.47 mg/100 g d.w. for *T. pulegioides ssp. montanus*) [1]. Essential oils from *T. glabrescens* samples, analyzed by GC/MS, contain a great spectrum of compounds. **Acknowledgements:** The work is sustained in the PNCDI-2 program financed by the Romanian Government – National R&D Agency. **References:** 1. Stahl-Biskup E., Sáez F., Thyme: The Genus Thymus, 1st edition, Taylor & Francis, London (2002), 330pp.

P058 Effect of sowing date and harvest frequency on flower yield, essential oil percent and composition of chamomile (*Matricaria recutita*) CV. Presov

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To determine the effect of sowing date and harvest frequency on flower yield, essential oil percent and composition of chamomile (Matricaria recutita) CV. Presov, prepared from Slovakia, an experiment was conducted. The experimental design was split-plot in the basic of randomized complete blocked design (RCBD) with three replications. Main plots consisted of three sowing dates (6 Nov, 5 Mar, and 4 Apr) and sub-plots included three harvest frequencies (first, second and third). Evaluated traits were dry flower yield, essential oil content and yield, yield of β-farnesene, α -bisabolol oxide B, α -bisabolol, chamazulene, α -bisabolol oxide A. The results showed that sowing date, harvest frequency and their interaction had significant effect as measured traits as concerned. On the basis of the results, the most dry flower yield (40 g/m2) was obtained from the second harvest of 6 of November. Also the highest essential oil content (0.72 percent w/w), essential oil yield (0.26 g/m2) and α-bisabolol yield (0.2375 g/m2) were obtained from the second harvest of March and the most chamazulene yield (0.0473 g/m2)

was obtained from the third harvest of March that it had a little difference with second harvest. According to the results, the best chamomile quality was attained in second harvest of March sowing date in Mashhad condition. **Keywords:** Chamomile, Harvesting time, Planting time, Medicinal plant **References:** 1. Marcum, D.B and Hanson, B.R.2006. Effect of irrigation and harvest timing on peppermint oil yield in California. Agricultural Water Management.82:118 – 128. 2. Naghdi Badi, H., Yazdani, D., Sajed Mohammad, A and Nazari, F.2004. Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in Thyme, Thymus vulgaris L. Industrial Crops and Products,19:231 – 236.

Biopiracy and bioprospecting

P059 Bioprospecting Icelandic liverworts for anticancer activity

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Liverworts (Marchantiophyta, Hepaticae) belong to the bryophytes and comprise of about six to eight thousand species which are distributed everywhere in the world. Although being the structurally most primitive of the terrestrial plants they produce a complex array of secondary metabolites, including a number of mono- sesqui- and diterpenoids and also aromatic compounds like bibenzyls and bis (bibenzyls). Studies have demonstrated that several of these compounds possess a wide range of interesting biological activities, for instance inhibitory activity against cancer cells. Herein, the inhibiting effects of extracts and fractions from two different Icelandic liverworts were investigated on cancer cell-lines in vitro. Chiloscyphus pallescens and Marchantia polymorpha were collected in Iceland. Diethyl ether extracts were prepared and they were further fractionated on VLC (Vacuum Liquid Chromatography) with a n-hexane:ethyl acetate gradient. The inhibitory effects of the extracts and selected fractions were tested in four concentrations 80, 40, 20 and 10 µg/ml, on the cancer cell-lines MCF-7 and T47D. Results were obtained as% viable cells by crystal violet staining. Extracts and fractions from both liverwort species demonstrated dose-dependent inhibitory effects onto cell growth, viability and colony formation ability of the cancer cells in vitro. One fraction from each liverwort showed the highest activity with approximate IC50 20 µg/ml. Bioguided isolation of active constituents from these fractions is in progress.

P060 Bioprospection of endemic species from Marquesas archipelago

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The present work is included in a research program focused on Marquesas archipelago aiming valorization of Marquesas heritage based on a bioprospection. We focussed our investigation on a study of traditional uses, phytochemistry and bioactivity of Nuku Hiva taxons. A list of endemic plants (53 species) from Nuku Hiva had been collected. An ethnobotanical framework on "Marquesas biodiversity" was held to gather naturalist knowledge from local population showing a partial loss of traditional uses. Extracts obtained from different parts (leaf, bark and root) were submitted to a biological screening using two in vitro bioassays: cytotoxicity (KB) and radical scavenging (DPPH). Three species extracts were found to present the highest cytotoxic activity at 1 µg/ml level: *Hernandia nukuhivensis* (root: 100%), *Maytenus crenatus* (bark: 100%) and *Myrsine nukuhivensis* (leaf: 72%). High antioxidant potential had been evaluated from three species extracts: *Wikstroemia coriacea*, *Myrsine nukuhivensis* and *Waltheria tomentosa*. Two species were found to contain interesting bioactive constituents such as alkaloids: *Hernandia nukuhivensis* and *Waltheria tomentosa*. We report herein the first phytochemical investigation on *Hernandia nukuhivensis* (family Hernandiaceae) which allowed the identification of three bioactive constituents: litseglutin A, reticulin and deoxypodophyllotoxin.

Enzyme inhibitors from plants

P061 High-performance countercurrent chromatography for the efficient isolation of alkaloid acetylcholinesterase inhibitors from *Nerine* species (Amaryllidaceae)

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In a general screening of South African Amaryllidaceae for inhibitors of the enzyme acetylcholinesterase, the genus *Nerine* was found to be a good source of novel alkaloids which exhibit this bioactivity [1]. In view of material losses when performing separations of these alkaloids by multiple chromatographic steps on silica gel, an all-liquid strategy was investigated. Bulbs of the plants were extracted with 90% ethanol and the resulting extracts were partitioned between chloroform and 1% ammonia solution. Chloroform fractions were chromatographed on a Dynamic Extractions Spectrum instrument with suitable 2-phase solvent systems, chosen by a TLC screening approach [2]. The high rotation speed (1500 rpm) of the high-performance countercurrent chromatography (HPCCC) instrument allowed separations to be completed within 2 – 3 hours. This procedure led to the one-step purification of numerous alkaloids, on injection of sample charges up to 2 g. Certain alkaloids, such as lycorine, could be isolated by direct crystallisation of the relevant HPCCC fractions. Other alkaloids required a Sephadex LH-20 gel filtration clean-up step. The interest in inhibitors of acetylcholinesterase lies in their possible contribution to the management of Alzheimer's disease by modifying levels of acetylcholine in the brain [3]. **References:** 1. Marston, A. et al. (2009) Planta Med. 75: 944. 2. Marston, A. and Hostettmann, K. (2006) J. Chromatogr. A 1112: 181 – 194. 3. Houghton, P. et al. (2006) Nat. Prod. Rep. 23: 181 – 199.

P062 Vanda coerulea stem's bio-markers inhibit COX-2 and prevent UV-induced MMP-9 and pro MMP-1 production in skin cells

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Background: According to our previous results [1], imbricatin (**1**) and methoxycoleonin (**2**), the two major dihydrophenanthrenes isolated from *V. coerulea* Griff ex. Lindl (Orchidaceae) stems, displayed radical scavenging activities and inhibited PGE-2 release from UV_B (60mJ/cm^2) irradiated human normal epidermal keratinocytes (NHEK). It has been reported that COX-2, which synthetised PGE-2, could be an effective target for the regulation of UV-induced skin disorders. Besides, UVB radiation promotes the degradation of dermal extracellular matrix (ECM) by matrix metalloproteinases (MMPs) [2]. **Principal findings:** To get further insights in the anti-inflammatory and skin protection effects of these bio-markers, we have measured their activities on COX-2 and on UV-induced MMP-9/pro-MMP-1 production on NHEK and Human Normal Dermal Fibroblast (HNDF), respectively. Quantification of PGE-2 produced was considered as an indicator of human recombinant COX-2 enzyme activity. UV induced MMP-9 and pro-MMP-1 released by irradiated NHEK and HNDF were quantified by Elisa. Compounds (**1**) and (**2**) were able to inhibit significantly COX-2 (IC_{50} 12.0 and 5.8 µM, respectively) when compared with positive reference indomethacin (1.6 µM). Besides, they were able to prevent cellular release of UV-induced pro MMP-1(IC_{50} 6.7 and 9.4 µM, respectively) and MMP-9 (IC_{50} 2.8 and 2.6 µM, respectively) in a dose dependant manner. **Conclusions:** PGE-2, implied in skin inflammation, is also known to trigger the release of MMPs, promoting dermal matrix damages but also cellular proliferation [3]. So, the antioxidant dihydro-phenanthrenes isolated from *V. coerulea* stems displayed complementary skin care and anti ageing effects by inhibiting PGE-2 and major MMPs production from skin cells. **References:** 1. Simmler C et al (2009) Planta Med. 2. Rittié L et al. (2002) Aging Res Rev 3. Tripp CS et al (2003) J Invest Dermatol.

P063 Inhibitory activity of the Chinese herbal formula Huang Lian Jie Du Tang and its single components on Leukotriene and Prostaglandin synthesis

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The Chinese herbal formula Huang Lian Jie Du Tang, consisting of Rhizoma Coptidis, Radix Scutellariae, Cortex Phellodendri, and Fructus Gardeniae, is used in China as a decoction to clear heat and to relieve toxicity. According to Western medicine it is used in the treatment of gastritis, hypertension, cerebrovascular diseases, liver diseases, against eczema, chronic inflammations of the intestine, as well as for psychiatric disorders like schizophrenia and depression. Previous investigations demonstrated antioxidant and anti-cancer activities of the mixture, as well as its abilities to inhibit the progression of arterios progression of arteriosclerosis and to lower plasma triglycerides [1,2,3,4,5,6]. clerosis and to lower plasma triglycerides [1,2,3,4,5,6]. The aim of our study was to compare the effects of the mixture and of the single herbs concerning their ability to inhibit leukotriene biosynthesis in human granulocytes and prostaglandin formation by COX-1 and COX-2. Decoctions of the mixture and of the single herbs were lyophilized and tested. Moreover, decoctions were fractionated using liquid-liquid chromatography with n-heptane, dichlormethane, ethylacetate and n-butanol. Leukotriene and prostaglandin biosynthesis inhibition assays were performed as previously described [7,8]. The investigation showed that Huang Lian Jie Du Tang and decoctions of the single herbs are inhibiting the formation of LTB4 in human granulocytes, as recently described [9], but have hardly any inhibitory effect on cyclooxygenases (Fig. 1)

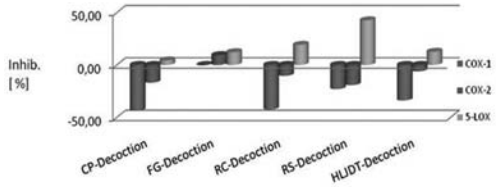

Fig. 1: Anti-inflammatory effects of decoctions of Huang Lian Jie Du Tang and of its single herbal components

Acknowledgements: The investigations were financially supported by the Austrian Federal Ministries of Health and of Science and Research within the research project "TCM and Age Related Diseases". **References:** 1. Sekiya, N. et al. (2003) Phytother. Res. 17: 147 – 151. 2. Ma, Z. et al. (2005) Blood 105: 3312 – 3318. 3. Otha, Y. et al. (2004) Journal of Ethnopharmacology 94: 323 – 329. 4. Sekiya, N. et al. (2005) Biol. Pharm. Bull. 28: 294 – 298. 5. Otha, Y. et al. (1999) Journal of Ethnopharmacology 67: 377 – 384. 6. Sekiya, N. et al. (2002) Phytomedicine 9: 455 – 460. 7. Fiebich, BL et al. (2005) Planta Med. 71: 12 – 19. 8. Adams, M. et al. (2004) Planta Med. 70: 904 – 908. 9. Zheng, H. (2009) J. Pharm. Pharmacol. 61:1699 – 1707.

P064 4(*1 H*)-Quinolones as inhibitors of LTB_4 formation *in vitro*

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Different 2-alkyl-4(*1 H*)-quinolones from the fruits of *Euodia rutaecarpa* Hook. f. & Thomson (Rutaceae) inhibited leukotriene biosynthesis in previous studies [1]. It was concluded that the characteristic lipophilic side chain might be essential for the observed activities. In order to substantiate this assumption, derivatives of 1-methyl-4(*1 H*)-quinolones with variations of the side chain at C-2 differing in length and unsaturation were synthesised and tested for inhibition of leukotriene B_4 formation in an *in vitro* assay using activated neutrophile granulocytes [2]. A saturated side chain as well as a *trans* double bond at C-1′ lead only to moderate inhibition at 50 μM (15.1 – 28.3% inhibition of LTB4 formation) whereas compounds with a *cis* double bond in combination with chain lengths between C-10 and C-14 showed the highest activities (42.1 – 93.0% inhibition at 50 μM). Increasing the chain length to C-20 significantly lowered the activity (9.8% inhibition; positive control zileuton 78.5% inhibition at 10 μM). The most active compound revealed no cytotoxicity at 300 μM even after 48 h incubation time in MRC-5 cells (XTT assay) [3]. It can be concluded that the length and saturation of the lipophilic side chain at C-2 in 1-methyl-4(*1 H*)-quinolones exert a significant influence on leukotriene biosynthesis in neutrophile granulocytes. **Acknowledgements:** Financial support by FWF (project no. P21152-B18 and NFN DNTI S10705-B03) is gratefully acknowledged. **References:** 1. Adams, M. et al. (2004) Planta Med 70:904 – 908. 2. Blunder, M. et al. (2010) Bioorg Med Chem. 18:2809 – 15. 3. Konkimalla, V.B. et al. (2010) Biochem Pharmacol 79:1573 – 1580.

P065 *Terminalia macroptera*, an African medicinal plant

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We have identified constituents of the leaves of the West African medicinal plant *Terminalia macroptera* Guill. & Perr. (Combretaceae) and studied their activity as antioxidants and enzyme inhibitors. The non-polar extract was inactive as a scavenger of the diphenylpicrylhydrazyl (DPPH) radical. The polar extract (methanol) had high activity in this assay (IC_{50} 6.2 μg/ml) and constituted a large portion, 35%, of the weight of the dry leaves. In another assay for antioxidant activity, inhibition of xanthine oxidase (which catalyzes the formation of superoxide radical), the methanol extract also showed activity (IC_{50} 52 μg/ml). Cis-polyisoprene (1) was the major non-polar constituent. Eight constituents were isolated from the methanol extract and characterized by spectroscopy: chebulic acid trimethyl ester (2), methyl gallate (3), corilagin (4), shikimic acid (5), chebulagic acid (6), chebulinic acid (7), rutin (8) and narcissin (9). The novel compound 2 (which may be an artifact formed from chebulic acid), showed high radical scavenging activity (IC_{50} 4.7 μg/ml), but was inactive as xanthine oxidase inhibitor. Compounds 1 – 5 have not been described previously for this plant. 8 and 9 have previously been found in the flowers of *T. macroptera*, but not in the leaves. The main substance corilagin, 4, a good radical scavenger (IC_{50} 2.7 μg/ml) and a moderate xanthin oxidase inhibitor (IC_{50} ca 105 μg/ml) has previously been reported to be antiinflammatory and antiviral. The antiinflammatory activity may be correlated to the antioxidant/radical scavenging effect. This may to some extent explain the medical use of this plant in West Africa.

P066 Cholinesterase inhibitors of *Rauvolfia serpentina* alkaloids

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At present, cholinesterase inhibitors are used in the treatment of memory impairment diseases. *Rauvolfia serpentina* (L.) Benth. ex. Kurz (RS) has been used as additional herb in some Thai traditional antirejuvenating and neurotonic remedies to potentiate hypnotic and tranquil effects within limited dose of dried root 50 mg t.i.d. or 150 mg/day. Dried root of RS (20 gm) was macerated in ethanol (48 hr x2 times) and the filtrate was freeze-dried to yield crude extract (RsC) 500 mg. Another RS (200 gm) was extracted for alkaloids by partition basic extract fraction with chloroform to obtain crude alkaloids, A (RsA) 345 mg and B (RsB)197 mg. Determination by TLC then detection under UV 365 and Dragendoff's reagent revealed that RsA was composed of 4 weak-base alkaloids whereas RsB was 2 alkaloids. Enzyme inhibitory activity of both acetylcholinesterase (AChE) and human butyrylcholinesterase (BuChE) were performed on RsC, RsA, RsB by the method of Elman (1,2,3). The results showed that the ethanolic extract of RsC, RsA, RsB at dose of 0.1 mg/ml had inhibitory effect more than 70% on both enzymes. The IC 50 values on BuChE were lower than on AChE. Moreover, RsB, RsA had more inhibitory activity on both enzyme than RsC. These results revealed the benefit effect of Rs in increasing neurotransmitter when given in limited dose.

Table 1 shows the inhibitory effect of RS extracts on acetylcholinesterase and butyrylcholinesterase represented as% inhibition and IC50 values in comparison with galantamine. N = 3; mean ± SD.

	AChEI activity		BuChuEI activity	
sample	% inh. (0.1 mg/ml)	IC50 (µg/ml)	% inh. (0.1 mg/ml)	IC50 (µg/ml)
RsC	75.78 (2.54)	35.02 (1.32)	75.02 (2.3)	20.65 (1.42)
RsA	72.33 (0.73)	28.22 (0.9)	87.20 (1.04)	10.79 (0.84)
RsB	73.33 (0.94)	27.05 (1.2)	90.16 (0.53)	9.07 (0.78)
Galantamine		0.217 (0.01)		1.06 (0.01)

References: 1. Elman G et al. (1961) Biochem Pharmacol 7: 88 – 95. 2. Ingkaninan K et al. (2003) J. Ethnopharmacol 89, 261 – 4. 3. Ingkaninun K, Changvijit K, Suwanborirux K. (2006) J.Pharm Pharmaco. 58(6):847 – 52.

P067 Phytochemical and biological investigations on *Carduus crispus* L.

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As people are becoming older different forms of dementia are a major public health concern. After a screening of 50 plants used in the Traditional Mongolian Medicine on their acetylcholinesterase inhibitory effect the MeOH extract of *Carduus crispus* L. (Welted Thistle; Asteraceae) showed a pronounced activity of 60.5% at a conc of 1.000 µg/ml. The biological test was performed by the method of Ellman [1, modif.]. Different plant parts (leaves, flowers, stem, roots) were extracted with solvents of different polarities. The highest activity was found in the MeOH mazerate of the leaves (60.9% at 1.000 µg/ml) whereas the traditionally used hot water extracts were not as active (32.4% at 1.000 µg/ml). The alcoholic extracts showed stronger effects ($MeOH_{cold}$ > $MeOH_{hot}$ > $EtOH_{hot}$ >> H_2O_{hot}). The differences between leaf, flower and stem extracts have not been significant. Separation of MeOH extracts by polarity differences showed a pronounced activity of the n-butanol fraction (stem 59.2%, flower 66.2%, leaf 81.6% at 1.000 µg/ml). Fractions with an activity up to 90.9% at 1.000 µg/ml were obtained by the use of SPE. The compounds from these fractions isolated by SPE and RP-HPLC could be identified by spectroscopic methods as chlorogenic acid; neochlorogenic acid; p-coumarylquinic acid and apigenin derivatives. These results may substantiate the traditional use of the investigated plant for the improvement of cognition. **Acknowledgements:** Honda Foundation, Japan and University of Graz for financial support. **References:** 1. Ellman, GL. et al. (1961) Biochem Pharmacol 7: 88.

P068 Screening of selected Mongolian medicinal plants for their acetylcholinesterase inhibitory activity

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In recent years the incidence of Alzheimer's is increasing especially in Western countries caused by the fact that people get older than in former times. Therefore it is important to find new compounds which have a significant effect on this special form of dementia. Therefore in this study 50 plants used in Traditional Mongolian Medicine were tested for their acetylcholinesterase inhibitory effect. MeOH extracts of different plant parts (leaves, flowers, aerial parts, roots) were tested by the modified photometric method of Ellman [1] *in vitro*. The methanolic extracts of six different plants showed an activity over 25% at a concentration of 1,000 µg/ml: Bergenia crassifolia Fritsch. (Saxifragaceae) 44.4%, Carduus crispus L. (Asteraceae) 60.5%, *Juniperus sibirica* Burgsd. (Juniperaceae) 28.4%, the two Lespedeza species *L. dahurica* (Laxm.) Schindl. 37.1% and *L. hedysaroides* (Pall.) Kitag. (Fabaceae) 36.0% and *Potentilla viscosa* G. Don. (Rosaceae) 26.0%. Further fractionation resulted in higher activities. Purification of the *Carduus crispus* extract led to an activity up to 90.9% at 1.000 µg/ml. The natural resources, especially plants combined with the knowledge of traditional medicine give a pool of potential new drugs. **Acknowledgements:** Honda Foundation, Japan and University of Graz for financial support. Reference: 1. Ellman, GL. et al. (1961) Biochem Pharmacol 7: 88.

P069 *Ligustrum vulgare* L. and its utilization as anti-inflammatory remedy in folks medicine

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The leaves of *L. vulgare* L. (common privet, Oleaceae) were well known in the Mediterranean historical medicine for their oropharyngeal anti-inflammatory effect against aphthae. Potential synergy between secoiridoidic (oleuropein) and flavonoidic components may play a central role in anti-inflammatory and probably antioxidative effect of this plant. It seems, anti-inflammatory activity depends on the total content of polyphenolic compounds. Lipoxygenase (LOX, EC 1.13.11.12) is a key enzyme in biosynthesis of leukotriens and lipoxins, which play an important role in pathophysiology of several inflammatory diseases. LOX catalyses the dioxygenation of polyunsaturated fatty acids with content of *cis*, *cis*-1,4-pentadiene structure by formation of hydroperoxides which are metabolised to mediators of inflammatory and allergic responses[1]. The aim of this study was to monitor the inhibitory effect of *L. vulgare* infusions (10 mg/10mL), oleuropein and echinacoside, a caffeoyl derivate ($3 - 10 \times 10^{-5}$ mol.l^{-1}) on LOX activity. The fresh extracts from leaves of *L. vulgare* and LOX isolated from cytosolic fraction of rat lungs were used. The activity of LOX was determinated using a spectrophotometric method, expressed in percentage of inhibition and as IC_{50}. Also the kinetic type of inhibition was determined. The result showed that all studied compounds had an inhibitory effect on LOX activity. The highest inhibition was observed at 10×10^{-5} mol/L. On the basis of this inhibitory effect, we suggest that these compounds have potential anti-inflammatory and antioxidative properties. Additionally, they provide support to the popular and ethnomedical descriptions of the utilization of privet as anti-inflammatory remedy in the Mediterranean area. **Acknowledgements:** VEGA grant MS SR 1/0145/10, MS SR 1/0145/10, 1/0089/10. **References:** 1. Funk, C.D. (2006) Arterioscler Thromb Vasc Biol 26 p. 1204 – 1206.

P070 Antioxidant and inhibitory activity of metalloproteinases 2 and 9 from *Potomorphe umbellata* fractions

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Natural antioxidants have been one perspective for the prevention of photodamage and photo-aging that develops in consequence to ultraviolet light exposure [1]. *Pothomorphe umbellata* (*Pu*) water-ethanolic root extract showed antioxidant, photo-protective and metalloproteinases (MMPs) inhibitory activity, in part attributed to 4-nerolidylcatechol (4-NC), the main compound. This suggests the presence of other compounds in the extract with these properties [2 – 4]. From *Pu* extract (7.9% of 4-NC) extraction was obtained: hexanic (Hex), n-butanolic (n-But) and aqueous (Aq) fractions. Only the Hex fraction showed the presence of 4-NC (38%), evaluated by TLC and HPLC-EC. The antioxidant analysis by ORAC, showed the 4-NC activity (48774,17) was higher than *Pu* extract, Hex, n-But and Aq (from 4494,810 to 342,667 µMol Eq.TROLOX/g). MMPs are associated to normal and pathological conditions, including tumor invasion and metastasis [6]. In an in vitro activity of MMP-9 from skin tissue homogenates incubated with 100 µg/mL of each sample, Hex fraction was able to inhibit the enzyme activity in 50% followed by 4-NC and *Pu* extract (30%), n-But (15%) and Aq fraction (10%). These results are in agreement with the presence of others compounds that could be responsible for inhibitory effects beside the 4-NC.

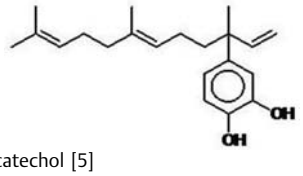

Fig. 1: 4-nerolidylcatechol [5]

References: 1. Adhami VM. et al. (2008). Photochem. Photobiol. 84:489 – 500. 2. Barros SBM. et al. (1996). Ciência e Cultura 48:114 – 116. 3. Desmerchelier C. et al. (1997) Planta Med. 63:561 – 563. 4. Ropke

CD. et al. (2006) Photochem. Photobiol. 82:439 – 442. 11. 5. Kijjoa A. et al. (1980). Planta Med.39:85 – 87. 6. Egeblad M & Werb Z. (2002). Nature Rev.Cancer 2:161 – 74.

P071 *In vitro* and *in vivo* evaluation of the anti-inflammatory effects of Arzanol from *Helichrysum italicum*

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Eicosanoids derived from arachidonic acid, mainly prostaglandins (PGs) and leukotrienes (LTs) function as powerful lipid mediators in inflammatory reactions. The dual inhibition of the LT-producing enzyme 5-lipoxygenase (5-LO) and of microsomal PGE_2 synthase-1 (mPGES-1, which forms pro-inflammatory PGE_2 from COX-2 derived PGH_2), is a novel and promising strategy for the therapy of inflammation. The acylphloroglucinol arzanol has recently been suggested as an active ingredient responsible for the anti-inflammatory effects of the plant *Helichrysum italicum*. Here we analysed the effect of arzanol on LT and PGE_2 formation in cell-free and cell-based models as well as in an experimental model of inflammation in rats. We find that arzanol inhibits 5-LO and mPGES-1 (IC_{50}= 5 μM and 0.5 μM, respectively) in cell-free assays and efficiently reduces mPGES-1-derived PGE_2 formation in LPS-stimulated human monocytes (at 3 μM) and LPS-stimulated human whole blood (at 10 μM) with a similar efficiency as the specific mPGES-1 inhibitor MD-52 (tested at 2 and 5 μM). In human neutrophils arzanol inhibited 5-LO product formation with an IC_{50}= 5 μM. The *in vivo* efficiency of arzanol (3.6 mg/kg i.p.) was demonstrated by the reduction of exudate volume (59%), number of inflammatory cells (48%) and PGE_2 (47%) and LTB_4 (31%) levels, in the pleural exudates of rats in a model of carrageenan-induced pleurisy. Taken together arzanol is a potent inhibitor of 5-LO and mPGES-1 in both cell-free and cell-based assays with significant anti-inflammatory effects *in vivo*.

P072 Inhibition of acetylcholinesterase by *Hedychium gardnerianum* from S. Miguel (Azores)

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Some species of Zingiberaceae have been the subject of a range of chemical and pharmacological investigations due to their significance in traditional medicine. The properties attributed to these plants are due to their richness in active compounds, such as terpenes and terpenoids. Cholinesterase inhibitors were introduced in the therapy of Alzheimer Disease in the 1990 s. The hopes and interest raised by these drugs are well demonstrated by the 41,370 references listed by PubMed under 'Acetylcholinesterase inhibitors'. In particular, the scientific community is searching for novel acetylcholinesterase inhibitors displaying less secondary effects. Extracts from Hedychium gardnerianum young leaves (Yl), mature leaves (Ml), stems (St), rhizomes (Rh), seeds (Se) and fruits (Fr) were prepared by sequentially extracting with dichloromethane (DCM) and metanol (MeOH) at room temperature. Essential oil from leaves was extracted by hydrodistillation using a modified Clavenger. Acetylcholinesterase (AChE) inhibition was assayed using a modification of the Ellman method [1]. All the fractions assayed inhibited AChE activity, although to different extents. Dichloromethane extracts were always more active than methanol extracts. The strongest inhibition was displayed by mature leaves, fruits and seeds (Ml>Fr>Se), with IC 50 values between 0.74 and 2.54 mg/mL, and by the essential oil, with IC 50 of 1.14 mg/mL, which are comparable to the value for alpha-pinene, a known AChE inhibitor (IC 50 = 1.43 mg/mL). Our results indicate that Hedychium gardnerianum, an abundant and fast-growing plant in the Azores, is a promising source of AChE inhibitors. More work is being carried out to fully characterize the active compounds and their mechanisms of action. **References:** 1. Ellman, G.L. Courtney, K.D. Andres, V. Feather-Stone, R.M. (1961) Biochem. Pharmacol. 7 88 – 95.

P073 Acetylcholinesterase inhibition properties of *Hypericum foliosum* Aiton

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Hypericum (Guttiferae) is a large genus (ca 450 species) of herbs or shrubs that has been used in folk medicine since the antiquity [1]. Their health benefits, demonstrated through the number of pharmacological and clinical trials [2], have been attracting attention to the scientific community for the study of the Hypericum genus. In spite of the intense research on both chemical constituency and biological activity, studies on acetylcholinesterase (AChE) inhibition are limited to a few species [3]. Therefore, in this study, methanolic extracts from eight anatomical parts of the endemic Azorean Hypericum foliosum Aiton (aerial parts; flowers; old leafs; young leafs; stems; stem bark; root and seed capsules) were evaluated for their potential anti-AChE activity by using a modified Ellman et al. [4] technique. The results reveal that six of the eight anatomical parts displayed significant AChE inhibition with an IC 50 value below 1000 μg/mL. From all samples assayed, the stems were the most effective with an IC 50 value of 130 μg/mL, followed by the root (170 g/mL) and the stem bark (200 μg/mL) samples. The leaf samples did not present any difference (840 μg/mL). Aerial parts displayed an IC 50 of 460 μg/mL. The flower and seed capsule samples were less effective with an IC 50 value above 2500 μg/mL. These results reveal a promising plant, particularly the stems, root and stem bark, containing powerful anti-AChE inhibitors that could provide novel poly-pharmacological leads for a more detailed future investigation. **References:** 1. Robson, N. K. B. (2003). Hypericum botany. In E. Ernst, Hypericum: the Genus Hypericum. Taylor and Francis. New York. 2. Barnes, J. et al. (2001) J. Pharm. Pharmacol. 53: 583 – 600. 3. Hernandez, M. et al. (2010) Food Chem. 120: 1076 – 1082. 4. Ellman, G. et al (1961) Biochem. Pharmacol. 7: 88 – 95.

P074 Inhibitory effects of *Lavandula* extracts on elastase and tyrosinase

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Nowadays, there is an increasing interest in the use of natural botanical herbal extracts as active ingredients of functional cosmetic and pharmaceutical products. *Lavandula x intermedia* Emeric ex Loisel. 'Budrovka' is an indigenous cultivar of lavandin and, together with the common lavender (*L. angustifolia*), it has been widely cultivated in Croatia. The present study was undertaken to evaluate and compare the possible inhibitory effects of *Lavandula* ethanolic extracts on elastase and tyrosinase which are important therapeutic targets. In terms of anti-ageing, finding inhibitors of elastase enzyme can be useful to prevent loss of skin elasticity and wrinkle formation, but also an anti-elastase agent may be useful in the treatment of inflammation. Inhibition of elastase activity by the leaf, flower and inflorescence stalk extracts of *Lavandula x intermedia* 'Budrovka' at 1 mg/mL was found to be 100%, 77.7% and 18.6%, respectively, while the inhibitory percentages for *L. angustifolia* extracts were 100% (leaf), 65.8% (flower) and 15.1% (inflorescence stalk). Investigation of tyrosinase inhibitors may lead to development of novel agents for the treatment of hyperpigmentation as well as skin-whitening agents. In the tyrosinase inhibition assay, flower extracts (1 mg/mL) of *L. x intermedia* 'Budrovka' and *L. angustifolia* were the most effective, exhibiting similar inhibitory effects of 70.4% and 72.9%, respectively, while the leaf and inflorescence stalk extracts exerted lower activity. According to the results of the present *in vitro* enzyme inhibition studies, flowers and leaves of both *Lavandula* taxa may be promising sources of active ingredients in dermatological preparations.

P075 Protease inhibition activity of semi-synthetic derivatives of Piperine isolated from *Piper tuberculatum* (Piperaceae) from Brazilian Flora

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Protease inhibition assays have been used as a model for screening fractions and secondary metabolites from plant species with biological activity. As part of our research on Brazilian flora species, we aimed at exploring the effects of piperine isolated from Piper tuberculatum and of 20 of it's semi-synthetic derivatives. FRET spectroscopy (350 nm excitation, 450 nm emission) were used for inhibition analyses of aspartate protease pepsin (17 nM, 1 mM acetate buffer, pH 4.4) and serinic protease subtilisin (37 nM, 1 mM phosphate buffer, pH 7.5). The substrate used was Arg-Glu-(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys-(DALB-CYL)-Arg (EDANS-DABCYL) (2 uM).

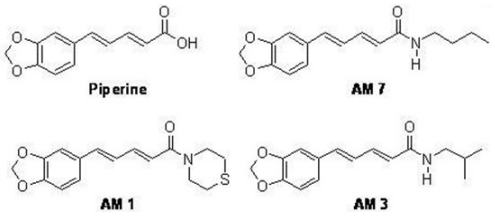

Fig. 1

The most effective semi-synthetic amide derivatives were AM1, AM3 and AM7, showing potent and selective inhibitory activity on aspartate protease pepsin, as shown in table 1. The experiments with the serinic protease subtilisin showed that only the compound AM 7 inhibit the enzyme activity. In conclusion, the results showed that the amides tested were selective for the inhibition of the aspartate protease. The bioactive semi-synthetic compounds will be submitted to further assays using other asparte proteases as HIV protease, rennin and beta-secretase.

Table 1: Results of piperine and its semi-synthetic derivatives in the protease inhibition assay

Pepsin		
Concentration (ug/mL)	**10**	**1**
	% Inhibition	
Piperine	65.03	54.12
AM 1	65.47	33.30
AM 7	52.89	37.98
AM 3	62.84	11.38
Subtilisin		
Piperine	nd	31.85
AM 1	−3.45	−2.04
AM 7	50.71	32.28
AM 3	−16.49	−7.37

Acknowledgements: FAPESP, CAPES and CNPq for financial support.

P076 *Olea europaea* L. leaf (Ph. Eur.) extract as well as several of its single phenolics inhibit the gout-related enzyme xanthine oxidase

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In Mediterranean folk medicine, olive leaf extract (*Olea oleuropaea* L.) is a common remedy for gout [1,2]. Therefore, in this *in-vitro* study kinetic measurements were performed to investigate its possible inhibitory effects on xanthine oxidase (XO), an enzyme well known to contribute to this pathological process [3]. In these experiments, both the whole leaf extract of *O. europaea* [4] as well as many of its characteristic phenolic constituents significantly inhibited the XO activity. Dixon and Lineweaver-Burk plot analyses were used to determine K_i values and inhibition modes for the single substances, the HPLC determination of which we discussed in an earlier study [4]. Among these, the flavone aglycone apigenin, which constitutes 0.033% of the extract, exhibited by far the strongest effect on XO with a K_i of 0.52 µM. In comparison, the phenolic secoiridoid oleuropein the main ingredient of the extract (24.8%) had a considerable higher K_i (53.0 µM). However, it still displayed a significant inhibition of XO. Caffeic acid (K_i11.5 µM/1.89%), luteolin-7-glucoside (K_i15.0 µM/0.86%) and luteolin (K_i2.9 µM/0.086%) also contributed to the XO inhibiting effect of *O. europaea* whole leaf extract. For oleuropein, a competitive mode of inhibition was found, while all other active substances displayed a mixed mode of inhibition. Tyrosol, hydroxytyrosol, verbascosid, and apigenin-7-glucoside were inactive in all tested concentrations but one has to take into consideration that apigenin-7-glucoside, which makes up for 0.3% of the extract, is transformed into active apigenin in the mammalian body [5], thus also contributing substantially to the anti-gout activity of olive leaf extract. **References:** 1. Leporatti ML et al. (1985) J Ethnopharmacol, 14, 65 – 68. 2. Cecchini T (1992) Enciclopedia de las hierbas medicinales. Edition de Vecchi. Barcelona. 3. Shoji A et al. (2004) Arthritis and Rheumatism 51 (3), 321 – 325. 4. Scheffler A, Rauwald HW et al. (2008) J Ethnopharmacol, 120, 233 – 240. 5. Hanske L et al. (2009) J Nutr 139: 1095 – 1102.

P077 The effect of structural characteristics of Lycopodane-type alkaloids on their acetylcholinesterase inhibitory activity in silico

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Club mosses are known to produce a variety of alkaloids with condensed polycyclic structures. The club moss Huperzia serrata, which has long been used in traditional Chinese medicine, produces huperzine A, a potent acetylcholinesterase inhibitor which is currently being evaluated for its clinical efficacy in management of Alzheimer's disease and vascular dementia. In a recent study by the present authors, 10 lycopodane-type alkaloids were isolated from an Icelandic collection of Lycopodium annotinum ssp. alpestre. Their structures were elucidated and the isolated alkaloids were evaluated for their in vitro inhibitory activity against acetylcholinesterase.1 Acetylcholinesterase inhibitory activities of natural lycopodane-type alkaloids isolated in the previous study were weak and the aim of this study was to investigate the structure-activity relationship further using in silico molecular modeling methods in order to find more active derivatives suitable for semi-synthesis. Structure-activity relationship was studied with the help of molecular modeling using Maestro, Glide and Pymol software. Novel binding orientation of annotine found in the docking study suggests the possibility of synthesis of analogues with increased potency. Docking studies of the 80 analogues of annotine that were designed using a model of EeAChE indicated that acylation of the tertiary hydroxy group of annotine may increase the activity via strong hydrogen bonds resembling those between the enzyme binding site and huperzine A. **Acknowledgements:** The Icelandic Research Fund, The University of Iceland Research Fund, The Icelandic Research Fund for Graduate Students, and Th. Scheving Thorsteinsson Fund. **References:** 1. Halldorsdottir, E.S. et al. (2009) Phytochemistry 71:149 – 157.

P078 Cancer chemopreventive activity of constituents from *Sedum dasyphyllum* L.

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Histone deacetylases (HDACs) mediate changes in nucleosome conformation and are important in the regulation of gene expression. HDACs are involved in cell cycle progression and differentiation, and their deregulation is associated with several cancers. HDAC inhibitors have emerged recently as promising chemotherapeutic agents because of their antitumor effects [1]. Many of them are currently studied in phase I and II clinical trials to establish a clear understanding of their effects. Data on the cancer prevention properties of epigenetic modulators, such as HDAC inhibitors, are emerging as there is now strong evidence that epigenetic alterations often are involved in the earliest stages of tumor progression. It appears that the real clinical home of cancer epigenetics may be prevention, where epigenetics potentially will have its greatest impact [2]. *Sedum dasyphyllum* L. (Crassulaceae) is a low compact plant growing from Central Europe to the Mediterranean coastlines. Some *Sedum* plants have been documented as either vegetables or folk medicines for the treatment of many diseases [3]. Previous studies on *S.*

dasyphyllum have shown the presence of a large variety of flavonoid glycosides with free radical scavenging activity. In our search for new cancer chemopreventive compounds from natural sources, flavonoid glycosides from *S. dasyphyllum* showed promising HDAC inhibitory activity. Further investigations are needed to evaluate the full potential of these compounds. **References:** 1. Villar-Garea A., Esteller M. (2004). Int. J. Cancer 112: 171 – 179. 2. Issa J.P. (2008) Cancer Prev Res 1: 219 – 222. 3. Stephenson R. (1994) Sedum cultivated stonecrops. Timber Press Inc. Portland.

P079 Anti-inflammatory activity of Imupret® – the combination product versus its single herbal extracts

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Imupret® is an alcoholic-aqueous extract of seven different herbal drugs: Radix Althaeae, Flores Chamomillae, Herba Equiseti, Folia Juglandis, Herba Millefolii, Cortex Quercus, and Herba Taraxaci. It is used for the treatment of recurrent infections of the respiratory tract, especially tonsillitis. The aim of the study was the assessment of the influence of the herbal extract combination Imupret® compared to selected single herbal extracts on its anti-inflammatory activity *in vitro*. Therefore pharmacological effects of Imupret® and its herbal components on arachidonic acid metabolism were investigated. The anti-inflammatory activity was measured by the inhibition of prostaglandin biosynthesis by cyclooxygenases (COX-1 and -2) [1] and the inhibition of leukotriene biosynthesis in human neutrophile granulocytes [2]. The inhibitory activity was calculated by the produced amount of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), respectively. This study compares different anti-inflammatory pharmacological effects in order to reveal whether there is an increase or decrease of enzyme inhibition caused by additional synergistic effects as a result of a multiple extract preparation. **References:** 1. Reininger, E. et al. (2006) Phytomedicine 13: 164 – 169. 2. Adams, M. et al. (2004) Planta Med. 70: 904 – 908.

P080 Selective serotonin re-uptake and acetylcholinesterase inhibitory activities of *Salvia officinalis*

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Herbal medicinal products (HMPs) are widely used alternatives to hormone replacement therapy (HRT) for the treatment of menopausal symptoms. Some HMPs mimic the effects of estrogen, but anti-menopausal effects of many HMPs may involve other or multiple mechanisms. For instance, selective serotonin re-uptake inhibitors (SSRI) have been shown to reduce hot flushes, possibly by increasing the thermoregulatory set point in the hypothalamus [1]. Furthermore, during their menopausal transition women suffer from an increased risk of developing Alzheimers disease [2], and this might be slowed down with acetylcholinesterase inhibitors (AChEI). AChE inhibitory activities of *Salvia* sp. have been described previously [3]. We recently reported moderate estrogenic activity of a *Salvia officinalis* tincture (SOT) and its subextracts (*n*-hexane-, chloroform and aq. EtOH) [4]. In continuation of our mechanistic studies into the anti-hot flush effect of this tincture, we have tested the SOT and subextracts for SSR and AChE inhibitory activities. For the SSRI assay, HEK-293 cells stably transfected with the human serotonin transporter (HEK-293-hSERT) were utilised and re-uptake was determined using scintillation counting of radio-labeled serotonin [5]. The AChE inhibition assay was performed based on a spectrophotometric method described by Ellman [6]. Neither SOT, nor the subextracts displayed significant inhibition in either assay at the highest test concentrations (125 µg/ml for SSRI and > 1 g/ml for AChEI assays). These findings indicate that SSR and AChE inhibition are not involved the mode of action of the SOT and therefore cannot explain its anti-menopausal activity. **Acknowledgements:** Funding from Bioforce AG Switzerland is gratefully acknowledged. **References:** 1. Curcio, J. et al. (2005) Altern Med Rev. 10: 216 – 221. 2. Mulnard, R. et al. (2000) JAMA 283: 1007 – 1015. 3. Houghton, P. et al. (2006) Nat Prod Rep. 23: 181 – 199. 4. Rahte S. et al (2008). Planta Med. 75: 1073. 5. Ramamoorthy, E. (1998) J Biol Chem, 273: 2458 – 2466. 6. Ellman, G. et al. (1961) Biochem Pharmacol 7: 88 – 95.

P081 Inhibition of P-glycoprotein, cytochrome P450, and glutathione-S-transferase in human cancer cells by *Polygonium cuspidatum*

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Activities of membrane ABC-transporters and other detoxifying enzymes play an important role in the bioavailability of drugs and control the success or failure of cancer chemotherapy. A synergistic interaction between the increased activity of efflux pumps, such as P-glycoprotein (P-gp/MDR1), the detoxification by phase I enzymes like cytochrome P-450 (CYP3A4), and phase II conjugating enzymes like glutathione S-transferases (GST) has been observed in multidrug resistance to many anticancer drugs [1 – 3]. We evaluated several TCM plants for their possible interaction with P-gp, CYP3A4, and GST. *Fallopia japonica (Houtt.) Ronse Decr. var. japonica* (FJ) (Polygonaceae) extract (10 – 100 µg/ml) significantly inhibited the active efflux of Rho123 in a dose and time dependent manner; P-gp inhibitory activity was 0.2 – 2.77-fold and 1.07 – 4.01-fold of verapamil in Caco-2 and CEM/ADR5000 cell lines, respectively. Moreover, (FJ) can significantly increase Calcein-AM accumulation in leukaemia cells to 1.19 – 6.36-fold using FACS assay. The cytotoxicity of doxorubicin was enhanced by using 100 µg/ml (FJ); IC50 values were decreased from 4.15 to 1.12 µM, and from 33.67 to 4.54 µM, respectively. RT-PCR reveals a significantly down regulation of MDR1 in Caco-2 cell lines ($P < 0.01$). 20 – 100 µg/ml (FJ) inhibited significantly GST and cytochrome P450 enzyme activity in a dose dependent manner. In conclusion, the inhibition of P-gp as well as metabolic enzymes could explain the anticarcinogenic effect of *Fallopia japonica* (Houtt.) Ronse Decr. var. *japonica* extract. **References:** 1. Harmsen, S. et al. (2007) Cancer Treat Rev. 33: 369 – 380. 2. Szakacs, G. et al. (2006) Nat. Rev Drug Discov. 5:219 – 234. 3. Meijerman, I. et al. (2008) Cancer Treat Rev. 34: 505 – 520.

P082 The inhibitory effect of the crude extract of *Centella asiatica* on α-amylase activity

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Inhibition of α-amylase activity can reduce the postprandial blood glucose level which can be achieved by drugs and medicinal plants (such as *Castanospermum austral* or black bean). Tannins in black bean have potential inhibitory effect on α-amylase activity [1]. Tannin is also found in *Centella asiatica* (L.) Urban. [2]. Therefore, the effect of ethanolic extract from *Centella asiatica* on α-amylase activity was investigated *in vitro*. The edible parts of *Centella asiatica* was extracted by 80% ethanolic solution. The total phenolic compound of sample extract was measured [3]. The α-amylase inhibition assay was applied [4]. To determine the effect of the *Centella asiatica* extract on the α-amylase activity, 10 to 50 mg/ml of extracts and 10 mg/ml of acarbose (α-amylase inhibitory drug), and active compounds found in *Centella asiatica* (tannin, rutin, and quercetin) were prepared. The results showed that the extract of *Centella asiatica* contained total phenolic compound equivalent to 97.45 mg of gallic acid/g dry weight. Acarbose and tannin could inhibit α-amylase activity (97.6% and 37.9%, respectively). Whereas, quercetin and rutin did not inhibit α-amylase activity which was similar to the study of Jo et al. [5]. Moreover, the extract of *Centella asiatica* had α-amylase inhibitory activity (11.4%) at the final concentration of 30 mg/ml while other concentrations of this extract had no inhibition. Although, the percentage of inhibition by *Centella asiatica* extract was less than acarbose, *Centella asiatica* extract could be a benefit candidate for treatment of diabetes mellitus. However, further *in vivo* studies are needed to be investigated. **References:** 1. Carmona, A. et al. (1996) J. Nutr. Biochem. 7:445 – 450. 2. Zheng, C. J. et al. (2007) J. Chin Intege Med. 5:348 – 351. 3. Paixao, N. et al. (2003) Food Chem. 105:204 – 214.

4. Nickavar, B. et al. (2008) Iran. J. Pharm. Res. 7:297 – 303. 5. Jo, S-H. et al. (2010) Int. J. Applied Res. Natural Products 2:52 – 60.

P083 Screening of some medicinal plants for antidiabetic activity

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Diabetes mellitus is one of the most serious chronic diseases characterized by chronic hyperglycemia. Traditional herbal remedies are still in use by diabetic patients especially in 3rd World Countries. The present work was carried out to investigate the potential antidiabetic effects of some Iranian medicinal plants using an *in vitro* α-amylase inhibition assay. The ethanol extracts obtained from ten plants (*Trigonella foenugraecum*, *Camellia sinensis*, *Urtica dioica*, *Vaccinium arctostaphylos*, *Urtica pilulifera*, *Calendula officinalis*, *Juglans regia*, *Olea europaea*, *Salvia officinalis*, *Arctium lappa*) were tested on inhibitory activity of α-amylase, expressed as IC_{50} values, calculated from Log concentration-response curves. As positive control acarbose was used. Among the tested samples, *Camellia sinensis* (Theaceae) leaf (IC_{50}= 1.54 mg/ml), *Trigonella foenum-graecum* (Fabacae) seed (IC_{50}= 1.87 mg/ml) and leaf (IC_{50}= 1.92 mg/ml), and *Urtica dioica* (Urticaceae) leaf (IC_{50}= 1.89 mg/ml) revealed appreciable α-amylase inhibitory activities in a concentration-dependent manner.

P084 An antidiabetic compound from *Vaccinium arctostaphylos* berries

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Vaccinium arctostaphylos L. (Ericaceae) is a compact shrub up to 3 m in height which grows wildly in the northern forests of Iran. Its berries are edible cherry and are used as an antidiabetic agent in Traditional Iranian Medicine. The present study deals with the bioassay guided fractionation of *V. arctostaphylos* berry extract and *in vitro* α-amylase enzyme inhibition assay of the extract and fractions for their antidiabetic activities. The crude extract showed a suitable dose-dependent inhibitory effect against α-amylase activity [IC_{50}= 1.91 (1.89 – 1.94) mg/mL]. The activity guided fractionation of the extract led to the isolation and purification of an anthocyanin from it. The compound was identified as malvidin-3-O-β-glucoside by determination of its hydrolytic and spectral data. The compound demonstrated a suitable dose-dependent enzyme inhibitory activity [IC_{50}= 0.16 (0.16 – 0.17) mg/mL].

Fertility management by natural products

P085 Study the abortifacient effects of pennyroyal decoction in pregnant mice

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Objective: Pennyroyal (*Mentha pulegium* L.) is a widely available herb that has long been used as an abortifacient. In this study, the effects of a Pennyroyal decoction on foetuses in pregnant Mice was assessed. **Methods:** 20 female BALB/c mice, weighting 25 – 30 grams, were breed in the animal house of the medical college. The first day of pregnancy was the day on which the vaginal plaque was observed. The pregnant mice were divided into 2 subgroups. Each pregnant animal was placed in a separate cage throughout the gestational period. Animals in the control group received tap water, animals of the test groups received an aqueous pennyroyal decoction in the 2nd trimester of the gestational period. In 18th day of pregnancy, animals were anesthetized and their foetuses were extracted through a cesarean section. The placenta was excised, weighed, and the number and placement of implantation sites, live, dead and resorbed foetuses were recorded. All foetuses were stereo microscopically examined for any morphological abnormalities. **Result:** There was no significant difference between the mean number of resorbed and dead fetuses of the test and control group. **Conclusion:** Although the number of included animals is very low, our data indicate that Pennyroyal has no negative effect on pregnancy.

P086 Effect of ethanolic extract *Anacyclus pyrethrum* DC on reproductive parameter of male rats

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Vajikaran is a speciality in Ayurvedic system of medicine in India dealing with herbs possessing rejuvenative and revitalizing properties for improving sexual dynamics and reproduction capacity. *Anacyclus pyrethrum* root is used traditionally used to enhance sexual power. An insight in the scientific literature appraised insufficient data to validate the rejuvinative property of *Anacyclus pyrethrum*. The study was therefore performed to evaluate the effectiveness of ethanolic extract of *Anacyclus pyrethrum* at a dose equivalent to 50, 100 and 150 mg/kg b.w. respectively in improving overall sexual behavior and reproductive parameters in male rats. Following parameters were evaluated the effect of extract on body and organ weights, change in histoarchitecture of testis, testosterone, FSH, LH level in blood, fructose level in seminal vesicles, sperm parameters and sexual behavior was studied. Administration of ethanolic extract had pronounced anabolic and spermatogenic effect in treated animals as evidenced by weight gains in the body and reproductive organs. Improvement in sexual behavior of animals as reflected in reduction of mount and intromission latency, an increase in mount and intromission frequency and enhanced attractability towards female. The increased spermatogenesis in treated group was confirmed by change in histoarchitecture and increase sperm count. The level of testosterone FSH, LH and fructose content in seminal vesicles was significantly increases in treated groups. These studies suggest the possible role of alkylamides of *Anacyclus pyrethrum* in improving sexual function by its action on HPG axis. These findings support the folk use of this plant as Vajikaran. **Acknowledgements:** AICTE, New Delhi for providing National Doctoral Fellowship. **References:** 1. Thakur M, Dixit VK,(2007) Sex Disabil. 25 (4): 203 – 207. 2. Chauhan NS, Sharma V, Dixit VK, (2008) Nat Prod Res. DOI: 10.1080/14786410802588493. 3. Chauhan NS, Dixit VK,(2009) Int J Impot Res. DOI:10.1038/ijir.2009.62.

Indigenous knowledge of traditional medicine and evidence based herbal medicine

P087 Nephroprotective and nephrocurative potential of *Momordica dioica* Roxb in streptozotocin induced diabetic nephropathy

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Momordica dioica Roxb (MD) is a climbing creeper and known as Kakora in Hindi. This plant possesses hypoglycemic gastroprotective and ulcer healing activities. Fruit is reported for hepatoprotective, nephroprotective and antidiabetic potential [1,2]. On the basis of traditional claim MD fruit was taken up for the study. Dried and powdered MD fruit was defatted and extracted with 95% ethanol. For screening of the activity, Wistar albino rats of either sex were divided into normal control, diabetic control and treated groups. Diabetes was induced with single dose of streptozotocin (55 mg/kg i.p.). In protective regimen, 4 – 30 days experimental period, ethanolic extract of MD fruit was administered as orally at 200 and 400 mg/kg/day, whereas curative regimen IT was administered onward 18 days upto 30 days. The final serum glucose, urea, creatinine and urine albumin levels were measured as functional profile of kidney function which was found to be significantly lower in treated groups than the diabetic control group. Malondialdehyde (MDA) was significantly ($p < 0.001$) lowered in treated groups compare to diabetic control group. Treated groups have shown significant increased ($p < 0.001$) in reduced glutathione (GSH) level which was reduced in diabetic control group compare to control groups. Change in body weight was found to be non significant. Results were supported by histopathological studies. Acknowledgments: Indian Council of Medical Research, New Delhi, India for financial support. **References:**1. Jain A,

Singahi AK (2008) Nat Prod Res (DOI: 10.1080/14786410802267569). 2. Reddy, GT, Kumar, BR, Mohan, GK, Mullangi, R, (2006) Asian J of Pharmacody Pharmacokin 6(4):327 – 329.

P088 Preventive and curative effects of *Phyllanthus acidus* (L.) Skeels extract against thioacetamide induced acute liver damage in Wistar rats

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Phyllanthus acidus (L.) Skeels has long been used in folk medicine to treat chronic liver diseases [1, 2]. In present study, protection of 70% ethanolic extracts of *Phyllanthus acidus* aerial parts (PAE) was evaluated in male Wistar albino rats against thioacetamide (TAA)-induced hepatic damage in preventive and curative models. Experimental rats received a single dose of TAA (100 mg/kg, s.c.) as hepatotoxin [3]. In preventive model, PAE was given orally at a dose of 125 and 250 mg/kg, 48, 24 and 2 h prior to TAA administration, meanwhile in curative model; rats were treated with PAE at a dose of 125 and 250 mg/kg, 2, 24 and 48 h after TAA intoxication. The protective effect was compared with Silymarin (100 mg/kg, p.o.). Rats pre-treated with PAE and Silymarin remarkably prevented the elevation of serum aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and malondialdehyde (MDA) in TAA-treated rats. Rats treated with PAE and Silymarin after the establishment of TAA liver injury showed significant ($p \leq 0.05$) protection of liver as evidenced from normal AST, ALT, LDH and MDA levels compared with toxin control rats. Hepatic glutathione (GSH) and other antioxidant enzymatic levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were significantly ($p \leq 0.05$) increased by extract treatment in both experimental groups. Histopathological observation of rat livers also indicated hepatoprotection against TAA liver damage. Phytochemical studies revealed the presence of saponins, triterpenes and phenolic compounds in PAE which could be responsible for the possible hepatoprotective action. **References:** 1. Kirtikar, K.R., Basu, B.D. (1987) Indian Medicinal Plants. Probasi Press. Calcutta. 2. Unander, D.W. et al. (1995) J. Ethno. 45: 1 – 18. 3. Kumar, G. et al. (2004) J. Ethno. 92:37 – 40.

P089 Application of *Leonurus cardiaca* L. oil extract for treatment of psycho neurological disorders in clinic

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Leonurus cardiaca L. has a long history of application in Russian traditional medicine and now it is described in a number of pharmacopoeias around the world. *L. cardiaca* is used in form of tinctures and tablets with dry extract and has been demonstrated to have sedative, hypotensive and cardiotonic effects. Recently an oil extract of *L. cardiaca* (OEL) was prepared by roto-pulsed extraction [1]. Its effects on animals were studied [2], but clinical application was not described. Fifty patients with stage 1 and stage 2 arterial hypertension accompanied by anxiety and sleep disorders were treated 28 days with 1200 mg (4 capsule) OEL per day. OEL was standardized in iridoids (0.15 mg/capsule) [3]. Subjective complaints of patients with stage 1 hypertension improved after 14 days, with stage 2 hypertension after 21 days of treatment and were significantly different from baseline after 28 days of treatment. Anxiety was reduced by 61% and 62%, emotional liability – by 53% and 20%, headache – by 41% and 34% and sleep disorders by 47% and 42% respectively. A statistically significant decrease and normalization in blood pressure was noted at 21 days and 28 days of treatment respectively. According to CGI scale large improvement and no side effects was observed for 32%, moderate effect for 48%, and weak effect for 8% of the patients. Only 12% of the patients did not respond to therapy. Thus this small uncontrolled pilot-study shows a positive effect of OEL on psycho-emotional status and arterial blood pressure of patients with psycho neurological disorders. **References:** 1. Shikov, A.N. (2006) Pharmaceutical Chemistry Journal 40(7):385 – 388. 2. Makarov, V.G. et al. (2006) Eksp Klin Farmakol. 69: 23 – 25. 3. Kosman, V.M. et al. (2002) Pharmaceutical Chemistry Journal 36:96 – 99.

P090 Gastroprotective effect of methanol extract of *Oxalis corniculata* Linn (whole plant) experimental animals

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In Indian traditional medicine, whole plant of Oxalis corniculata Linn (family: Oxalidaceae) is used to treat gastric and related disorders [1]. However, there are no scientific evidences available on its anti-ulcer activity. Hence, present study was carried out to investigate anti-ulcer effect of O. corniculata (whole plant) methanol extract using pyloric ligation and indomethacin induced gastric ulceration in the Sparque-Dawely rats (180 – 200 g) at the dose levels of 125, 250 and 500 mg/kg. In pylorus ligation induced ulceration model, various parameters were studied such as gastric secretions, its pH, total acidity, free acidity, and ulcer index, where as in indomethacin induced ulceration model, ulcer index and percentage inhibition of ulceration were calculated. Ranitidine, a standard anti-ulcer/antisecretory agent was used as positive control at the dose 100 mg/kg. Results were analyzed by One-way analysis of variance (ANOVA) followed by Dunnet's t-test. Oral treatment of O. corniculata (whole plant) showed, significant ($p < 0.01$) decrease in the gastric secretions, total acidity and free acidity. However, pH of the gastric content was significantly ($p < 0.05$) increased only at higher dose, 500 mg/kg. It showed also significant ($p < 0.01$) decrease in number of ulcers and ulcer score index in pyloric ligation and indomethacin induced ulceration models. Overall, results of the study showed that, the methanol extract of O. corniculata possess significant anti-ulcer properties in a dose dependent manner. Preliminary phytochemical evaluation of the extract showed the presence of flavonoids, which are among the cytoprotective materials for which anti-ulcerogenic efficacy has been confirmed. Hence, the present anti-ulcer activity of *O. corniculata* extract could be attributed to the presence of flavonoids. **References:** 1. Kiritikar K., Basu B., (1984) Indian medicinal plants, Lalit Mohan Basu. Dehradun. 437.

P091 Lupeol derivatives from the Eastern Nigeria mistletoe, *Loranthus micranthus* Linn. (Loranthaceae) with enhanced cell proliferative potentials

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Bioassay-guided fractionation of the crude extract of Eastern Nigerian mistletoe Loranthus micranthus Linn. (Loranthaceae) showed that the hexane fraction was very active in terms of immunostimulation with enhanced macrophage activation and cell proliferative abilities [1, 2]. Further fractionation and purification of the hexane fraction led to the isolation of a known triterpenoid, lupeol and some derivatives which were esters of either a C 14 or C 16 fatty acid. Characterization and unequivocal structural assignments were achieved by a combination of UV/visible, IR, NMR, MS, COSY experiments including, HMBC, HQSC, NOEs, NOESY. The characterized compounds exhibited mild immunostimulatory activity on cell line (C57Bl/6 splenocytes) and flow cytometry techniques against Lipopolysaccharide and Concanavalin A standards. This is the first report of the presence and immune stimulation potentials of the triterpenoid, Lupeol and derivatives isolated from our local mistletoe. **Acknowledgements:** The authors specially acknowledge Dr. C. S Nworu for the Pharmacological tests. **References:** 1. Osadebe PO, et al (2009) J Ethnopharmacology, 125:287 – 293. 2. Osadebe et al (2008), RPMP Vol 27: 473 – 485.

P092 Investigation of Thai traditional way for detoxification of aloe

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In Thai traditional medicine, dried juice extract (DJE) of *Aloe vera* (L.) Burm has been used in many formulations for a long time [1]. The Thai traditional way recommends detoxifying the aloe DJE before combining with other herbal constituents. The reason for detoxification is to decrease the gastrointestinal tract irritation of aloe. The traditional method to detoxify aloe is adding small amount of water into the DJE powder and heating in clay pot until the dried and crispy mixture is formed. The objective of this study is to verify the chemical conversion of major ingredients in aloe DJE using HPTLC analysis (Silica gel 60 GF254, solvent system; ethyl acetate: methanol: water 100: 13.5: 10, detection at 420 nm). Our study found that the amount of a major compound aloin (Rf 0.43) in the DJE was decreased after heating while the amount of its aglycone aloe-emodin (Rf 0.80) was increased from 50 μg/mL to 473.2 μg/mL. This result explains that hydrolysis and oxidation reactions can occur and some aloin is conversed to aloe-emodin resulted in decreasing anthraquinone glycoside intensity, which can cause irritation of the gastrointestinal tract and increase the chance for gripping action [2]. Further study of other glycosides and aglycones in the DJE of aloe is in progress. **References:** 1. Poomchusri, NT. (1973) Ayurvedic study. 2nd edn, Bangkok; Promjakkanpimp. 2. Robber, J E.; Speedie, M K.; Tyler, V E. (1996) Pharmacognosy and Pharmacobiotechnology. Pennsylvania; Williams & Wilkins, p 52.

P093 *Hypericum* species in Estonian folk traditions and in local scientific studies

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Both *Hypericum perforatum* and *H. maculatum* are common species throughout Estonia and neighbouring countries. The study used folklore texts from the HERBA (1) database collected between 1868 – 1994 on medicinal plants in Estonia. 117 texts describing 137 usage cases of plants bearing vernacular names related to *naistepuna* [*Hypericum* in Estonian, direct translation – women's red] were selected and analyzed using textological methods. Almost 30% of the usage cases were related to gynecological problems (as the name implies), followed by 14% for stomach problems, 10% for bladder and kidney problems and 5% each for stomachache caused by overexertion, skin diseases and pain. Although altogether 8 species belonging to 7 genera or even families bore such vernacular names in different places, the most frequently used plants were two species of genus *Hypericum*. Those two externally very similar species were not distinguished by ordinary people and were used in folk medicine as one. The content of hypericin (2) and total flavonoids before (3) and after (4) hydrolysis of glucosides was determined spectrophotometrically in both *Hypericum* species. The standard deviations of these methods did not exceed 0.5%, 8.7% and 5.4%, respectively (n = 4). The flowering tops (15 cm) and flowers in three different blooming stages (at the beginning of blooming, in full bloom and at the end of blooming) of *H. perforatum* and *H. maculatum*, collected in 2008 from South-Estonia (Antsla parish), were used as plant material. *H. maculatum* contained about a 2.5 times more hypericin (141 – 228 mg%) than *H. perforatum* (75 – 81 mg%). The amount of hypericin detected was higher in the flowers and lower in the flowering tops of both analyzed *Hypericum* species, but the comparative analysis of *H. perforatum* ja H. *maculatum* showed no similar tendencies for flavonoid content. Both species contained practically the same amount of total flavonoids (5,6 – 6,2% and 4,6 – 6,0%, respectively), but *H. perforatum* contained somewhat more flavonoid aglycones (1,1 – 2,0%) than *H. maculatum* (0,7 – 1,5%). It is necessary to continue this work. **References:** 1. Sõukand, R., Kalle, R. (2008) HERBA: Historical Estonian Folkmedical Herbal Database – http://herba.folklore.ee. 2. Ceskoslovensky lékopis (1987), vol. 4. Avicenum, Praha. 3. U.S.S.R. Pharmacopoeia (1987), vol. 11. Meditsina, Moscow [in Russian]. 4. Georgijevski, V.P. et al. (1990) Hayka, Novosibirsk [in Russian].

P094 Structure-based design of new analogs of Withaferin A as antitumor agents

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The endemic plant from Canary Islands (Spain), Withania aristata (Aiton) Pauqui (Solanaceae), popularly known as "orobal" [1] is widely used in folk medicine as antitumor, antispasmodic, antirheumatic agent; further for eye and otitis problems, as well as for insomnia and urinary pathologies [2]. We have found that the acetone extract from the leaves of this species is a rich source of the known whitanolide, withaferin A (more than 0.2% dried weight). The therapeutic potential of Withania species has been attributed to the presence of withanolides [3] and, in particular, withaferin A which showed anti-cancer activity in vivo and has been reported to induce cytoskeletal disruption, to inhibit cell mobility and NFkB activation [4], and to induce apoptosis in vitro [5]. Taking this into account, we have prepared analogs of withaferin A for structure-activity relationship studies. We have performed several chemical transformations on ring A, on the epoxy group of ring B, and on the lactone and hydroxyl groups of the side chain of withaferin A, including oxidations, reductions and halogenations. All semi-synthetic analogs were tested for their potential anticancer activity against four tumor cell lines. The biological results obtained from these analogs allowed us to deduce a number of structure-activity relationships, which confirmed the potential of withaferin A in cancer chemotherapy. The semi-synthesis, the bioactivity data and the SAR studies will be presented in the accompanying poster. **References:** 1. Anderson GJ et al (2006) Am. J. Bot. 93:1295 – 1305. 2. Benjumea D et al (2009) J Ethnopharmacol 123: 351 – 355. 3. Veleiro AS et al (2005) Studies in Natural Products Chemistry, Bioactive Natural Products Atta-Ur-Raman, Elsevier Science Publishers, Amsterdam, p 1019 – 52. 4. Ichikawa H. (2006) Mol. Cancer Ther. 5: 1434. 5. Malik F et al (2007) Apoptosis 12: 2115 – 2133.

P095 Investigation on possible interaction of cranberry polyphenols and warfarin using in vitro and in vivo studies

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Cranberry (*Vaccinium macrocarpon* Ait., Ericaceae), distributed in North America, has traditionally been used in the treatment and prevention of urinary tract infections. Various types of constituents including terpenoids, iridoids, and polyphenols, such as proanthocyanidins, anthocyanins, flavonoids, and organic acids are contained in cranberry. Among them, A-type proanthocyanidins have been revealed to inhibit the adherence of uropathogenic P-fimbriated *Escherichia coli* to eukaryotic cells, and this is thought to be the mechanism underlying its role in preventing urinary tract infection. However, some case reports showed the interaction between cranberry and warfarin. [1, 2] The possible interaction of cranberry with warfarin was suggested to depend on the inhibition of cytochrome P450 (CYP) activities, which is involved in most metabolism-mediated drug interaction. We recently reported the inhibitory effect of 60 polyphenols on CYP3A4 and CYP2C9 activity, indicating that some cranberry flavonoids can significantly inhibit these CYPs [3] In this study, we investigated CYP3A4 and CYP2C9 inhibitory activities of cranberry juice constituents and their metabolites. The bioassay-guided fractionation of cranberry extracts gave the cranberry-derived proanthocyanidin polymer (CPP) as the most potent inhibitor of CYP3A4 and CYP2C9. Furthermore, we investigated the plasma level of warfarin and alternation in international normalized ratio after ingestion of CPP or cranberry juice (CJ) to rats, indicating that co-administration of CJ was unlikely to affect the pharmacokinetics and pharmacodynamics of warfarin in rats. Further investigation and improved case studies are needed to address these issues. **References:** 1. Committee on Safety of Medicines, Medicines and Healthcare Products Regulatory Agency. Possible interaction between warfarin and cranberry juice. (2003) Curr. Probl. Pharmacovigil. 29:8. 2. Suvarna, R. et al. Possible

interaction between warfarin and cranberry juice. (2003) BMJ. 327:1454. 3. Kimura, Y. et al. (2010) Food Chem. Toxicol. 48:429 – 435.

P096 Antiplasmodial remedies from European renaissance herbals: HPLC based activity profiling of *Alisma plantago-aquatica* extract for antiplasmodial activity, and isolation of active dammarane triterpenoids

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The common water plantain *Alisma plantago-aquatica* L. (Alismataceae) was used in the 16th and 17th centuries in Central Europe to treat *Plasmodium vivax* malaria (tertian fever). The Renaissance herbals by Bock (1532), Brunfels (1532), Mathioli (1560), and Zwinger (1696) described the internal use of alcohol extracts of the tubers to treat this disease, which was quite common in German speaking areas then [1]. In the course of a screen of remedies from Renaissance herbals an extract of water plantain tubers was active against *P. falciparum* (77% inhibition at 4.9 μg/ml). With analytical scale time-based HPLC separation and testing of one-minute fractions in combination with HPLC hyphenated methods (HPLC-PDA, -MSn, HR-MS, off line microprobe NMR) the active substances were identified as acetylated dammarane triterpenes. Seven of these compounds were isolated using normal phase medium pressure column chromatography and semi-preparative HPLC. Structure elucidation was achieved by extensive 1 H and 13C NMR analysis. The dammaranes had IC_{50}s ranging from 3.3 to 7.0 μM. This is the first report of antiplasmodial activity of this triterpenoid class, and the first result of our ongoing project of screening for antiprotozoal natural products from remedies used in European Renaissance medicine. **References:** 1. Adams M, et al. (2010) J Ethnopharm, submitted. 2. Adams M, et al.(2009) Nat Prod Comm., 10:1377 – 81.

P097 Effect of *Rhoicissus digitata* extracts on acetylcholine-mediated contraction in rat uterus

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Rhoicissus digitata (L.f.) Gilg & M. Brandt (Vitaceae) is used in traditional medicine in Southern Africa to tone the uterus during pregnancy and to facilitate delivery. Aqueous and methanolic extracts were prepared from powdered dry leaves and roots of *R. digitata*. The uterine horns were surgically removed from female Sprague Dawley rats in estrous, placed in de Jalon solution (NaCl: 9 g/l, KCl: 0,42 g/l, NaHCO3: 1,50 g/l, Glucose: 0,5 g/l, CaCl2: 0,12 g/l) and hung in the experimental setup in the uterus contraction assay. After mounting, the uterine horns were allowed to equilibrate in the organ bath for 30 min, renewing the solution every 10 min by flushing. The organ strips were incubated with extract in a concentration of 1.3 mg/ml for 5 min before starting cumulative addition of acetylcholine. The water extract of the root and the methanol extract of the leaves increased the response significantly ($p < 0.0001$ for both (two-way ANOVA)), and the methanol extract of the root inhibited the response significantly ($p < 0.0001$). The results suggests that the use of water extracts (decoctions) of *R. digitata* roots in South African traditional medicine as pregnancy related medicine can be justified, as they increase the acetylcholine-mediated uterine contractions in rats. The uterus contracting effect of the methanol extract of the leaves of *R. digitata* suggests that alcoholic leaf extracts could be used as substitutes for root extracts, which as a renewable source could help with sustainable usage of these species.

P098 Investigation of the antidiabetic activity of *Morus alba* leaf extract *in vitro* and *in vivo*

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The leaves of white mulberry (*Morus alba* L.) are widely used in the traditional medicine of the type II diabetes mellitus. Several groups of plant metabolites are held to be responsible for the complex antidiabetic effect of the drug: iminosugars, flavonoids and other phenolic compounds, ecdysteroids, glycoproteins, and, as we recently suggested, megastigmanes and glycosylated volatile compounds [1,2]. As an initial step of a research project aiming to clarify the importance of different antidiabetic constituents, here we report the *in vitro* and *in vivo* activity and chemical evaluation of the 70% EtOH extract of mulberry leaves. In vitro, fully differentiated adipocytes were used. 50 μg/mL of extract was administrated into the culture medium containing 15 mM glucose, with or without the addition of 0.32 μM insulin, and the antidiabetic activity was determined by the remaining glucose concentration in the medium after 24 hours. A significant effect was found in both systems. The *in vivo* antidiabetic effect was evaluated in non insulin dependent diabetic SPRD rats. In this model, diabetes was induced by intraperitoneal administration of streptozotocin (150 mg/kg) to newborn rats. Oral treatment of fasted animals with the extract (750 mg/kg) resulted in a significant decrease of postprandial glucose level. Composition of the extract was characterized by gradient RP-HPLC, and the potentially active chlorogenic acid [3], rutin and isoquercitrin were determined as major constituents, while quercetin, apigenin, morin and 20-hydroxyecdysone were present in much lower amounts. The contribution of each compound to the measured antidiabetic activity needs further investigation. **Acknowledgements:** This project was supported by the Hungarian National Research Fund (OTKA; PD 75383), the New Hungary Development Plan (TÁMOP-4.2.2 – 08/1 – 2008 – 0013) and by the grant from the National Science Council of Taiwan (NSC 98 – 2314-B-037 – 011-MY3). **References:** 1. Hunyadi A et al. (2007) Planta Med. 73: 941. 2. Hunyadi A et al. (2008) Planta Med. 74: 1117. 3. Andrade-Cetto et al. (2001) J. Ethnopharmacol. 78:145 – 149.

P099 Evaluation of antioxidant and DNA protection activities in the extracts of the *Terminalia catappa* leaves

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Terminalia catappa (Combretaceae) is used commonly as a folk medicine in Taiwan, and has been claimed to have therapeutic effects in hepatitis and liver related diseases [1, 2]. The leaves, bark and fruit of the *Terminalia catappa* have also been commonly used as a folk medicine for antidiarrheic, antipyretic and haemostatic purposes in India, Philippines, Malaysia and Indonesia [3]. The extracts from the *Terminalia catappa* leaves were used in this study. Antioxidant activities were measured by both 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH·) scavenging and 2, 2-azino-bis (3-ethylbenz- thiazoline-6-sulfonic acid) (ABTS·+) decolourisation methods. To further evaluate the effect of *Terminalia catappa* extracts on UV induced DNA damages, the DNA protection assay was employed. *Terminalia catappa* leaf extracts had effective DPPH bleaching activity and ABTS·+ radical scavenging activity in a concentration dependent manner. Furthermore, the *Terminalia catappa* leaf extracts to inhibit the oxidative DNA damages were assessed by measuring the conversion of supercoiled pUC 119 plasmid DNA to the linear forms. UV irradiation of DNA with hydrogen peroxide resulted in the formation of linear forms of DNA, indicating double-strand DNA breaks. Addition of *Terminalia catappa* plant extracts to DNA resulted in a partial inhibition of the conversion of supercoiled DNA to linear forms, indicating that the *Terminalia catappa* plant extract was able to protect plasmid DNA against hydroxyl radical induced oxidative damage. The inhibition of hydroxyl radical induced DNA strand breaks by *Terminalia catappa* plant extracts exhibited a concentration dependent relationship. **Acknowledgements:** Committee on Chinese Medicine and Pharmacy (Grant No. CCMP 98-RD-006), Chia Nan University of Pharmacy and Science. **References:**

1. Chyau, CC. et al. (2002) Food Chemistry 78: 483 – 488. 2. Kinoshita, S. et al. (2007) Phytomedicine 14: 755 – 762. 3. Chyau, CC. et al. (2006) LWT 39:1099 – 1108.

P100 Glucose transport activity on adipocytes by extracts form Thai medicinal plants used to treat diabetic patients

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Ten Thai medicinal plants which have been used by Thai folk doctors for treatment of diabetic patients were selected for test hypoglycemic activity by promoting the glucose transport in adipocyte cells. The uptake of radioactive 2-deoxyglucose in 3T3-L1 adipocytes was evaluated in this assay [1]. The antioxidant activity by DPPH assay was also investigated [2]. The extraction method of all plants in analogy to the method of folk doctors was the maceration in 95% ethanol. The results showed that Nelumbo nucifera and Tacca chantrieri showed the highest values for increase of glucose uptake in adipocytes at 0.1 μg/ml (1.7and 1.6 fold of basal) and also have good antioxidant activity (EC50 = 5.33 and 10.24 μg/ml). Piper sarmentosum also showed the highest hypoglycemic activity in the glucose uptake assay at 1.0 μg/ml (1.7 fold of basal) but its extract exhibited less antioxidant activity (EC50 = 83.18 μg/ml). The study found that all plant extracts showed high glucose uptake to adipocyte cells but the low dose of all extracts exhibited more effective glucose uptake than the high dose. In addition, most plant extracts (7 in 10 plants) also showed relation between hypoglycemic and antioxidant activity. In conclusion, these results should support the use of Thai folk doctors for treatment of diabetic patients. **Acknowledgements:** Faculty of Medicine, Thammasart University for financial support **References:** 1. Hong SJ et al (2000) J Med Sci;16:445 – 51. 2. Yamazaki K, et al (1994) Chem Pharm Bull; 42:1663 – 5.

P101 Standardization and pharmacological activities of a *Curcuma comosa* traditional formula for menopausal women

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Menopause causes changes in lipid metabolism that increase the risk for cardiovascular disease [1]. In Thai traditional medicine, there are a number of traditional formulas for menopausal symptoms. One of those is a formula (CCZ) containing *Curcuma comosa*, *Curcuma aromatica*, and *Zingiber montanum*. Our studies were undertaken to standardize and evaluate pharmacological activities of the fomula. Pharmacognostic characters and TLC fingerprintings of each plant and CCZ were used for standardizing the formula according to WHO recommendation [2]. CCZ demonstrated estrogenic and antiosteoporotic activities in preliminary in vivo experiments. Oral administration of CCZ (0.5, 1 and 2 g/kg/day) for 8 weeks decreased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in hypercholesterolemic rats, but lower than simvastatin. No effect on triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and atherogenic index (AI) was observed. Furthermore, CCZ decreased alkaline phosphatase (ALP) and lesion development in aorta, heart, and liver. Moreover, CCZ (1 and 2 g/kg/day) possessed a protective effect on increased TC, LDL-C, and AI. In conclusion, the result supported the traditional use of this formula in the management of menopausal symptoms related to cardiovascular disease. **Acknowledgements:** Mahidol University Research Grant **References:** 1. Carr, M.C. (2003) J Clin Endocrinol Metab 88: 2404 – 2411. 2. World Health Organization (1998) Quality Control Methods for Medicinal Plant Materials. World Health Organization. Geneva.

P102 Formulation and stability test of BJ adaptogen tablets for cancer treatment

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BJ adaptogen is a Thai Traditional medicine preparation, used for balanced health and cancer treatment by Thai folk doctors. This preparation is composed of five plants; Piper longum, Piper samentosum, Piper interuptum, Plumbago indica and Zingiber officinale. Its ethanolic extract exhibited high cytotoxic activity against lung cancer cells or COR-L23 (IC_{50}= 19.80 μg/ml) by SRB assay [1,2]. Its main compound was determined as piperine. In this investigation, we formulated a BJ adaptogen extract tablet and tested its stability under accelerated condition (45 °C, 75%RH, 4 months) by determination of piperine content in the tablets by HPLC. A wet granulation method was used in developing the tablets. The suitable excipients were lactose, starch, Explotab® and magnesium stearate. The physical properties of tablets were evaluated following the USP25 requirements. The results of stability testing found that content of piperine from BJ adaptogen extract tablets was reduced by 10% within 4 months. **Acknowledgements:** Faculty of medicine, Thammasart University for financial support **References:** 1. Itharat, A. et al. (2004) J. of Ethnopharmacology: 90:33 – 38. 2. Skehan P et al.(1990) J Natl Cancer Inst 1990: 82(13): 1107 – 12.

P103 Determination of total phenolic compounds and phytochemical screening from Thai medicinal plants

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The present study deals with the total phenolic contents and phytochemical screening the traditionally used as improved tendon functions, the relief of muscle fatique, the nourishment of bone joint and bone tissue and also tonic from 3 Thai medicinal plants such as *Sambucus javanica* Reinw., *Smilax corbularia* Kunth., and *S. glabra* Wall. ex. Roxb [1]. Different solvents including dichloromethane, ethyl acetate and ethanol were used to prepare extracts from these plants. Total phenolic contents were determined using a spectrophotometric technique, based on the Folin-Ciocalteu reagent and calculate as gallic acid equivalents GAE/g extract [2]. The high total phenolic contents of dichloromethane, ethyl acetate and ethanolic extracts were found that *S. corbularia* Kunth. ranged from 4.15, 18.95 and 20.64 mg gallic acid/g extract, respectively. Additionally, the preliminary screening, qualitative thin layer chromatography of secondary metabolites were studied [3] and showed the presence of flavonoids, phenolic and alkaloids in these plants. Thus, the total phenolic contents and phytochemical screening tests may be used to investigate the bioactive compounds and subsequently may lead to screening of these plants for antiosteoporotic. Further, the preliminary screening of these plants for bone formation and bone resorption activities have been studied. **Acknowledgements:** This study was supported in grants MRG51 from The Thailand Research Fund and NRCT. **References:** 1. Maneekun, U. et al. (1997) General traditional medicines: Pharmacy. Medical Registration Division. Office of the Permanent Secretary of MOPH. Pharmacy. 2. Javanmardi, J. et al (2003) Food Chemistry. 83:547 – 550. 3. Mallikharjuna, PB. Et al (2007) E-Journal of Chemistry 4(4):510 – 518.

P104 Phenolic compounds from the leaves of *Ouratea gilgiana* and their biological activities

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The genus *Ouratea* (Ochnaceae) comprises 300 tropical species [1] and 34 of them are present in Africa dense forest [2]. *Ouratea* plants have been reported to be used in Cameroonian folk medicine for the treatment of dysentery, rheumatism and gastric distress [3]. The chemistry of Cameroonian *Ouratea* genus is poorly studied. A methanolic extract of the aerial parts of *Ouratea gilgiana* H.J. Winkl. was fractionated by column chromatography, leading to the isolation of one sterol glycoside and five phenolic compounds. All those compounds were analysed by HPLC-UV-DAD after the purification process. Their structures were established by spectroscopic methods, including two-dimensional NMR and mass spectrometry. Some of them (amenthoflavone (1) and 6″-*O*-acetylvitexine (2)) showed radical scavenging properties in a DPPH assay. The study of their phytoestrogenic activity is on going.

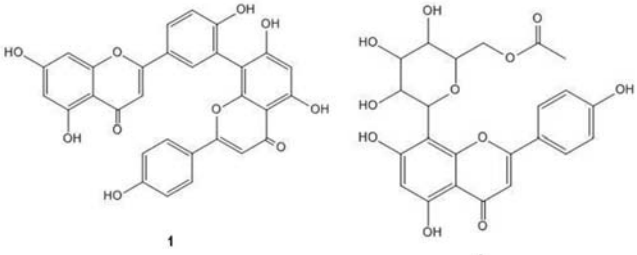

Fig. 1

Acknowledgements: Gaétan Bayiha Ba Njock acknowledges the Swiss Department of Foreign Affairs for the International Scholarship. **References:** 1. Heywood V H, (1978) Flowering plants of the world. (Oxford University Press, London). 2. Hutchinson J, Dalziel J M, (1954) Flora of West Africa, Vol 1, p 224. 3. Bouquet A, (1969) Féticheurs et médecines traditionnelles du Congo Brazzaville. (ORSTOM, Paris).

P105 Chemical and pharmacological evaluation of fractions potentially active obtained of the aqueous extract of *Capraria biflora* L.

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Capraria biflora L. is a wild plant very used in traditional medicine in American continent and in the Caribbean area (1). This plant is active as analgesic, diuretic and anti-inflammatory, equivalent to the reference drugs according to studies developed previously (2,3,4) but no detailed chemical study had been reported. The present work had as objective to identify the active fractions obtained from the aqueous extract of the leaves of this species and to deepen in the chemical composition of the active fractions. Aqueous extract was partitioned successively with chloroform and butanol to give the chloroform, butanol and water fractions, which were evaluated as anti-inflammatory using zebras fish like pharmacological model (5). The chloroform and butanol fraction was found the most active. The combination of thin layer chromatography and column chromatography and the spectroscopic evaluation of the isolated compounds allowed suggesting the presence of naringenin, mannitol, derived of apigenin and luteolin in the most active fractions. The identified compounds are informed for the first time in the plant, and they allow justifying the anti-inflammatory effects and diuretics shown by the aqueous extract of this species and the uses in traditional medicine. **References:** 1. Roig JT. (1988) Plantas medicinales, aromáticas o venenosas de Cuba. Editorial Científico-Técnico. La Habana.; 510 – 11. 2. Loy S, et al. (2003) Fitoterapia 74, 686 – 8. 3. Vicet L, et. al. (2004) Cuban Jornal of Pharmacy., 38: 660 – 665. 4. Acosta SL et al (2003) Acta Farm. Bonaerense, 22 53 – 5. 5. Lieschke, et al. (2001) BLOOD, 98, 3087 – 96.

P106 Antimicrobial and cytotoxic activities of five Thai plants used as antipyretic drug

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From selective interviews Thai folk doctors found that there were five Thai medicinal plants popularly used as antipyretic drug and to reduce fever in cancer patients. These are roots of *Harrisonia perforata* Merr (HP), *Capparis micracantha* DC (CM), *Clerodendrum petasites* S. Moore (CP), *Ficus racemosa* L. (FR) and *Tiliacora triandra* Diels (TT)[1]. The objective of this work wasto investigatetheir anti-microbial activities against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* using the disc diffusion method [2]. Cytotoxic activity against lung (COR-L23) and breast (MCF-7)cancer cells and lung normal cells (MRC-5) was also tested by SRB assay [3,4]. The ethanolic extracts were tested for their activity. TT showed the highest inhibitory activity against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* (clear zone = 11.12, 13.8, 9.5 and 20.5 mm respectively). HP and CP showed antibacterial activity against gram positive organisms only. TT and HP showed high cytotoxicity against MCF-7 (IC_{50}= 7.9 and 27.7 µg/ml respectively) and CORL-23 (IC_{50}= 5.5 and 32.07 µg/ml respectively). Surprisingly, there was no cytotoxic activity against lung normal cells or MRC-5 (IC_{50}> 50 µg/ml). These results showed that TT should be investigated further to isolate active antimicrobial and cytotoxic compounds. These results support the use of these plants by Thai folk doctors as antipyretic drugs. **Acknowledgements:** Faculty of Medicine, Thammasart University for financial support **References:** 1. Foundation for Thai Traditional Medicine Resuscitation and encourage (2005) Thai Pharmaceutical Science. Pikanate Printing Center Cooperation p225 – 226. 2. Jorgensen J et al. (1999) Manual of clinical microbiology, 6th ed. Washington DC: ASM Press. P1526 – 1543. 3. Itharat, A. et al. (2004) J. of Ethnopharmacology: 90:33 – 38. 4. Skehan P et al.(1990) J Natl Cancer Inst 1990: 82(13): 1107 – 12.

P107 Anthraquinones with potent quinone reductase-inducing activity from the stem bark of *Morinda citrifolia* (Rubiaceae)

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In French Polynesia, *Nono* (*Morinda citrifolia* L.) is the given name to a small shrub belonging to the Rubiaceae family. It grows at low altitude and generally isolated from other plants. *M. citrifolia* is widespread in southern Asia and the Pacific islands. In the past, the Polynesians used the roots as a dye [1]. Moreover, there is a long history of the use of *M. citrifolia* as an important medicinal plant for the treatment of asthma, bone fractures, cancer, urinary difficulties and many other ailments [2 – 3]. Phytochemical studies have shown that various parts of *Nono* contain anthraquinones, fatty acid glycosides, xanthones, iridoids and benzophenones [2 – 4]. Recent studies of compounds isolated from the fruit and root of *M. citrifolia* have shown a wide range of biological activities, such as the inhibition of angiogenesis [5], cyclooxygenase-1 [6], tyrosine kinase [7] and the induction of quinone reductase[4]. As part of a project directed toward the discovery of new cancer chemopreventive agents from plants, the CHCl3-soluble extract of the stem bark of *M. citrifolia* was found to induce the enzyme quinone reductase. Bioassay-guided fractionation together with chromatographic methods led to the identification of potent quinone reductase inducting anthraquinones.

References: 1. Pétard P. (1986). Plantes Utiles de la Polynésie-Raau Tahiti. Editions Haere Po No Tahiti. Papeete. 2. Potterat O. et al. (2007) Planta Med., 73: 191 – 199. 3. Pawlus A.D. et al. (2007) J. Pharm. Pharmacol., 59: 1587 – 1609. 4. Deng Y. et al. (2007) J. Nat. Prod., 70: 2049 – 2052. 5. Hornick C.A. et al. (2003) Angiogenesis, 6: 143 – 149. 6. Li R.W. et al. (2003) J. Ethnopharmacol., 85: 25 – 32. 7. Hiwasa T. et al. (1999) FEBS Lett., 444: 173 – 176.

P108 Research of chemical composition of cloudberry fruit

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Rubus chamaemorus L. commonly known as cloudberry is a widespread plant of the northern latitudes which had been used in folk medicine of these countries as a source of vitamins. The aim of this work was to research the bioactive compounds of cloudberry fruit gathered in fruitage. The qualitative composition and quantitative content of chemical compounds were determined by TLC, spectrophotometry and chromatography-mass spectrometry by using standard samples. Essential oil was gotten by micro steam distillation/solvent extraction (SD/SE). The trace element composition of investigated species was established by means of inductively coupled plasma mass-spectroscopy. As a result of the given research, a presence of some vitamins (ascorbic acid, carotenoids, phylloquinone, retinol, tocopherol) has been established the structure of bioactive compounds of cloudberry fruit. The quantitative content of some compounds has also been defined as follows: ascorbic acid – 240 mg%, β- carotene – 2.5 mg%, rutin – 6.7 %, water-soluble polysaccharides and pectin – 6.5 %, essential oil – 0.05 %. Additionally it was established a great content of potassium ~ 15.0 mg/g and unbound organic acids, which were determined as follows: citric, tartaric, oxalic and malic acids. The total content of acids was defined ~ 5.0 %. Cloudberry fruit can is an excellent source of polyvitamins and microelements.

P109 Immunomodulatory activity of adlay bran and its phenolic components

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Adlay (Chinese pearl barley, soft-shelled Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a grass crop that has long been used as a traditional Chinese medicine and as a nourishing food. Under the β-hexosaminodase release from RBL-2H3 stimulated with A23187-guided fractionation, a series of flavonoids: 3,5,7,4′-tetramethoxyflavone, 3,5,7,3′,4′-pentamethoxyflavone, 5,6,7,8,4′-pentamethoxyflavone, 3,5,6,7,8,3′,4′-heptamethoxyflavone, 5,6,7,8,3′,4′-hexamethoxyflavone, parvisoflavone, formononetin, isoliquiritigenin, liquiritigenin, homoeriodictyol, 5,7,3′-trihydroxy-4-methoxyflavanone, 4,2′,4′-trihydroxychalcone, 4,2′,4′-trihydroxydihydrochalcone and a new aurone derivative, and two chromones: 5-hydroxy-7-methoxychromone and 5,7-dihydroxychromone were isolated from the active fraction.

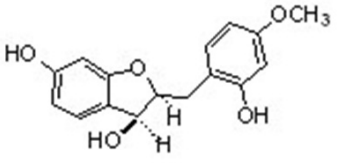

Fig. 1

Their structures were elucidated by means of spectroscopic data analysis, including 1D and 2D-NMR. All of them are the first isolates from adlay bran and LC/MS analysis was used as a quality control platform.

P110 Hypoglycemic effect of a leaf extract of *Pseuderanthemum palatiferum* (Nees) Radlk.

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The hypoglycemic effect of an 80% ethanolic leaf extract from *Pseuderanthemum palatiferum* (PPE) was investigated in normal and Streptozotocin (STZ)-induced diabetic rats. The PPE was administered daily and orally to the rats at the doses of 250, 500, and 1000 mg/kg body weight (b.w.) for 14 days. The levels of fasting plasma glucose (FPG), serum insulin, and biochemical data such as blood urea nitrogen (BUN), triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), and alkaline phosphatase (ALP) were evaluated. The hypoglycemic effect of PPE was compared to that of the known anti-diabetic drug glibenclamide (0.25 mg/kg b.w.). FPG and serum insulin in normal rats were not significantly different from the control and test groups in all dosages. The treated diabetic rats which had received PPE and glibenclamide showed significantly ($p < 0.05$) decreased FPG and increased serum insulin levels at the end of experiment. The hypoglycemic effect of PPE at the dose of 250 mg/kg b.w. was significantly ($p < 0.05$) more effective than that of glibenclamide. The serum insulin in PPE fed diabetic rats at the dose of 250 mg/kg b.w. was not different from those which had received glibenclamide, and this dose was significantly ($p < 0.05$) more effective than PPE at the doses of 500 and 1000 mg/kg b.w. while PPE increased HDL and decreased TC, TG, LDL, BUN and ALP in the diabetic rats. In conclusion, PPE has a beneficial effect in hypoglycemic rats and may prevent the complication of diabetes. **Acknowledgements:** This work was partially supported by the Development Research Division, Mahasarakham University and Faculty of Pharmacy, Mahasarakham University. We thanks Prof. Dr. Adolf Nahrstedt for valuable discussion and critical review of the manuscript. We also thank to Roche Diagnostics Co., Ltd. (Thailand) for support with glucose strips and meter.

P111 Large scale multi-centre randomized placebo controlled clinical study proves the efficacy of pumpkin seed in symptomatic benign prostatic hyperplasia (BPH) – G.R.A.N.U. study

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Of all known herbal drugs in the therapy of BPH, pumpkin seed has the longest tradition. The high level of unique delta-7 sterols is discussed as the active principle. This is supported by a former study where prostate tissue of patients taking pumpkin sterols contained much less dihydrotestosterone, compared to the control group [1]. The aim in the treatment of symptomatic BPH is to improve lower urinary tract symptoms (LUTS) and patient's quality of life (QoL) [2]. This study was carried out to confirm the efficacy of pumpkin seed in a design referring to the recommendations of the International Consultation on BPH and WHO criteria. **Method:** three treatment groups. 1. pumpkin seed extract (2 x 500 mg capsule per day, PROSTA FINK® FORTE), 2. placebo capsule. 3. pumpkin seed (2 x 5 g per day), The pumpkin is a special variety: GRANU FINK® medicinal pumpkin. Treatment period: 12 months. 1431 patients were randomised. A noticeable decrease in the international prostate symptom score (IPSS) by 3 to 4 points was observed after 3 months followed by gradually continuous decrease until month 12. The QoL improved continuously over time with the remarkable improvement by 36% in the pumpkin seed group. Treatment with pumpkin seed results a strong improvement of micturition complaints. Level of IPSS improvement (%) over 12 months is comparable to most prescription medicines in this indication [3]. The symptom relief is accompanied by a clinically significant improvement of Quality-of-Life by 36%. Pumpkin seed is an effective and safe option for the long-term therapy in men with LUTS due to BPH. **References:** 1. Schilcher H et al. (1987) Urologe B 27:316 – 319. 2. Berges R et al. (2009) Urologe A 48:12:1503 – 1516. 3. Madersbacher S et al. (2007) European Urology 51:1522 – 1533.

P112 Demographic survey of traditional healers and their practices in Mushin local government area of Lagos state and Ifedore local government area of Ondo state, Nigeria

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Traditional Healers (THs) are known to play an important role in health care delivery system in Nigeria and the majority of the population patronise them because of easy accessibility and affordability. However, traditional healing methods are generally associated with occultism, unhygienic environment and lack of documentation of mode of practice. It is thus imperative that their present level of practice should be upgraded to an acceptable standard which will eventually lead to their corecognition or full integration into the health care delivery system in Nigeria. A demographic survey was conducted on some of the THs and their practices in Mushin (within 7 out of 8 health districts) and Ifedore (within the 7 health districts) Local government areas (LGA) of Lagos and Osun States respectively. The surveys revealed that while the majority (about 76%) of the practitioners in Ifedore LGA were operating their clinics on part-time basis. The majority (about 98%) were operating on full-time basis in Mushin LGA. About 52% (in Ifedore) and 52% (in Mushin) used only medical methods for treating their patients while 26% (in Mushin) and 48% (in Ifedore) used both medical and occultic methods. The survey showed that the THs are contributing their quota in the management of diseases especially at the primary health care level. However, there is a need for the government to establish traditional healers' schools to retrain the existing healers in basic medical practices and to train more practitioners that will increase the number of health manpower at primary care level.

P113 The antidiabetic effect of W9, a medicinal plant from the pharmacopea of the Cree of the Eastern James Bay region in Canada

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W9 is a Boreal forest plant identified among species used by the Cree of Eeyou Istchee of northern Quebec to treat symptoms of diabetes. In a previous study, the ethanol extract of W9 enhanced glucose uptake in C2C12 muscle cells via stimulation of AMP-activated protein kinase (AMPK) pathway. In this study, we investigated the effect of this product on the translocation of insulin-sensitive GLUT4 transporters in skeletal muscle cells in culture. Treatment of L6 myotubes with W9 for 18 h significantly increased glucose uptake and GLUT4 translocation to the cell membrane. W9 increased phosphorylation of AMPK and P38 MAPK with no indication of increased phosphorylation of Akt. To validate the effect of W9 in vivo, the extract (1% in drinking water) was administered to KKAy diabetic mice for 10 days. Glycemia and fluid intakes were significantly reduced by W9. Moreover, W9-treatment increased skeletal muscle GLUT4 content, stimulated ACC phosphorylation and increased hepatic levels of PPAR-α of KKAy mice. Administration of W9 to normal C57BL/6 had no effect on glycemia. The results of the present study expand the understanding of the molecular mechanisms underlying the antidiabetic potential of W9. **Acknowledgements:** Canadian Institutes of Health Research

P114 Phytochemical components and bioactive effects of traditional Chinese medicine and the relationship with their characteristics

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Eighty-eight Traditional Chinese Medicines were extracted with 50% methanol, and relationships between Chinese characteristics of nature (hot, warm, cool and cold), flavors (sour, bitter, sweet, pungent and salty) v.s. phytochemical contents and bioactives effects were explored by nonparametric correlation analysis. The individual phytochemical components of 50% MeOH extracts were listed below: total polyphenol, 97.68 μg gallic acid equivalent/mg (15.18~606.86); total flavonl, 28.53 μg cathechin/mg (0.47~247.41), total saponin, 110.16 μg diosgenin/mg (20.44~254.74) and total coumarin, 110.16 μg coumarin/mg (33.27~1875.5) in 88 kinds of TCMs. According to the TCM characteristics, the bitter and pungent groups were rich in saponin and strong anti-Staphylococcus aureus; the warm and cold groups, too. There was good relationship found between the anti-Staphylococcus aureus and total saponin contents. (R = 0.519) The results supported the correlation between natures and flavors in TCMs, which means specific natures and flavors usually match to each other. As the data shown, the herb medicine with cold nature often has bitter taste, and also, the warm-nature medicine has pungent flavor in most cases. On the other hand, total Polyphenol was less in hot and sweet groups and didn't scavenge DPPH radical effects. The results indicated the high correlation between total polyphenol and DPPH scavenge effects. (R = 0.763)

P115 Anti-inflammatory compounds of the traditional Hungarian medicinal plant *Centaurea sadleriana*

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The decoction of the aerial parts of *Centaurea sadleriana* JANKA (Asteraceae), a plant native to Hungary, is traditionally used to treat the wounds of sheep in the Southern Great Plain region. Phytochemical and pharmacological studies on this plant have not been performed so far. Only we have recently confirmed the wound healing effect of the plant on rats [1]. The objective of the present work was the in vivo and in vitro investigation of the anti-inflammatory effect of *C. sadleriana* and the isolation and identification of its active compounds. The concentrated methanol extract of aerial parts of *C. sadleriana* was partitioned using n-hexane and chloroform. These two fractions were further fractionated via VLC and the anti-inflammatory effects of the fractions were studied by *in vitro* (COX-1, COX-2 and LTB_4 formation inhibitory activity) and *in vivo* (intraperitoneal and oral administration to rats) methods. Some of the fractions gained from the n-hexane extract possessed marked *in vitro* (70 – 85% LTB_4 formation inhibition, 59 – 83% COX-1 inhibition, 80 – 92% COX-2 inhibition at a concentration of 50 μg/ml) and *in vivo* anti-inflammatory effects (25 – 50% oedema volume reduction). Chromatographic purification (VLC, MPLC, HPLC, preparative TLC, CPC and gel filtration) of the active fractions resulted in the isolation of flavonoids, triterpenoids and lignans. Our present study confirmed the marked anti-inflammatory effect of *C. sadleriana* which may play role in the ethnomedicinal application of the plant. **Acknowledgements:** The financial support of the Hungarian Scientific Research Fund (OTKA PD 71724) is gratefully acknowledged. **References:** 1. Csupor, D. et al. (2010) J Etnopharmacol 127: 193 – 195.

P116 Phytochemical investigations of *Piper sarmentosum* and *Zanthoxyllum gilletii*

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In the course of our work on anti-inflammatory herbs we investigated extracts of *Piper sarmentosum* Roxb. (syn. *Piper lolot* C. DC; Piperaceae) and *Zanthoxyllum gilletii* (De Wild.) Waterman (syn. *Fagara macrophylla* (Oliv.) Engl.; Rutaceae). Extracts of *Piper sarmentosum* are used in traditional Thai and Chinese medicine for the treatment of toothache, oedemas, fever, common cold and rheumatism. [1] In African and traditional Chinese medicine *Zanthoxyllum gilletii* is applied for the treatment of rheumatism, headache, stomach-ache, and toothache. [2] The dichloromethane extracts of these plants were subjected to phytochemical analysis by combining different chromatographic means like LC, SPE and prep HPLC. A variety of secondary constituents could be isolated and

was structurally elucidated using UV, 1D (^{1}H, ^{13}C) and 2D NMR (DQF-COSY, HMBC, HSQC) experiments and mass spectroscopy. From *Piper sarmentosum* extracts of both, herba and radix et caulis, were prepared. Among the isolated compounds pyrrole and pyrrolidine amides, phenolic compounds, and a new ester of 3-(4′-methoxy-phenyl)-propionic acid were identified. The investigation of the dichloromethane extract of the bark of *Zanthoxyllum gilletii* revealed the presence of aromatic amides like pyrrole amide (e.g. fagaramide), alkylamides, lignans (e.g. sesamin) and coumarins. **References:** 1. Li, C.-Y. et al. (2007) J. Agric. Food Chem. 55:9436 – 9442. 2. Wansi, J.D. et al. (2009) Planta Med. 75:517 – 521.

P117 Ethnobotanical study of plants used in managing depression within Lagos metropolis Nigeria

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Antidepressant drugs continue to raise concerns about their side effects, which include suicide, clinical worsening of depression, and unusual changes in behavior of adolescents and children. Recently, the FDA instructed all drug manufacturers to add black box warnings (the most serious warning label for a prescription medicine) to their antidepressant drugs. In light of these findings, doctors and patients are seeking safer alternative therapies. An ethnobotanical survey of medicinal and poisonous plants was conducted amongst herb sellers in major open markets in Lagos metropolis, Nigeria. Data was collected by the authors using classical descriptive ethnobotanical techniques (i.e. no quantitative measures) through an unstructured open ended interview. A total of 48 ethnomedicinal plant species distributed in 12 families are documented in this study. The medicinal plants used are listed with Latin name, family, local name, parts used, mode of preparation and duration of treatment. The majority of the remedies were prepared from freshly collected plant material from the wild. They are mainly taken orally as single plants, but some applications were prepared with a mixture of plants or ingredients such as honey, salt, palm oil and pepper. Decoction of the leaves was the main form of preparation (68%) and leaf powder was mostly used for the preparation of infusions (16%). The need for the use of stem and root barks increases when the leaves are not available. These plants can serve as sources of new alternatives for managing depressive symptoms through exploitation of novel pharmacological agents.

P118 Adaptogenic and central nervous system effects of *Potentilla alba* L extract in mice

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Potentilla alba L. (Rosaceae) rhizomes are used in Russian and Ukrainian folk medicine to reduce the thyroxin level in blood plasma as an important drug against thyroid gland diseases. The preparation shows anti-inflammatory, antioxidant, and adaptogenic effect [1,2]. *P. alba* contains a significant amount of proanthocyanidins, procyanidins, polyphenolics, some flavonoids, polysaccharides etc. [3]. The data about adaptogenic and central nervous system (CNS) activities of *P. alba* are fragmentary. The adaptogenic and CNS effects of *P. alba* were examined in the swimming to exhaustion, light/dark exploration, and open field tests on mice. The treatment groups were orally administered dry extract of *P. alba* (in doses of 0.3, 0.9 and 1.8 mg/kg), control group received distilled water. The swimming time after 7 days of treatment was increased by 3.3 – 5.5 fold. Minimal glycogen level was observed in the group treated by 0.9 mg/kg *P. alba*. It was estimated that glycogen was hydrolyzed to glucose, glucose level was increased in the blood plasma, and lactic acidosis was prevented by aerobic oxidation of glucose (Table 1).

Table 1: Results of fswimming test in mice

Group	Swimming time, s	Glucose, mmol/L	Lactate, mmol/L	Glycogen in liver, mg/g
Control	730 ± 90	6.2 ± 0.5	5.8 ±.0.5	0.61 ±.0.15
P. alba, 0.3 mg/kg	2393 ± 371*	2.5 ± 0.4*	6.1 ± 0.8	0.18 ±.0.05*
P. alba, 0.9 mg/kg	4015 ± 795*	4.9 ± 0.8	4.9 ±.0.4	0.12.± 0.06*
P. alba. 1.8 mg/kg	2597 ± 572*	2.8 ±.0.4*	5.6 ± 0.6	0.20 ±.0.10*

A dose dependant tendency for increased time spent in the light compartment (light/dark exploration) in the treated groups was observed. However, no changes were found in the number of entries into each compartment. Statistical analysis revealed that in the open field test the extract at a dose of 0.3 mg/kg induced a significant increase in locomotor activity but alterations in the anxiety of the treated mice were not marked. **References:** 1. Smyk. K.G. (1982) Chimia I Zhizn 3: 15 – 17. 2. Tomczyk. M. Latte. K.P. (2009) J. Ethnopharmacol. 122: 184 – 204. 3. Shikov. A.N. et al. (2009) Book of abstracts of 57 GA Congress: A5.

P119 Azadirachtin-rich Neem extracts against ectoparasites

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Leaves, seeds, bark and other parts of the Neem tree *Azadirachta indica* A. JUSS have been used in traditional medicine in India (Ayurveda, Unani and Homeopathy)[1] since centuries against different diseases especially skin disorders and ectoparasites like pediculosis, scabies, fleas, ticks etc. With respect to these experiences a standardised method for extracting neem seed kernels was developed and patented. Currently the ingredients of this extract called NeemAzal® are characterized and Azadirachtin A is found as the main ingredient and analytical leading substance.

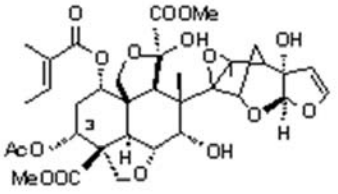

Fig. 1: Azadirachtin A

Investigations show a favourable toxicological profile of NeemAzal® (acute oral toxicity in rat LD 50 > 5000 mg/kg bw, acute dermal toxicity in rabbit LD 50 > 3000 mg/kg bw; NOAEL 3.7 mg/kg bw/d)[2], good compatibility and effectiveness. Different standardised formulations are available and examined: – Sheep and goats are successfully treated with NeemPro®-Sheep (in accordance with Biocide Directive 98/8/EC and approved for organic farming) against Anopheles spec., Culicoides spec., Damalinia spec.[3,4], Wolfarthia magnifica). – Ectoparasites can be controlled effective by ContrAcar®Pet (in accordance with Biocide Directive 98/8/EC and approved for organic farming) in the pet surrounding. – Bernauer-Jacob reported NeemPro®-Dog kills adult fleas (Ctenocephalides felis) on dogs at doses of 83 mg AzA/kg bw and stops completely the egg production of adult fleas.[5] – The good compatiblity[6] for humans of NeemExtract-FT shampoo was shown by a safety assessment including stability, physico-chemical and bacteriological tests, Het Cam Test and a 48 hour Patch-test with the finished product. In Italy a registration procedure for marketing authorisation (Presidi Medico Chirugici) is ongoing. **References:** 1. Ketkar, A.Y. et al. (2002) The Neem Tree. Schmutterer, H. Mumbai. 2. Niemann, L. (2002) The Neem Tree. Schmutterer, H. Mumbai. 3. Habluetzel, A. (2006) Vet Parasitol 144(3 – 4): 328 – 337. 4. Guerrini, V.H. (2000) OJVR 4: 133 – 138. 5. Bernauer-Jacob, V. (1999) Orientierende Untersuchungen zur Wirksamkeit von NeemAzal auf den Katzenfloh Ctenocephalides felis felis (Bouché) und den tropischen Rattenfloh Xenopsylla cheopsis (Rothschild), Berlin. 6. Saboureau, D. (2006) Safety Assessment for human health's safety evaluation of a cosmetic product, unpublished.

P120 Quantification of some phenolic acid and rutin in the leaves of rosemary from Turkey and Croatia

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Rosemary contains a number of potentially biologically active compounds, such as carnosic acid and rosmarinic acid (RA), camphor, caffeic acid (CA), ursolic acid, betulinic acid, rosmaridiphenol and rosmanol. OBJECTIVES: In this study, using HPLC-ED system, analysis of gallic acid

(GA), RA, CA, and rutin was carried out in water extracts of rosemary. **Methods:** Analyses of GA, RA, CA and rutin were performed of leaves rosemary. The drug (1 g) was powdered and extracted with pure water (9 ml). Afterward 1 ml of that extract was decanted and centrifuged. Supernatant was used for analysis. The standard solutions were of GA, RA, and CA dissolved in mobile phase, and rutin was dissolved pure water. HPLC conditions were following: Mobile phase methanol-acetonitrile-water-acetic acid (20+10+70+1); ED detector with range 50nA, potential +0.840 V, filter 0.02 Hz; flow rate 1 ml/min; temperature 25 °C, Column: ODS hypersil. **Results:** The content in (mg/g) of GA was in the rosemary from Turkey 1.54, RA 11.31, CA 1.12, and rutin 2.45. The content in (mg/g) of GA was in the rosemary from Croatia 0.77, RA 14.62, CA 0.96, and rutin 1.61. **Conclusion:** The highest content of RA was found in leaves of rosemary from Croatia, and highest content of GA, CA and rutin was found in leaves of rosemary from Turkey.

P121 Traditional medicine in Banat region (Romania): results of an ethnobotanical survey

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Empirical knowledge about the medicinal properties of plants is a fundamental source for drug discovery. Due to urbanisation and industrialization, orally transmitted folk knowledge on medicinal plants (MPs) is dramatically decreasing in most European populations. This situation is bound to affect profoundly the scientific community interested in natural products, and demands written preservation of traditional knowledge. We aimed to contribute to this task by documenting the MPs preparations and usages from Banat county (Western Romania). Data were collected in the small town of Buzias, based on spontaneous citation of plant species, followed by structured interviews following predesigned questionnaires. Nineteen members of the local community were interviewed. Records were made of the main usages, extractive process, doses, and degree of contentment with the results of the treatment. The results of the ethnopharmacological fieldwork were interpreted by means of semi-quantitative methodologies. We employed the Use Value (UV) method and the Corrected Percentage of Agreement related to the Main Uses (cAMU), with the purpose of quantifying the importance of medicinal species and families [1]. Thirty species, belonging to 21 families were identified. Infusion (70%) and maceration (16%) are the most frequent extractive methods; macerations employed water, petroleum, lard, and water-ethanol mixtures. Species with the highest cAMU values are used to treat digestive disorders, skin lesions, diabetes, rheumatism and hypertension. Particular attention was drawn to the stems of *Cuscuta* sp., successfully used to treat rheumatism (as petroleum macerate). This natural product may inspire further research in the quest for new anti-inflammatory compounds. **References:** 1. Phillips O., Gentry A.H. (1993) Economic Botany 47: 15 – 32.

P122 Organic production of German chamomile (*Matricaria recutita* L.) intercropped with pot marigold (*Calendula officinalis* L.)

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Effects of organic production of German Chamomile intercropped with Pot Marigold, on agronomic criteria and chemical composition of German Chamomile was studied in a split plot design with three applications. Treatments were four levels of animal manure (0, 30, 40 and 50 tons/ha) as main plots and five seeding ratio of German Chamomile with Pot Marigold (100:0, 75:25, 50:50, 25:75 and 0:100) as sub plots. Results showed that animal manure has no effects on total dry matter of *Matricaria chamomilla*. However with decreasing proportion of seeding rates of German Chamomile in the mixture, leaf area, total dry matter yield and seed yield of German Chamomile was reduced. Chamazulene content of essential oil was affected by leaf area and seed yield. Seeding ratios of 50:50 or 25:75 were the most promising seeding rates in terms of Chamazulene content. **References:** 1. Emonger, V.E. et al. (1989) Discovery and Innovation 1: 18 – 25. 2. Hornok, L. (1992) Cultivation and processing of medicinal Plants. Academic Pub. Budapest. 3. Emonger, V.E. (1988). Effects of nitrogen and phosphorus on growth, yield of flowers and essential oil of camomile grown under Kenya conditions. M.Sc. Thesis, Faculty of Agriculture, University of Nairobi. 4. Kuepper, G. (2000). Manure for organic crop production. ATTRA, Fayetteville, AR 72702. Available Online (July 2004): www.attra.org/attra-pub/manure.htm. 5. Wallace, J. (2001) Organic Field Crop Handbook. Pub. Canadian Organic Growers. Ottana, Ontario.

P123 Ethnopharmacological investigation of different parts of *Perovskia abrotanoides* Karel.

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Perovskia abrotanoides Karel belongs to the Lamiaceae family, local name "Visk", and is one of the most important medicinal herbs growing wild in the mountainous road of Golestan and North Khorasan Province. It has been used by the rural people in traditional medicine to treat several ailments. In this research the flowering aerial parts of the plant were collected in two natural habitats (1000 and 2300 m). Ethnopharmacological data were obtained from rural healers and sheepers, due to its important ecological effects and its effects as a tonic, antiseptic, anti-inflammatory rheumatic pain, to treat of leishmaniasis, especially in combination with *Artemisia sieberi*, *Thymus carmanicus* and *Artemisia annua*. The contents of flavonoids, total phenol and anthocyanins were increased at altitudes of 2300 m in Golestan province. These findings confirm the rural use of this plant to treat local ailments, especially as sedative, to expel worms and as an antileishmaniasis treatment.

P124 The most useful herbs of Iranian traditional medicine prescribed in xerosis

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Xerosis (dry skin) is an irritating skin condition affects almost everyone during lifespan. An abnormality of the moisture and oil balance of the dermis layers that caused by general dehydration, atopic dermatitis, Vitamin A deficiency, or diabetes is what happens in this condition. Xerosis is a well known disorder in Iranian traditional medicine (ITM). Avicenna and some other famous Iranian traditional physicians described this condition in their manuscripts precisely. Treatmens which were used by Iranian scholars included pharmacotherapy (herbal or animal), diet therapy, and changing lifestyle. Five Iranian ancient medical texts i.e. Canon of Medicine (Avicena 980 – 1037), alhavi (Razes 865 – 925AD)Tohfat ul- Mo'menin (Mo men tonekaboni), Makhzan ul-Advia (Aghili), and Ekhtiarat Badi'i (Ansari 1329 – 1404 AD) were studied for anti- xerosis herbal medicines (1 – 4). The main anti-xerosis herbal medicines used in ITM were scored based on the frequency of their prescriptibility. Sesamum indicum, Vicia ervilia, Urginea maritima, Ficus carica, and Olea europea were the most frequent herbs mentioned in ITM prescriptions. Due to the prevalence of xerosis, finding new and safe remedies for dry skin is favorable. Among the herbs ment ioned above, olive oil (olea europea) is now used in some moisturizing products (5) to cure xerosis. Based on our findings we intend to introduce new natural products of anti-xerosis by caring out evidence based researches on other herbs mentioned in ITM text book in close future. **References:** 1. Avicena.cano n(5)195.canon(2). 2. Tohfat ul- Mo'menin. 3. Makhzan ul- Advia 4. Ekhtiarat Badi'i. 5. Kiechl-Kohlendorfer U, Berger C, Inzinger R.Pediatr Dermatol. 2008 Mar-Apr;25(2):174 – 8.

P125 Long-term follow-up of patients with phytotherapy as only causal treatment for malignant and benign tumors

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Purpose: To determine the long-term clinical course of patients with advanced cancer and benign brain tumors with expansive growth (meningiomas) who achieved remission with angiosperms from arid regions of northern Mexico that show antineoplastic activity [1] [2] [3]. **Patients and Methods:** We evaluated the efficacy and safety of several angiosperms as unique causal treatment in patients with malignant and benign tumors by clinical monitoring from 2005 to 2009 [4]. To assess the long-term prognosis, we reviewed our experience with the oral administration of aqueous plant extracts. Characteristics of long-term survivors were evaluated, and hazard rates for progression were calculated. **Results:** Cases: A 42 -year-old woman with papillary thyroid carcinoma and cervical nodal metastases, evolved without evidence of malignant growths. Two female patients, 71 and 72 years of age, with meningioma of the brain, developed calcified meningioma after phytotherapy. A female, 38 years of age, with invasive cervical squamous cell carcinoma with metastases in abdominal cavity, her condition improved. A 44-year-old male, with tumor of clear cells in the right kidney with lung metastases, his condition and survival improved for several years. A 96-year-old male with bladder transitional cell carcinoma evolved after phytotherapy with no signs of tumor. A female patient, 47 years of age, previously treated with chemotherapy and mastectomy for end-stage breast carcinoma with multiple lung metastases, have presented obvious improvement after phytotherapy. **Conclusion:** Patients with advanced cancer and benign brain tumors, achieved remission and improvement with the oral administration of aqueous mixture of plants with antineoplastic activity. No side effects were observed. **References:** 1. López-MCA, et al. (2000, Oktober 18 – 20). International Symposium: Oncology. Schliersee, Deutschland. 2. López-MCA, et al. International PSE Symposium on Natural Products in Cancer Therapy 23 – 26 September 2008 Naples, Italy http://www.phytochemicalsociety.org/naples. 3. López-MCA, et al. 5a Reunión Nacional de Investigación en Productos Naturales 28 al 31 de Mayo de 2008 Guadalajara Jal. México. 4. López-MCA, et al 57th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research Geneva 16th to 20th August 2009. 5. WHO Traditional Medicine Strategy 2002 – 2005 WHO/EDM/TRM/2002.1 Original: English Distribution: General. World Health Organization Geneva.

P126 Content of total polyphenols, tannins and flavonoids in *Epilobium* species growing in Estonia

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Epilobium species (Onagraceae) are commonly used as herbal remedies in traditional, adjuvant therapy of benign prostate hyperplasia; the most well known species in ethnomedicine is *E. parviflorum* Schreb. [1]. Extracts of different *Epilobium* species (*E. rosmarinifolium* Haenke, *E. spicatum* Lam., and *E. tetragonum* L.) inhibit proliferation of prostate cells in a nonspecific manner [2]. *E. angustifolium* L. extracts may be a potential remedy in diseases connected with the disturbed metabolism of signaling peptides [3]. Six *Epilobium* species (Table 1) growing in Estonia were collected in 2007 from South-Estonia (Otepää parish) and, after drying, separated into leaves, flowers, stems, and roots. The quantitative content of total polyphenols, tannins, and flavonoids, determined spectrophotometrically as described in [4], was rather similar in the leaves of all six *Epilobium* species (Table 1). The flowers contained more tannins (up to 32.3%) and flavonoids (up to 0.58%) than the leaves. The tannin and flavonoid content in stems and roots was rather low. All analysed species contained practically the same amount of polyphenols in all investigated plant parts. Since *E. angustifolium* grows widely throughout Estonia, it can probably be used as a substitute for *E. parviflorum*. Thus, it appears necessary to continue this work using HPLC, analysing the content of individual biologically active compounds, especially the amount of oenothein B in different *Epilobium* species of Estonian origin.

Table 1: Content (%) of total polyphenols, tannins, and flavonoids in leaves of *Epilobium* species

Phenols	E. parviflorum Schreb.	E. hirsutum L.	E. montanum L.	E. palustre L.	E. ciliatum Raf. (syn. E. adenocaulon Hausskn.)	E. angustifolium L.
Polyphenols	10.6 ± 0.8	19.3 ± 1.5	10.3 ± 0.7	11.9 ± 0.9	11.0 ± 0.8	6.3 ± 0.5
Tannins	18.2 ± 1.8	21.7 ± 2.1	16.1 ± 1.6	17.9 ± 1.8	19.4 ± 1.9	16.0 ± 1.6
Flavonoids	0.32 ± 0.02	0,29 ± 0.02	0.30 ± 0.02	0.46 ± 0.02	0.69 ± 0.04	0.24 ± 0.01

References: 1. Toth, B.H. et al. (2009) J. Pharm. Biomed. Anal 49:26 – 31. 2. Vitalone, A. et al. (2003) Pharmacology 69:79 – 87. 3. Kiss, A. et al. (2006) Pharmazie 61:66 – 69. 4. Raal, A., Jürgenson, S. (2009) Eur. J. Pharm. Sci. 38/1S:152 – 153.

P127 Anti-inflammatory activity and toxicity of the standardised water extract of *Phyllanthus emblica* L.

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Phyllanthus emblica L. is an herbal plant commonly used in Asian traditional medicine. Its fresh or dry fruits were reported as an alternative treatment of diarrhea, jaundice, and inflammatory disorder [1, 2]. The aim of this study was to investigate anti-inflammatory activity and toxicity of a standardised water extract of *P. emblica* fruits prepared according to Thai Herbal Pharmacopoeia. Its anti-inflammatory activity was tested in rats using carrageenan-induced paw edema and cotton pellet-induced granuloma models. Acute (5,000 mg/kg) and chronic oral toxicities (300, 600, and 1,200 mg/kg) were also evaluated in rats. Oral administration of*P. emblica* extract at the doses of 150, 300, and 600 mg/kg caused dose-dependent inhibition of carrageenan-induced acute inflammation (Table 1). In chronic inflammation, *P. emblica* (600 mg/kg) did not reduce both transudative and proliferative phases, body weight gain and thymus weight in cotton pellet-induced granuloma formation (data not show). The pharmacological mechanism of activity of the standardised water extract of *P. emblica* seems to be more similar to NSAIDs rather than to steroidal drugs. Inhibitory effect on the synthesis and/or release of inflammatory mediators, especially prostaglandins, may be the main mechanisms of action of *P. emblica* water extract. In addition, *P. emblica* water extract did not produce acute (LD 50 > 5,000 mg/kg) and chronic oral toxicity. The extrapolation of these results to humans suggests that *Phyllanthus emblica* L. water extract should be acceptably safety level for usage at the doses of 300, 600, and 1,200 mg/kg/day. **Acknowledgements:** Royal Golden Jubilee Ph.D. Program and the National Research Council of Thailand **References:** 1. Santisuk, T. et al. (2005) Floral of Thailand. Vol. 8 Part 1 (Euphorbiaceae). Prachachon. Bangkok. 2. Khan, KH. (2009). Bot Res Intl 2(4): 218 – 28.

P128 Cytotoxicity of some Nigerian plants used in traditional cancer treatment

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The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Five plants-[Sapium ellipticum Hochst. ex Krauss Pax (Euphorbiaceae), Combretum paniculatum Vent. (Combretaceae), Celosia trigyna L. (Amaranthaceae), Drymaria cordata (L.) Willd. ex Roem. & Schult. (Caryophyllaceae) and Cyathula prostata (L.) Blume (Amaranthaceae)] are used traditionally in the treatment of cancer [1]. The ethanolic extracts of the plants were evaluated for cytotoxic activity using the MTT assay on the colon (HT29) and breast (MCF-7) cancer cell lines at concentration ranging between 125 – 500 µg/ml to validate the ethno-

medicinal uses and compare activities [2]. The percentage inhibition of the extracts in the MCF-7 cell line were in order of Sapium (74%) > Cyathula (48%) > Celosia (30%) > Combretum (28%) > Drymaria (22%) at 500 μg/ml respectively. Sapium showed inhibition comparable to the reference compound Cisplatin. However, all the plants showed less than 50% inhibition at 500 μg/ml in the HT29 cell line. The results showed that Sapium showed greater cytotoxic activity than all the plants tested and this validates the traditional use of the plant. **References:** 1. Burkill, H.M. (1994). Useful Plants of West Tropical Africa. Royal Botanic Gardens, Surrey. 2. Mossman, T. (1983). J. Immunol. Methods 65: 55 – 63.

P129 Immunomodulatory and antioxidant constituents of Eastern Nigeria mistletoe, *Loranthus micranthus* Linn. (Loranthaceae) parasitic on *Cola acuminata* Schott et Endl.

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Our recent reported data established mistletoe harvested from Cola acuminata Schott et Endl. as the most potent in terms of immunostimulation [1, 2]. Further bioassay-guided fractionation of the crude extract showed potency in the order of chloroform fraction > > ethylacetate fraction > n-hexane fraction [3]. Our continued efforts to isolate and characterize these active constituents led to the isolation of nine major compounds, epimeric β-carotenoids and eight lipophilic fractions. Two notable steroids, 5α-16, 16-dimethyl-androstan-17-one and 6β-hydroxy-17-oxo-4, 5-secoandrostan-4-oic acid were identified in the lipophilic fractions. A novel sesquiterpene-like compound; 2, 3-dimethoxy-benzo [a, b] cyclopentenyl-31, 31, 51-trimethyl pyran-4-carboxylic acid and an alkaloid, CFO were isolated from the chloroform fraction. Stigmast-7, 20 (21)-diene-3β-hydroxy-6-one, 3β-Hydroxy- stigmast- 23-ene (Stigmast-23-ene-3β-ol), 21β-hydroxy- 5, 9(10), 24-triene-21-nor-dammaren-3-one, lupeol and other steroids, HF4, HF6, HF7 were isolated from the n-hexane fraction while dibutyl phthalate and a flavonoidal EA2 were confirmed in the ethylacetate fraction. All isolated compounds were subjected to cell proliferation studies using cell line (C57Bl/6 splenocytes) and flow cytometry techniques against Lipopolysaccharide and Concanavalin A standards. The results showed that of all the major compounds isolated; only CFO, EA2 and HF7 exhibited immunostimulatory activity. Specifically, EA2 and CFO were the most potent with activity index of 91.49 ± 0.22% and 69.84 ± 0.19% respectively compared to 34.01 ± 0.32% recorded for the two standards. The antioxidant potentials of these compounds showed that EA2 was most potent with an effective concentration (EC 50) value of 55.42 ± 0.99 mg/ml against ascorbic acid value of 17.6 ± 1.78 mg/ml. **Acknowledgements:** The authors acknowledge Mr. A. Ozioko of BDCP, Nsukka, Enugu State Nigeria for providing the plant material. **References:** 1. Osadebe PO, et al (2009)J Ethnopharmacol., 125:287 – 293. 2. Osadebe et al (2008), RPRM Vol 27: 473 – 485. 3. Omeje EO et al (2009) Asian Pacific J.Tropical Med., 2(4): 11 – 18.

P130 Anthraquinone content and toxicity test of *Cassia fistula* pod extracts

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Cassia fistula Linn. (Caesalpiniaceae) can be easily found in all parts of Thailand as an ornamental plant and its ripe pods are almost treated as a waste. In Thai traditional medicine, the ripe pods of *C. fistula* have long been used as a laxative drug. They contain several anthraquinones such as rhein, aloe-emodin and sennosides [1], These anthraquinones promote laxative effect of which their glycosides contents indicate the laxative potency [2]. In this study, the ripe pods of *C. fistula* were collected from 10 different provinces of Thailand. The pod pulp was separated and extracted with distilled water by decoction. The water extracts were determined for the contents of total anthraquinones and total anthraquinone glycosides by a UV-vis spectrophotometric method at 515 nm and the acute toxicity of the extracts was investigated in mice and rats. Extract ratio (crude drug: 1 g crude extract) of all the extracts are 1 – 2: 1. The contents of total anthraquinones and total anthraquinone glycosides in the extracts calculated as a major anthraquinone rhein were 1.45 – 1.85% w/w (average 1.63% w/w) and 0.38 – 0.71% w/w (average 0.53% w/w), respectively while in the fresh pod pulp contained 0.89 – 1.03% w/w (average 0.94% w/w) and 0.22 – 0.39% w/w (average 0.31% w/w), respectively. After tested mice and rats were administered with the extract at dose level 5 g/kg, no mortality or any sign of toxicity were found within 14 days. The body weight gain of all animals was not significantly different between the treated groups and the respective control group. This indicats that *C. fistula* pod extract was grouped in a slightly toxic (LD 50 > 5 g/kg). In Thai traditional medicine, the fresh pod pulp 4 – 8 g has been used as a dose for laxative drug. This dose amount was equal to 37.6 – 75.2 mg of total anthraquinones and 12.4 – 24.8 mg of total anthraquinone glycosides. From our preliminary study, it suggests that the decoction extract of *C. fistula* pod might be used as an alternative source of natural laxative drugs and should be further developed as a modern pharmaceutical laxative preparation. **References:** 1. Indian Council of Medical Reseach (2005) Quality standards of Indian medicinal plants Vol.2, Indraprastha Press (CBT), New Delhi: 47 – 53. 2. Bruniton J (1995) Pharmacognosy, Phytochemistry, Medicinal plants, Lavoisier Publishing, Paris: 349 – 354.

P131 Antigenotoxic study of rice extracts from Thai Sung-Yod red rice cultivar in human lymphocytes *in vitro*

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The antigenotoxic effect of rice extracts from Sung-Yod red rice cultivar produced in Thailand have been investigated in human lymphocytes for a sister chromatid exchange (SCE) assay *in vitro*. Pretreatment of 2 different fractions of the rice extracts, the rinsed water fraction (SYGW) and the aqueous extract of freeze dried boiled rice bran (SYBBOIL) at the concentrations of 6.25 – 100 μg/ml alone for 2 h followed by 0.1 μg/ml doxorubicin as chemotherapeutic agent for 2 h could not significantly reduce SCE level induced by doxorubicin ($p < 0.05$). Our data indicated that rice extracts in the form of SYGW and SYBBOIL fractions can not protect human lymphocytes from genotoxic damage induced by 0.1 μg/ml doxorubicin. In conclusion, our Sung-Yod rice extracts in the form of SYGW and SYBBOIL have no antigenotoxic potential against doxorubicin. However, their antigenotoxic potential is further investigated using other genotoxic compounds to investigate its benefit as herbal medicine. **Acknowledgements:** This study was supported by Research Fund, National Research Council of Thailand (NRCT) **References:** 1. Phutthaphadoong, S. et al. (2010) Oncol.Rep. 23: 53 – 59. 2. Kannan A. et.al. (2008) J Agric Food Chem 56:11643 – 7. 3. Nam, SH. et al. (2005)J Agric Food Chem. 53:816 – 22.

P132 Chemical composition and antioxidant activity of essential oil and aqueous extract of *Pelargonium graveolens* L'Her.

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Pelargonium graveolens L'Her is an aromatic, rose-scented herb which is indigenous to various parts of southern Africa, and nowadays cultivated worldwide. The whole plant has relaxant, anti-depressant and antiseptic effects, reduces inflammation and controls bleeding. The essential oil from the leaves is used in aromatherapy and is also applied locally to cervical cancer [1]. This work presents the first phytochemical investigation of *P. graveolens* of Bosnian origin. The essential oil as well as waste water after hydrodistillation from stems and leaves of *P. graveolens* was studied. The volatile profile of odorous parts of the plant was analyzed by GC/MS. More than eighty compounds were identified in both samples, representing 92.3% and 96.4% in total, for essential oil

obtained from the leaves and stems, respectively. The major compounds in essential oils were oxygenated monoterpenes (64.3 – 74.2%), with geraniol (27.5 – 50.2%) and citronellol (14.2 – 19.0%) as the main representatives. Our results are comparable with those found in the literature [2 – 3]. The content of phenolic compounds in a water extract was examined by a slightly modified Folin-Ciocalteu method [4] and was 34.88 ± 2.00 mg/g GAE in leaves and 102.44 ± 1.63 mg/g GAE in stems including flavonoid compounds of 32.35 ± 0.81 mg/g to 101.87 ± 1.03 mg/g GAE. The radical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [5]. Scavenging activities indicated as IC_{50} values ranged from 0.19 ± 0.05 mg/mL (stems) to 0.39 ± 0.04 mg/mL (leaves) for essential oils, and from 63.70 ± 1.56 mg/mL (leaves) to 64.88 ± 1.12 mg/mL (stems). Our findings are in agreement with those found in literature [6]. **References:** 1. Bown. D. (1995) Encyclopedia of Herbs and their Uses. Dorling Kindersley, London. 2. Shellie, R.A., Marriott, P.J. (2003) Analyst 128:879 – 883. 3. Gomes, P.B. et al. (2007) J. Supercrit. Fluids 41:50 – 60. 4. Singleton, V.L., Rossi, J.A. (1965) Am. J. Enol. Vitic. 16:144 – 158. 5. Brand-Williams, W. et al. (1995) Lebensm. Wiss. Technol. 28:25 – 38. 6. Sun, W. et al. (2005) J. Chinese Med. Materials 28:87 – 89.

P133 Determination of phenolic compounds, flavonoids, and antioxidant activities in crude water extracts of Thai red and white rice cultivars

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Pigmented rice varieties have been shown to exhibit strong antioxidant activity and to reduce oxidative stress implicated in various chronic diseases [1, 2]. The antioxidant activities of the water extract of brown rice or bran from two Thai rice cultivars: (i) Sang Yod, a red pigmented rice typically grown in Southern Thailand, and (ii) Dawk Mali 105, a commercial white-colored rice, were evaluated through chemical assays: DPPH radical-scavenging and inhibition of lipid peroxidation assays, as well as through cell-based assays: scavenging capacity of intracellular ROS in HL-60 cells using the fluorescent DCF and the NBT reduction. These assays have their specific mechanisms in detecting a particular mode of antioxidant actions. In the two chemical assays, all the rice extracts showed free radical scavenging and free radical chain breaking activities with EC_{50} values ranging from 26 to 357 µg/ml. Moreover, the cell-based assays detected ROS scavenging activities of these extracts with EC50 values in the range of 0.6 – 5 mg/ml. All these assays indicated that the water extracts of Sang Yod exerted significantly higher antioxidant activity than those of Dawk Mali 105, which exhibited only moderate to low activity. Furthermore, high levels of antioxidant activity of the water extracts of Sang Yod were closely correlated to their flavonoid and phenolic contents, which were approximately 2.5 and 3 times higher, respectively, than those of Dawk Mali 105. **References:** 1. Higashi-Okai, K. et al. (2008) J Uoeh. 30(4):37589. 2. Xia, M. et al. (2003) J Nutr. 133(3):74451.

P134 Anti-inflammatory diterpenoids and other secondary metabolites from *Dodonaea polyandra*; a traditional medicine used by Kaanju people in North Eastern Australia

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Collaborative research guided by the traditional Indigenous knowledge of Northern Kaanju people, Cape York Peninsula, Australia has recently led to the isolation of four new clerodane diterpenoids from the leaves of Dodonaea polyandra (Sapindaceae). These compounds have shown high levels of anti-inflammatory activity in a TPA mouse ear oedema model of inflammation. Dose-response studies revealed compound CS004 (below) was the most potent of these displaying a linear dose-response relationship.

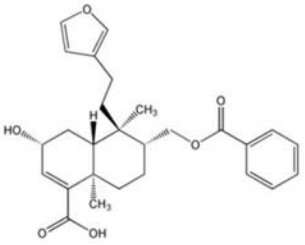

Fig. 1: CS004

Percentage inhibition of oedema for this compound ranged from 44 to 85% over the dose range 0.022 to 1.77 µmol. The maximum percentage of 85% was comparable at an equimolar dose to the known synthetic steroid betamethasone (90%). The dose-response profiles of the other diterpenoids isolated which are structural analogues of CS004 were also evaluated, however the dose-response characteristics of these compounds were found to be non-linear, with a tendency towards being U-shaped. Additional chemical investigations of the same plant revealed the presence of some flavonoid constituents. These findings add Western scientific evidence supporting the traditional use of the plant in traditional Northern Kaanju medicine.

P135 Inhibitory effect of the Thai rejuvenating medicinal plant in acetylcholinesterase activity

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Twenty-two ethanolic rejuvenating medicinal plant extracts were investigated for acetylcholinesterase (AChE) inhibitory activity using Ellman's colorimetric method in 96-welled microplates. Results indicated that four sample; *Stephania erecta* 1 (tuber), *Stephania venosa* 2 (tuber), *Tinospora crispa* 3 (stem) and *Kaempferia parviflora* 4 (tuber) exhibited the strong potent AChE inhibitory activity. At 0.1 mg/ml plant extracts, the percentage of inhibition values obtained for these extracts were 97.16, 93.36, 72.81 and 70.29, respectively. The results demonstrated that 4 is a plant with strong AChE inhibitory activity present in the traditional Thai remedies for rejuvenating purposes. **Acknowledgements:** The authors thank the Office of Research and Academic Service, Suan Sunandha Rajabhat University, Department of Biology and Faculty of Pharmaceutical Sciences, Naresuan University for grant support. **References:** 1. Ingkaninan, K. Temkitthawon, P., Chuenchom, K., Yuyaem, T., Thongnoi, W. (2003). Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J. Ethnopharmacol 89: 261 – 264. 2. Vinutha, B. et al. (2007). Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J. Ethnopharmacol 109: 359 – 36.

P136 Correlation of cytotoxicity, antioxidant activities, gamma-Oryzanol content and extraction methods of Hommali 105 rice bran (*Oryza sativa*)

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The objectives of this research are the investigation for the correlations of cytotoxicity against cancer cells, antioxidant activity, gamma oryzanol and extraction methods of Homali 105 rice bran (*Oryza sativa*) extracts. Sulphorhodamine (**SRB**) assay [1] was used to test cytotoxic activity against four human cancer cell lines: lung (**CORL23**), cervical (**Hela**), prostate (**PC3**) and breast (**MCF-7**) cancer cell lines and one normal human cell line (MRC5)[1,2]. DPPH assay [3] was carried out for antioxidant activity and gamma oryzenol content was determined by HPLC [4]. The methods of extraction were maceration by ethanol 95% (**ME**)

supercritical fluid extraction (**SFE**), boiling in water and dry by freeze dry (**BF**), expression method (**EX**) and soxhlet extraction method by using different solvent as hexane, chloroform and methanol (**SXH**, **SXC** and **SXM** respectively). The results found that **BF** showed the highest percentage of yield (21.73%). **SXC** showed the highest percentage of selective cytotoxicity against only human cancer cells dependent on hormone at a concentration of 100 µg/ml such as PC3, followed by Hela and MCF-7 (46.9, 29.6 and 21.58 respectively) but it had no cytotoxic activity against both COR-L23 and MRC5. **ME** method gave the highest gamma oryzenol content followed by **EX** and **SXH** (31.64, 27.90 and 25.88 mg/g of extracts, respectively). There were no correlations between gamma oryzanol content and cytotoxicity, antioxidant activities and the methods of extraction. However, these results are very useful for the selection of the extraction method of rice bran hommali 105 extracts for treatment of cancer patients especially with types of cancer dependent on hormones. **Keywords:** Hommali 105 rice bran, *Oryza sativa*, the extraction, cytotoxicity, SRB, Gamma Oryzanol, HPLC. **Acknowledgements:** Faculty of medicine, Thammasart University and National Research Council of Thailand for financial support **References:** 1. Itharat, A. et al. (2004) J. of Ethnopharmacology: 90:33 – 38. 2. Skehan P et al.(1990) J Natl Cancer Inst 1990: 82(13): 1107 – 12. 3. Yamazaki K, et al (1994) Chem Pharm Bull 1994; 42:1663 – 5. 4. Chotimakorn, C et al. (2008). Food Chemistry, 111, 636 – 641.

P137 Antioxidant activity of two *Satureja* species

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Satureja is a genus of the well-known medicinal plants of Lamiaceae family that comprises numerous species growing wild in Mediterranean area. *Satureja visianii* Šilic is a stenoendemic species while *Satureja montana* L., is a well known aromatic and medicinal herb [1]. The purpose of this study was to compare the antioxidant capacities and total phenolic content of essential oil and aqueous tea infusion extracts of the two investigated species. The radical scavenging activity was measured using the DPPH method [2]. IC_{50} values for aqueous tea infusion extracts were similar and ranged from 0.33 to 0.37 mg/mL for *S. visianni* and *S. montana* respectively, while values for essential oil showed significant differences and ranged from 5.18 for *S. montana* to 26.0 mg/mL for *S. visianii*. In addition, antioxidant capacity was measured using ORAC test [3]. Two different reactive oxygen species were used: Cu^{2+}-H_2O_2 as a hydroxy radical generator and AAPH as a peroxy radical generator. Obtained values for aqueous extracts reanged from 10.3 to 13.3 and for essential oil from 66.3 to 61.6 mmolTE/g against OH for *S. montana* and *S. visianii* respectively, while values against OOH varied from 4.13 to 58.7 mmolTE/g for all samples. Total phenols were determined according to the slightly modified Folin-Ciocalteu method in tea infusion samples and varied from 225 mgGAE/g for *S. visianii* to 275 mgGAE/g for *S. montana* [4]. Results from the present study suggested further analyses on the chemical composition of the plant extracts in order to identify phenolic compounds that might be responsible for their antioxidant activity. **References:** 1. Vidic, D. et al. (2009), J. Essnt. Oil Bear. Plants 12 (3):273 – 281. 2. Brand-Williams, W. et al. (1995) Lebensm. Wiss. Technol. 28:2538. 3. Sofic, E. et. al. (2005) J. Neural Transm. 112 (3):349 – 358. 4. Katalinic, V. et al. (2006) Food Chem. 94 (4):550 – 57.

P138 Phytochemical profiling of the Mongolian medicinal plant *Myricaria longifolia* EHRENB.

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Myricaria longifolia EHRENB. (Tamaricaceae) is used in traditional Mongolian medicine to heal fever, poisoning [1, 2], and liver diseases. It is an ingredient of various prescriptions consisting of several herbal components. Aqueous extracts have been shown to inhibit the growth of liver carcinoma cells ($HepG_2$), breast cancer cells (MCF-7) [3], and primary rat hepatocytes [4]. The same extracts caused damage of the isolated rat liver during perfusion experiments [5]. Data about this plant are scarce in literature. Sterols, flavonoid aglycones, their glycosides, and derivatives of gallic and cinnamic acid have been detected so far [6, 7]. For our analyses, an aqueous extract was prepared and separated by column chromatography on Sephadex LH-20. The phytochemical screening was performed employing HPLC-UV-DAD and LC-MS^n. Ellagic acid, gallic acid, rhamnetin, and rutin were identified by comparison to reference substances. In addition, MS revealed the presence of various sulphates of rhamnetin, isorhamnetin, and quercetin.

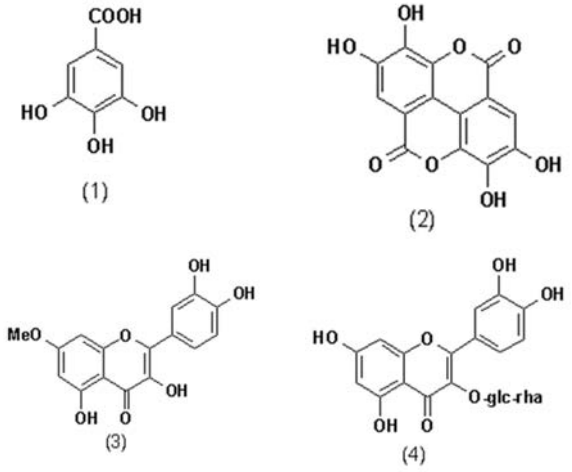

Fig. 1: Gallic acid (1), ellagic acid (2), rhamnetin (3), rutin (4)

References: 1. Boldsaikhan B. (2004) Encyclopedia of Mongolian medicinal plants. Ulaanbaatar. 2. Ligaa, U., Davaasuren, B., Ninjil, N. (2005) Medicinal plants of Mongolia used in western and eastern medicine. Ulaanbaatar. 3. Holec, N. (2005) Diploma thesis. University of Vienna. 4. Vogl, C. et al. (2009) Sci. Pharm. 77: 268. 5. Kletter, Ch. et al. (2008) Sci. Pharm. 76: 49 – 63. 6. Donath, O. et al. (2006) Sci. Pharm. 74: S 93. 7. Semenova, L.S. (1993) Rastit. Res. 29: 40 – 42.

P139 Antioxidant activity of some *Scorzonera* species and quantitative analysis of chlorogenic acid

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The genus Scorzonera L. (Compositae) is mainly originated from the Mediterranean Region and distributed from the Central Europe to Central Asia with more than 175 members [1]. In Europe there is 28 species of Scorzonera which are distributed all over the continent, from Northern Russia to Spain and Crete [2]. In Turkey Scorzonera genus is represented by 49 species [3]. This genus plants are mainly used as a vegetable in Europe as well as in Turkey. Some species of the Scorzonera genus have also medicinal usage in Turkish additionally in Europe, China and Mongolia folk medicines. In Turkish folk medicine Scorzonera species are used to treat a variety of illnesses, including arteriosclerosis, kidney diseases, hypertension, diabetes mellitus and rheumatism as well as for pain relief [4]. In previous studies, triterpenes, sesquiterpenes, sesquiterpene lactones, flavonoids, lignans, dihydroisocoumarins and phenolic acids have been isolated from Scorzonera species. In present study antioxidant activities of some Scorzonera species were evaluated by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and superoxide anion scavenging methods [5]. All of the extracts exhibited a scavenging effect on the DPPH and superoxide anion radical with various potencies. S. parviflora extract was established the most active with an IC50 value of 42 g/ml and 2.25 mg/ml respectively. Furthermore quantitative analysis of chlorogenic acid were performed by HPLC on all tested extracts. **References:** 1. Bohm, B.A., Stuessy, T.F. (2007) Flavonoids of the Sunflower Family (Asteraceae). Springer Wien. New York. 2. Paraschos, S. et al. (2001) J. Nat. Prod. 64:1585 – 1587. 3. Davis, P.H. (1975) Flora of Turkey and The East Aegean Islands. University Press. Edinburgh. 4. Baytop, T. (1999) Treatment with Plants in Turkey. Nobel. Ankara. 5. Altun, M.L. et al. (2007) Int. J. Food Sci. Nutr. 59:175 – 180.

P140 A cytotoxic compound against lung cancer cells from *Dioscorea birmanica* Prain&Burkill

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The rhizome of Dioscorea birmanica Prain & Burkill was commonly used by Thai folk doctors to treat cancer patients. The previous report was found that its extract exhibit high cytotoxic against lung cancer cells. Thus, the objective of this research was to investigate the cytotoxic activity against lung cancer cells using Dioscorea birmanica Prain & Burkill extract and an isolated cytotoxic compound therein. The ethanolic extract of Dioscorea birmanica Prain & Burkill showed cytotoxic activity against two types of lung cancer cell lines (CORL23 and A549) and against normal lung cells (MRC5) (IC50 = 8.7, 7.5 and 94.8 μg/ml respectively) in the sulphorhodamine B assay [1,2]. Bioassay guided fractionation was used to isolate the cytotoxic saponin diosgenin-3-O-α-l-rhamnosyl (1 → 2)-β-d-glucopyranoside or Prosapogenin A of dioscin (DBS1). DBS1 showed high cytotoxic activity against two types of lung cancer cells; CORL23 and A 549 (IC50 = 1.8., 1.8 μg/ml respectively) but less cytotoxic activity against normal lung cells MRC5 (IC50 = 37.1 μg/ml). These results can support using this plant to treat cancer by Thai folk doctors. **Acknowledgements:** Thammasart University for financial support **References:** 1. Tharat, A. et al. (2004) J. of Ethnopharmacology 90:33 – 38. 2. Skehan P. et al.(1990) J.Natl. Cancer Inst. 82(13): 1107 – 12.

P141 Bioactivity profile of compounds isolated from *Hymenocardia acida* Tul. leaves

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In the course of phytochemical investigations to determine the different constituents of *H. acida* we isolated di (2-ethylhexyl) phthalate (DEPH) and homoorientin. These compounds were subjected to several assays for bioactivity profiling viz: antioxidant, in vitro anti-inflammatory, cytotoxicity and antimicrobial studies. Homoorientin inhibited hydroxyl radicals generation in horse radish assay but failed to inhibit superoxide in xanthine-xanthine assay, while DEPH did not show any antioxidant property. DEPH and Homoorientin showed moderate inhibition of 3α-hydroxysteroid dehydrogenase with an IC_{50} of 10 and 16 μg/ml respectively. The compounds did not show good activity against the microorganisms tested. The results of this study lend credence to the ethnomedicinal use of *H. acida* as an anti-inflammatory agent (1) and expand knowledge on the biological activity of its secondary metabolites. **References:** 1. Burkill, H.M. (1994). Royal Botanic Gardens. Surrey. 2. Afonin S, Glaser RW, Berditchevskaja, M, Wadhwani P, Gührs K-H, Möllmann U, Perner A, Ulrich AS (2003). 4-Fluoro phenylglycine as a label for 19F-NMR structure analysis of membrane associated peptides. Chem BioChem 4: 1151 – 1163. 3. Angeh JE, Huang X, Sattler I, Swan GE, Dahse H, Härtl A, Eloff, JN (2007) Antimicrobial and anti-inflammatory activity of four known and one new triterpenoid from Combretum imberbe (Combretaceae). J of Ethnopharmacol ogy110: 56 – 60. 4. Dahse HM, Schlegel B, Gräfe U (2001) Differentiation between inducers of apoptosis and nonspecific cytotoxic drugs by means of cell analyzer and immunoassay using the cell lines cell lines K-562 (human chronic myeloid leukemia), and L-929 (mouse fibroblast) for antiproliferative effects and HeLa (human cervix carcinom) for cytotoxic effects. Pharmazie 56: 489 – 491. 5. Sud'ina, G.F., Mirzoeva, O.K., Pushkareva, M.A., Korshunova, G.A., Sumbatyan, N.V., and Vafolomeev, S.D. (1993). Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. Federation of European Biochemical Societies 329, 21 – 24.

P142 The screening of antihypertensive and antioxidant activities of tropical fruit seeds

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The aim of this study is to investigate antioxidant activity of methanolic extracts from fruit seeds by DPPH radical scavenging and linoleic acid emulsion methods [1], and to evaluate ACE inhibitory property [2,3] among them. Seeds of Thai fruits were selected for this study. The methanolic extracts (1 – 20 mg/ml) showed antioxidant potential by scavenging DPPH radicals as follows: jambolan plum seeds (0.05 – 11.05%), litchi seeds (1.71 – 16.39%), litchi longan seeds (25.49 – 80.40%), tamarind seeds (20.49 – 93.14%) and rambutan seeds (67.26 – 93.48%). The methanolic extracts (1 – 20 mg/ml) also inhibited linoleic acid peroxidation as follows: Indian gooseberry seeds (94 – 96%), litchi seeds (95 – 97%), longan seeds (94 – 97%), rambutan seeds (94 – 95%) and jambolan plum seeds (85 – 95%), respectively. The ACE inhibition% of longan seeds, litchi seeds, tamarind seeds were 48.57%, 48.57% and 82.86%, respectively. The degree of ACE inhibition was not related to the concentrations of the extracts. Because it depended on flavanols and high molecular weight procyanidin components, they acted as ACE inhibitors [4]. It is needed to characterize and quantify the phenolic compounds and confirm antioxidant activity and antihypertensive activity of methanolic extract of seeds with other biological methods. **References:** 1. Yen, GC & Duh, PD. (1994) J Agric Food Chem. 42:629 – 632. 2. Cushman, DW & Cheung, HS (1971) Biochem Pharm. 20:1637 – 1648. 3. Tomokuni K & Ogata, M. (1972) Clin Chem. 18:349 – 351. 4. Actis-Goretta, L. et al. (2003) FEBS Lett. 555:597 – 600.

P143 The medicinal plant known as Negramina (*Siparuna guianensis* Aubl. – Siparunaceae) in Southwestern Mato Grosso, Brazil

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Negramina (*Siparuna guianensis* Aubl. – Siparunaceae) is a bush that occurs in the municipality of Cáceres, Mato Grosso, Brazil. This species grows in "cerrado" areas, developing in "reboleiras" which are clump of trees in an open camp. These in general are located in areas semi-shadowed by higher trees and at the margins of fragments of wood areas. This demonstrates that this species prefer environmental conditions of sub-wood. The Negramina grows predominantly in well drained soils and that present low fertility. It is necessary to protect the environments of occurrence of this species to prevent its extinction. Its leaves and stem exhale a typical strong smell. It is used by the local community for medicinal, insecticide and mystical applications. For medicinal use, the vegetable material is boiled in water, which is left to cool and is used: (a) in external applications for washing of female genitalia aiming asepsis; (b) for head washing to alleviate cephalalgia. For insect control, the residents of the local communities use leaves and stem in bomfire to produce smoke that has a repellent effect. It is also crushed and rubbed in arms, legs, neck and face to keep the undesirable insects, mainly mosquitoes and hematophagous flies, away. Traditional residents of the local communities believe the typical odor of Negramina leaves and its aqueous extract obtained by boiling possesses mystic powers, "cleansing" the soul and keeping "bad spirits" away. **Acknowledgements:** UNEMAT institutional support, and FAPEMAT EMPAERMT; support afforded by the whole project team PLAMED: Bonila MGO, Carniello M, Ramos PR et al.

P144 Evaluation of the burn healing properties of five Iranian medicinal plants in diabetic rats

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Some of the medicinal plants in Iranian traditional medicine (Unani) whose have been used as remedy against edema, burn, wound and for their carminative, antimicrobial and anti-inflammatory activities [1, 2]. For example, Malva sylvestris L. (Malvaceae), Punica granatum L. (Punicaceae), Prunus amygdalus L. (Rosaceae), Arnebia euchroma Rolye (Johnst) (Boraginaceae) and Scrophularia deserti Del. (Scrophulariaceae) used for wound and burn healing by tribal communities in Iran[3]. The ethanol extracts of Malva sylvestris and P. granatum flowers, P. amygdalus leaves, A. euchroma roots and S. deserti were used to evaluate the burn healing activity at 200 mg/kg/day dose in alloxan-induced diabetic rats. Burns were induced in Wistar rats divided into nine groups as following; Group-1, normal rats were treated with simple ointment base. Group-2, diabetic rats were treated with simple ointment base (control). Groups-3 and -7, diabetic rats were treated with simple ointment base containing of extracts (diabetic animals), Groups 8, diabetic rats were treated with simple ointment base containing of mixed extracts, Group-9, diabetic rats received the standard drug (Silver sulphadiazine). The efficacy of treatment was evaluated based on burn wound area relative and histopathological characteristics. The extract-treated diabetic animals showed significant reduction in the wound area when compared with control. Also, histopathological studies of the tissue obtained on days 9th and 16th from the extract-treated by extracts showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells. **References:** 1. Ghasemi Pirbalouti A, et al. (2009) Pharmacognosy Mag. 5: 433 – 437. 2. Zargari A. (1989 – 1992) Medicinal Plants. University Publication of Tehran, Iran. 3. Ghasemi Pirbalouti A. (2009) Herba Polonica. 55: 69 – 75.

P145 Antinociceptive effects of *Stachys laxa*

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The Labiatae family (Lamiaceae) is one of the largest and most distinctive families of flowering plants, with about 200 genera and almost 4000 species worldwide. This family has an almost cosmopolitan distribution. Many stachys plants- a genus of this family- have been used as medicinal plants because of their broad spectrum biological activities. Some stachys have been used as remedy for painful or inflammatory conditions in folk medicine. Anti-inflammatory and analgesic effects of some species of *Stachys* e.g. *S. inflata*, *S. byzantina* and *S. Schtschegleevii* have been reported. In this study, the antinociceptive properties of total methanolic extracts of the aerial parts of *S. laxa* was investigated by formalin test in mice. Intraperitoneal injection of the methanolic extract of *S. laxa* at the doses of 100, 200 & 400 mg/kg, 15 min before formalin test, significantly inhibited the chronic phase of formalin- induced pain. Intraperitoneal injection of opioid antagonist (naloxone) significantly reversed the analgesic effect of extract in chronic phases of formalin test. This results suggest that that analgesic activity of this plant may be partly mediated by opiate system. **References:** 1. v. Mozaffarian (2006) A dictionary of Iranian plant names, farhang moaser, tehran. 2. A. Zargari (1996) Medicinal plants, vol 4, Tehran university, Tehran. 3. v. Mozaffarian (2005) classification of plants, vol 2, amirkabir, tehran. 4. M. Semnani, M. Saeidi, M.R. Mahdavi, F.Rahimi, J of Mazandaran University Medicinal science, 2007, 17(57):57 – 66. Antimicrobial effects of metanolic extracts of some species of stachys &philoms. 5. M. khanvi: M. Hajimahmoodi, M. Cheraghi-Niroomand, Z. Kargar, Y. Ajani, A. Hajiakhoondi, M.R. Oveisi. 6. African Journal of Biotechnology Vol. 8 (6), pp. 1143 – 1147, 20 March, 2009 Comparison of the antioxidant activity and total phenolic contents in some stachys species.

P146 Phytoecdysteroids from arial parts of *Ajuga chamaecistus subsp. tomentella*

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The genus *Ajuga* (Lamiaceae) is represented by five species in the flora of Iran, of which *Ajuga chamaecistus* has several exclusive subspecies include subsp. *tomentella* [1]. The plants of this genus are used traditionally for treatment of joints pain, gout and jaundice [2]. In this study diethylether fraction of defatted methanolic extract (80%) from arial parts of A. *chamaecistus subsp. Tomentella* was chromatographed on silicagel using a $CHCL_3$-EtoAc-MeOH gradient system to give compound 1 and 2. The structure of compound 1 and 2 were determined to be 20-Hydroxyecdysone and Cyasterone, respectively, by means of spectroscopic analysis (^{13}C, HNMR, IR, Mass Spectroscopy). Ecdysteriods are a large group of natural polyhydroxysteroids with a wide spectrum of biological activities, which are isolated from the animal and plant kingdoms [3]. In plants of genus *Ajuga*, a variety of phytoecdysteroids has been isolated. Among them, 20-Hydroxyecdysone or Cyasterone are usually the most abundant [4]

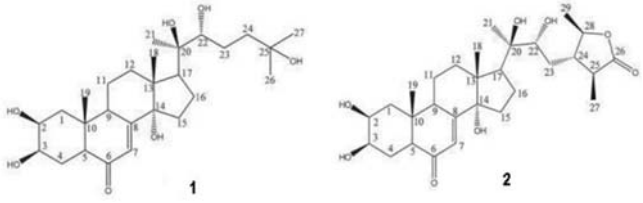

Fig. 1: Structure of compound 1 and 2

References: 1. Mozaffarian, V (2007) A Dictionary of Iranian Plant Names. Farhang Moaser. Tehran. 2. Naghibi, F. et al. (2005) IJPR. 2:63 – 79. 3. Ramazanov, N Sh. (2005) Chemistry of Nat. Comp. 41(4): 361 – 369. 4. Calcagno, M.-Pia. et al. (1995) Tetrahedr. 51(44): 12119 – 12126.

P147 Evaluation of chemical composition and antioxidant activity of total extract of *Cuscuta chinensis* Lam. used in traditional medicine

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Cuscuta sp. (Convolvulaceae), known as dodder, has been used in Iranian traditional medicine for many gastrointestinal, respiratory, endocrine, skin and neurological diseases and is locally named "Davaa"-ol-Jonoon" (Drug of mania) [1, 2, 3]. In recent experimented traditional medicine some species are used for a wide variety of conditions such as bipolar disorder, depression, anxiety, etc. According to previous studies, oxidative stress is related to some neurological disorders such as schizophrenia, psychosis and bipolar disorder [4, 5]. C. chinensis is one of Cuscuta sp. used widely in traditional medicine that is rich in flavonoids as one of the major antioxidant compounds [6]. In this study we evaluated the chemical composition and examined the antioxidant activity of this species to define one of the possible mechanisms for its use in traditional medicine. The total extract of C. chinensis (whole plant) was screened for saponins, alkaloids, flavonoids, tannins and sterols. The free radical DPPH scavenging activity was compared with the synthetic antioxidant (BHA) as a positive control. Total extract showed the presence of alkaloids, tannins, saponins, sterols and flavonoids. In a concentration of 500 μg/mL it showed antioxidant activity comparable with that of BHA (220 μg/mL, $p < 0.05$). The IC_{50}'s of extract and BHA were 235 μg/mL and 7.9 μg/mL respectively. These data suggest that one of the mechanisms

of action of Cuscuta chinensis used for neurological disorders could be via its antioxidant activity. **References:** 1. Hussayni, M. M., (2008), Tohfat-ol-momenin, Nashr-e-Shahr, Tehran. 2. Razi, M. Z., (826 – 930), Alhavi, Vol. 20. 3. Ansarishirazi, A., (1996) Ekhtiarat Badiee, 1st ed. The drug distributing company of Razi, Tehran. 4. Aghili-Alavi, M. H., (1996) Gharabadin kabir, 10th ed., Marvi, Tehran. 5. Yumru, M. et al. (2009), Oxidative imbalance in bipolar disorder subtypes: a comparative study, Progress in Neuro-Psychopharmacology & Biological psychiatry, Vol. 33, p. 1070 – 1074. 6. Parellada, M. (2010), Antioxanat status in first episodes of early onset psychosis compared with asperger and healthy control adolescents, doi: 10.1\‰16/j.schres.2010.02.6 61. 7. Ye, M., et al. (2002), studies on chemical constituents of Cuscuta chinensis, Zhongguo Zhong Yao Za Zhi, Vol. 27 (2): 115 – 117.

Miscellaneous

P148 Pre-myrsinanes and deoxygenated phorboids from the Iranian spurge *Euphorbia macroclada* Boiss.

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VEGFR antagonism was recently reported for some myrsinane polyesters [1], a very surprising observation since kinase inhibitors are generally flat molecules that act as an ATP-mimics, while myrsinanes diterpenoids are exuberant in terms of tridimensional chirality and structural complexity. This report prompted us to systematically investigate a series of spurges for the presence of myrsinane-type polyesters, and we report that *Euphorbia macroclada* Boiss, a spurge endemic to the Iranian plateau, is a good source of this type of compounds. It had previously been reported to contain cytotoxic [2] principles; besides the new pre-myrsinane polyesters 1a, 1b and 2, a series of deoxygenated phorboids of general formula 3 (R1 = H, Ac, 4 H = -β or -α; R2 = short alkyl chain) were obtained.

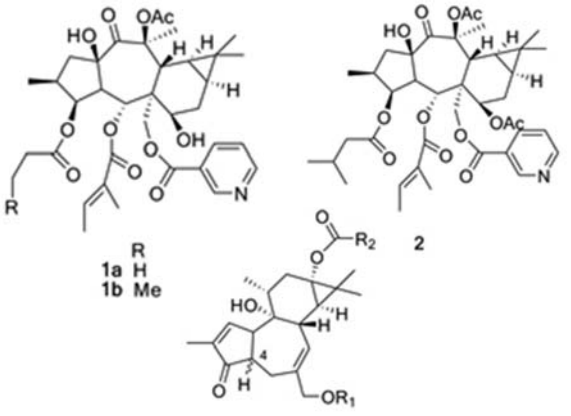

Fig. 1 3

All new compounds were structurally elucidated by spectroscopic methods, and especially 2D NMR measuremets. **References:** 1. Hussain, S. et al. (2008). BMC Cell Biology.9:7. 2. Sadeghi-Aliabadi, H. et al. (2009) IJPR. 5:103 – 108.

P149 Analysis of essential oil of *Zhumeria majdae* extracted by SFE

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Traditional methods for isolating essential oils from plant materials, such as steam distillation and solvent extraction have some draw backs due to the heat instability of essential oil and the presence of residual organic solvent in the extract. Thus, the use of super critical fluid extractions (SFE) for extraction of essential oils has received increasing attention to this traditional techniques [1, 2]. An experimental flow-type apparatus has been designed for the extraction of the essential oil from *Zhumeria majdae* (Lamiaceae) with super critical carbon dioxide. In the present study, the operating conditions for extraction were conducted at the pressure of 200 bar, the temperature of 37 °C, the carbon dioxide rate of 0.3 liter per min, the dynamic extraction time of 20 min with different static times of 30, 45 and 60 min. Main compounds were found to be linalool (73.8, 72.5, 41.5 %, respectively) after analysis by GC/MS with a HP5-MS column. In comparison with other samples obtained by hydro distillation, SFE oil (static times of 30, 45 min) contains higher amount of linalool. **References:** 1. Mchugh M A., Krukonis V J. (1986) Supercritical Fluid Extraction Principles and Practice, Butter Worths, Boston, MA. 2. Goodarznia I, Esmaeilzadeh F. (2002) J. Chem. Eng. Data 47:333.

P150 Antioxidant activity of *Cladophoropsis* sp. alga, from North of Persian Gulf

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Antioxidant compounds play an important role against different diseases. They are employed in medicine, cosmetic industry and food production (1). In searching for safe and effective antioxidants from natural resources, marine organisms are a new source (2). In recent years, algae have attracted great attention of researchers as their primary or secondary metabolites can be biologically active (3). They are also rich in proteins, minerals and polysaccharides (2). There are about 153 species of algae in Persian Gulf and Arabian Sea that can be a new source of natural antioxidants (4). The goal of this study was assessing the antioxidant activity and phenolic content of Cladophoropsis sp., from north of Persian Gulf. At the first step, the algae was lyophilized and extracted by 90 % ethanol. For examination the antioxidant activity, two methods including radical scavenging test by using DPPH and reducing activity test, were carried out. Phenolic content (antioxidant compound) was also examined by using folin ciocalteu. The results showed radical scavenging activity with IC 50 = 0.9 mg/ml and reducing activity with absorbance of 0.5 at the concentration of 1.48 mg/ml. The IC 50 of Gallic acid (0.82 µg/ml) as powerful antioxidant standard was less than algal extract ($P < 0.0001$). Its total phenolic content was 2.1 mg/g of extract. The results indicate moderate radical scavenging activity and reducing power of alga. In comparison with the others studies, this algae are rich in phenolic compounds. **References:** 1. M, Zubia. et al. (2007) J. Appl. Phycol. 19:449 – 458. 2. M, Zubia. et al. (2009) J. Food Chemistry. 116:693 – 701. 3. S, Takamatsu. et al. (2003) J. Nat. Prod. 66:605 – 608. 4. J, Sohrabipour. et al. Iran. Journal. Bot. 8:131 – 162.

P151 Standardized *Cassia fistula* pod extracts

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Cassia fistula L. pods have been used as a laxative drug in Thai traditional medicine for a long time. The pods of *C. fistula* were collected in summer (April, 2007) from 10 provinces in the North, North-East, Central, and South of Thailand. The pods were extracted by decoction which was found to be a suitable method for extracting anthraquinone glycosides from *C. fistula* [1]. The pod extracts are brownish-black color with characteristic odour and mild sweet taste. The extract ratio (crude drug: 1 g crude extract) were about 1 – 2: 1. All extracts contained anthraquinone compounds identified by Borntrager's test and TLC. Rhein was a major anthraquinone compound in the pod extracts. Loss on drying of the extracts was not more than 1% w/w. The extracts were freely soluble in water and slightly soluble in 95% ethanol. By UV-vis spectrophotometric analysis, total anthraquinones in the pod extracts were more than 1% w/w, while total anthraquinone glycosides were more than 0.4% w/w calculated as rhein. According to HPLC analysis, the content of rhein in pod extracts was not less than 0.5% whereas aloe-emodin content was not less than 0.0003% w/w. The pod extracts did not contain heavy metals and pesticide residues. Bacterial contamination of the extract was not more than 10 cfu/g while fungi were not more than 11 cfu/g. Also, pathogenic bacteria was not found in the extracts. These informations will be useful for quality assessment of the pod extracts of *C. fistula* as a raw material for herbal laxative drug. **References:** 1. Sakulpanich A, Gritsanapan W. (2008) J Health Res. 22(4): 167 – 172.

P152 Genomics-based approach towards the discovery of a lipopeptide from *Herpetosiphon aurantiacus*

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Microbes provide a rich source for compounds of therapeutic importance. These metabolites are synthesized by enzymes encoded by associated biosynthetic gene clusters. Genome sequencing data from microbes revealed the presence of many gene clusters, for which the secondary metabolites are not known. These gene clusters are often referred to as orphan gene clusters (1) whereby the structure of the metabolites originating from this genetic information can be predicted by alignments with conserved regions of biosynthetic genes of known metabolites. Recently the genome of *Herpetosiphon aurantiacus*, a gram-negative, gliding bacterium (2),(3) was sequenced. From its genomic sequence, a putative PKS-NRPS (polyketide synthase-non-ribosomal peptide synthetase) orphan gene cluster involved in the biosynthesis of a lipopeptide has been identified. Bioinformatic analysis of this PKS-NRPS cluster revealed that the lipopeptide is composed of six amino acids, including the unusual amino acid p-hydroxy phenylglycine, and a carboxylic acid moiety. One of the NRPS adenylation domains was heterologously expressed and first experiments showed that p-hydroxy phenylglycine is activated. Expression analysis at different growth conditions showed that the PKS-NRPS genes are expressing in casitone-yeast based medium. Furthermore, the bacterium was cultivated in casitone-yeast based medium and extracted. The extract showed antibacterial activity and LCMS analysis revealed a compound with a molecular mass of 774, coinciding with that of the proposed lipopeptide. Bioactivity and LC-MS guided purification and structure elucidation of the compound is being carried on. **References:** 1. Gross H., Stockwell V. O., Henckels M. D., Nowak-Thompson B., Loper J. E., Gerwick W. H. (2007) Chem Biol., 14: 53 – 63. 2. Nett M., Erol Ö., Kehraus S., Köck M., Krick A., Eguereva E., Neu E., König G.M. (2006) Angew. Chem.Int.Ed., 45: 3863 – 3867. 3. Reichenbach H., Golecki J.R. (1975) Arch. Microbiol., 102: 281 – 291.

P153 Composition of the volatiles and fixed oils of the seeds of *Nigella* species

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Nigella L. s.l. (Ranunculaceae) is represented in the world by 24 species comprising 31 taxa. In the flora of Turkey, there are 15 species and altogether 19 taxa [1 – 7]. As part of a project involving a revision of the genus Nigella we are investigating the chemical composition of Nigella seeds. In the course of the present work, microdistillation and the n-hexane extracted fixed oils of the seeds of the following Nigella taxa have been investigated by gas chromatography/mass spectrometry (GC/MS) and gas chromatography-flame ionization detector (GC-FID): *N. arvensis* L. subsp. *arvensis*, *N. arvensis* L. subsp. *brevifolia* Strid, *N. arvensis* L. var. *glauca* Boiss., *N. arvensis* L. var. *involucrata* Boiss., *N. arvensis* L. var. *tauricola* P.H. Davis, *N. arvensis* L. *assyriaca* (Boiss.) Boiss., *N. ciliaris* DC., N. *damascena* L., *N. degenii* Vierh. subsp. *degenii*, *N. elata* Boiss., *N. lancifolia* Hub-Mor., *N. latisecta* P.H. Davis, *N. orientalis* L., *N. nigellastrum* (L.) Willk., *N. oxypetala* Boiss., *N. sativa* L., *N. segetalis* Bieb., *N. turcica* Dönmez & Mutlu, *N. unguicularis* (Lam.) Spenner collected from Greece, Syria and Turkey. In general, mono- and sesquiterpenoids appeared to be the main constituents of the volatiles. In all the fixed oils, linoleic and oleic acids were the main fatty acids. **References:** 1. Davis, P.H., (1965). Nigella L. Flora of Turkey, 1, 98 – 105. 2. Dönmez A.A. and Mutlu, B. (2004). A new species of Nigella L. (Ranunculaceae) from Turkey, The Linnean Society of London, Botanical Journal of the Linnean Society. 3. Mabberley DJ (2008). Nigella L. (Ranunculaceae), Mabberley's Plant-Book. A Portable Dictionary of Plants, Their Classification and Uses. Cambridge: Cambridge University Press. 4. Strid, A. (2002). Nigella L. Flora Hellenica, 2: 3 – 13. 5. Tamura 1993, Nigelleae in The Families and Genera of Vascular Plants (eds. Kubitzki and Bittrich), 2: 574. 6. Tutin, T.G and Akeroyd J.R., 1993. Nigella L., Flora of Europaea, 1: 251 – 253 (2nd ed.). 7. Zohary, M. 1983. The Genus Nigella (Ranunculaceae) – A Taxonomic revision, Pl. Syst. Evol. 142: 71 – 107.

P154 Cytotoxicity and mutagenicity investigation of extracts of common South African ethnoveterinary plants

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Rural livestock keepers in southern Africa have access to an extraordinary diversity of plants to provide treatments for various diseases in their animals, particularly infections and wounds. Many of these plants have been shown in our previous studies to possess promising antibacterial, antifungal and antiviral properties, but have rarely been investigated for toxicity. Cytotoxic and genotoxic effects of acetone extracts of sixteen plants used widely in South African ethnoveterinary medicine (EVM) were studied. Cytotoxicity was determined against Vero kidney cells and bovine dermis cells using various assays. Mutagenic effects were investigated using the Ames test with Salmonella typhimurium strains TA98 and TA100. Bovine dermis cells were generally more sensitive to the extracts than Vero cells. Combretum caffrum was the most cytotoxic, with LC50 less than 50 ug/ml against both cell types. Markhamia zanzibarica was also fairly cytotoxic, but most of the plant extracts had LC50 values between 0.1 and 1 mg/ml. Sclerocarya birrea was not cytotoxic to the cell lines up to the highest test concentration of 1 mg/ml. None of the plant extracts exhibited mutagenic effects against the strains tested. Obtaining an indication of toxicity of ethnoveterinary plant extracts aids in the selection of plants for isolation studies of anti-infective compounds lacking non-specific toxicity. These studies also provide useful indications of toxicity of presently used traditional remedies particularly in the case of topical applications, bearing in mind the limitations of in vitro tests.

P155 Impact of plant extracts on drug transport across intestinal mucosa

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Plant materials in the form of fruit, vegetables or herbal medicines are often taken in conjunction with allopathic medicines. Pharmacodynamic and/or pharmacokinetic interactions may occur between phytoconstituents and the co-administered drug [1]. Pharmacokinetic interactions include interferences with drug delivery and distribution by means of altered absorption, metabolism, distribution and/or elimination [2]. This work reports on the effects of extracts from traditional medicinal plants (*Hypoxis hemerocallidea* and *Sutherlandia frutescens*), fruit (*Sclerocarya birrea, Psidium guajava, Dovyalis caffra, Prunus persica, Fragaria ananassa, Prunus domestica*), herbs (*Aspalathus linearis*) and vegetables (*Daucus carota, Beta vulgaris*) on drug transport. The transport was measured in both directions (apical to basolateral and basolateral to apical) across Caco-2 cell monolayers and pig intestinal tissues to identify effects on the active efflux of the drug in the basolateral to apical direction. The results showed that some of the investigated plant extracts decreased drug efflux probably by inhibition of P-glycoprotein resulting in increased drug absorption, while others increased the efflux with a subsequent decrease in absorption. Although *in vitro* pharmacokinetic interactions are not always clinically significant in the *in vivo* situation [3], it may indicate potential changes in the bioavailability of co-administered drugs. **References:** 1. Harris R.Z. et al. (2003) Clin. Pharmacokinet. 42:1071 – 1088. 2. Manzi S.F. et al. (2005) Clin. Pediatr. Emerg. Med. 6:93 – 102. 3. Farkas et al. (2008) Expert Opin. Drug Metab. Toxicol. 4:381 – 39.

P156 Shikonin derivatives from the roots of *Onosma nigricaule* (Boraginaceae)

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The genus *Onosma* (Boraginaceae) is represented by 87 species in Turkey [1]. The roots of some *Onosma* species are used for wound healing and burns [2,3] in Turkey. *Onosma nigricaule* is an endemic species for Turkey [1] and its roots are used for wound healing and burns in Erzurum Province. In this sudy, the roots of *O. nigricaule* (500 g) were extracted with n-hexane:dichloromethane (1:1) (2L x 3) and then filtered. The filtrate was evaporated at 40 °C. Three shikonin derivatives (deoxyshikonin (**1**), β,β-dimethylacryl shikonin (**2**), and acetyl shikonin (**3**)) were isolated from this extract by using several chromatographic methods. The structures of the pure compounds were elucidated by using ^{1}H-NMR and ^{13}C-NMR Spectroscopy, and ESI-MS. **Acknowledgements:** The authors would like to thank Prof. Dr. Mehmet Koyuncu (Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara) for identification of the plant material. **References:** 1. Riedl H. (1978) *Onosma* L. Flora of Turkey and the East Aegean Islands. Vol. 6, pp. 326 – 376. Edinburgh University Press, UK. 2. Ozgen U., Ikbal M., Hacimuftuoglu A., Houghton P.J., Gocer F., Dogan H., Coskun M. (2006) J. Ethnopharmacol. 104:100 – 103. 3. Cadirci E., Suleyman H., Aksoy H., Halici Z., Ozgen U., Koc A., Ozturk N. (2007) Chem. Biol. Interact. 170(1):40 – 48.

P157 Evaluation of the efficiency of CROPWAT model for determining water requirement of cumin (*Cuminum cyminum* L.) in arid regions

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Shortage of water resources and increasing demand to consumption of this scarce resource, leads to some noticeable limitations. On the other hand, population growth and consequently, increasing demand for water in arid and semi arid regions, needs production in exchange of little amount of water consumption. To approach this objective, an experiment in the complete randomized blocks carried out in four replications for cumin plant growing in Zabol, southeastern Iran. Experimental treatments included irrigation periods at three levels. Then using CROPWAT model, the water requirement of the plant is met. Analyzing the data resulted from production gathered in different times of irrigation and consumption of water in the three times irrigation case with sound efficiency (1750 m3/ha), is more little than the water amount which is simulated by the CROPWAT model in 2003 (6070 m3/ha) and (5363 m3/ha) in 2004. It then showed that this model is not effective in determining the water requirement of cumin at this region. **Keywords:** Water requirement; Cumin; CROPWAT model; Efficiency; Simulation

P158 The influence of planting time on development and yield of various cultivars of medicinal flax

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In order to determine the optimum planting date for different medicinal flax cultivars, a split plot experiment with completely randomized block design with 12 treatments was conducted in experimental farm of Yasooj Islamic Azad University. The treatments were replicated 3 times. The treatments were composed of four different combinations of planting dates ((Feb. 20, Mar. 6, Mar. 21, and Apr. 4) and 3 flax cultivars (Somaco, India, and Foster). The measured traits were plant height, 1000-kernel weight, harvest index, grain yield (with 12% MC), Dry Matter, Leaf Area Index (LAI), Crop Growth Rate (CGR), Relative Growth Rate (RGR), and Net Photosynthesis Rate (NFR). The result showed that the planting date has a significant effect on all the measured traits. The first and second planting dates resulted in the highest production (1230 and 1170 kg/ha) and the lowest production (600 kg/ha) was obtained by the fourth date, respectively. The maximum and minimum yields were obtained by Somaco and Foster cultivars, respectively. The maximum 1000-kernel weight (5.853 g) was obtained in the first planting date, and the Somaco cultivar by itself produced the highest 1000-kernel weight (6.162 g). **References:** 1. Beighi, O.R. (2005). Production and processing medicinal plants. Vol. 1. Astane-Ghodse Rezavi Publication. P.347. 2. Zarghari, Gh. (2004). Medicinal plants. Tehran: Tehran University Press. P.342.

P159 Quality control of *Hoodia gordonii* raw material and products

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Hoodia gordonii is a popular weight loss product highly susceptible to adulteration. This highlights the need for rapid and simple quality control methods for authentication of raw material and quantification of the perceived active ingredient, P57, a steroidal glycoside. High performance thin layer chromatography (HPTLC) analysis was used to authenticate raw material and near infrared (NIR) spectroscopy and Raman spectroscopy combined with chemometric techniques were used to attempt the quantification of P57 in raw material. The concentration of P57 determined with liquid chromatography coupled to mass spectrometry (LC-MS) was used as reference data to develop calibration models based on the partial least squares projections to latent structures (PLS) regression algorithm. The performance of each calibration model was evaluated according to the correlation coefficient (R^2) and root mean square error of prediction (RMSEP). The HPTLC system produced good separation of compounds including that of the P57 band which was confirmed with preparative TLC. For the FT-NIR spectroscopy data the PLS model with 2nd derivative pre-processing predicted P57 content with an R^2 value of 0.9629 and an RMSEP of 0.03%. Pre-processing of the Raman data with orthogonal signal correction (OSC) yielded a PLS model with an R2 value of 0.9986 and an RMSEP of 0.004%. The HPTLC analysis provided a chemical fingerprint for authentication and confirmation of the presence of P57 in *H. gordonii* raw material and products. The parameters of the calibration model demonstrated that both NIR and Raman spectroscopy shows potential to rapidly quantify P57 in *H. gordonii* raw material.

P160 Antioxidant, anti-inflammatory activities and HPLC analysis of South African *Salvia* species

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The antioxidant and antiinflammatory activities of the methanol:chloroform (1:1) extracts of 16 *Salvia* species indigenous to South Africa were evaluated. Antioxidant activity was measured using the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the 2,2,-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays and compared to the control values obtained with Trolox®. Nearly all the solvent extracts displayed antioxidant activity with the IC_{50} values ranging from 1.61 to 74.50 µg/ml using the DPPH·, while the IC_{50} values ranged from 11.88 to 69.26 µg/ml when tested with the ABTS·+. The extract of *S. schlechteri*, with an IC_{50} value of 1.61 µg/ml, was three times more active than the reference compound, Trolox® (IC_{50} value: 2.51 µg/ml). The anti-inflammatory activity was evaluated using the 5-lipoxygenase assay. With the exception of *S. radula* (IC_{50} value: 78.78 µg/ml), the extracts displayed poor inhibition of the 5-lipoxygenase enzyme with all IC_{50} values being greater than 100 µg/ml. The total phenolic content based on gallic acid equivalents (GAE) confirmed the presence of total soluble phenolics in the various extracts from 45 to 211 mg of GAE dry sample and showed strong association (r^2 = 0.90) with antioxidant activity. High performance liquid chromatography (HPLC) was used to identify various compounds in the extracts. Betulafolientriol oxide and rosmarinic acid were detected in all the species investigated and rosmarinic acid, carnosic acid, carnosol and oleanolic acid/ursolic acid were abundant in many species.

P161 *In vitro* evaluation of the effect of selected herbal extracts on drug transport across porcine jejunal tissue

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P-glycoprotein (P-gp) is a membrane-bound transporter protein, which causes multidrug-resistance in tumour cells [1]. It is also localized in the apical membrane of normal intestinal epithelial cells [2] where it actively pumps substrates from within the cell back into the intestinal lumen with a consequent reduction in their bioavailability [3]. Many drugs, herbs and food substances may inhibit or induce P-gp, which may consequently influence the absorption of co-administered drugs [4]. The aim of this study was to investigate the effect of selected herbal extracts on the bidirectional transport of cimetidine, a P-gp substrate, across porcine jejunal tissue segments. The herbs were selected on the basis of having medicinal properties, being commonly used daily as flavourants in food as well as being easily accessible. The water extracts of selected herbs (garlic, cranberry, buchu and rosemary) were used to determine their effects on the transport of cimetidine across porcine jejunal segments in both the apical-basolateral and basolateral-apical directions. Cimetidine alone and verapamil were included as negative and positive control groups, respectively. The apparent permeability coefficient (P_{app}) and flux (J) values [5] were calculated for cimetidine. *Vaccinium macrocarpon* (cranberry), *Allium sativum* (garlic) and *Agathosma betulina* (buchu) extracts inhibited P-gp efflux of cimetidine and thereby enhanced cimetidine transport in the apical-basolateral direction, while *Rosmarinus officinalis* (rosemary) induced P-gp efflux and thereby enhanced cimetidine transport in the basolateral-apical direction. The results clearly indicate that the four selected herbal extracts contain phytoconstituents that modulate P-gp mediated drug efflux. **References:** 1. Sun et al. (2004). Med. Sci. Monitor, 10:RA5 -RA14. 2. Doppenschmitt et al. (1999). J. Pharm. Sci. 88:1067 – 1072. 3. Koren et al. (1998). Vet. Human Toxicol. 40:45 – 46. 4. Laitinen et al. (2004). Pharm. Res. 21:1904 – 1916. 5. Hansen et al. (2009). Phytother. Res. 23:86 – 91.

P162 Intestinal drug transport enhancement by *Aloe vera*

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Many drugs are poorly absorbed from the gastrointestinal tract when administered by the oral route. One approach to overcome the restriction of the physical barrier to drug absorption is the co-administration of absorption enhancing agents [1]. In this study the effect of *Aloe vera* (L.) Burm. f. (*Aloe barbadensis Miller*) gel and whole leaf extract on the permeability of intestinal epithelial cell monolayers (Caco-2) was determined. Solutions of gel and the whole leaf extract were applied to the cell monolayers and the transepithelial electrical resistance was monitored, which was continued after removal of the test solutions to measure reversibility of the effect. The transport of model compounds in the presence and absence of the *A. vera* gel and whole leaf extract solutions was also investigated. Both the *A. vera* gel and whole leaf extract were able to significantly reduce the transepithelial electrical resistance of the Caco-2 cell monolayers at concentrations above 0.5% w/v, which was fully reversible. The *A. vera* gel and whole leaf extract solutions significantly enhanced the transport of model compounds across the Caco-2 cell monolayers compared with the control. The results suggest that these plant products have a high potential to be used as absorption enhancers in novel dosage forms for drugs with poor bioavailabilities when administered orally. On the other hand, an uncontrolled increase in the bioavailability of drugs that are taken simultaneously with A. vera gel and whole leaf extract products may result in adverse effects. **References:** 1. Whitehead et al. (2008). Pharm Res, 25:1782 – 1788.

P163 Qualitative and quantitative analysis of *Paris quadrifolia* mother tincture

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Paris quadrifolia L. (Trilliaceae) is a small herb occurring locally in temperate and cool areas throughout Europe and Asia. The plant is known to contain steroidal saponins, which seem to be responsible for its toxicity [1, 2]. Ethanolic tinctures of the whole plant are used in homeopathy to treat headache and neuralgic pain [2]. The homeopathic tincture of *P. quadrifolia* is monographed in the current German Homeopathic Pharmacopoeia (HAB 2007). A phytochemical reinvestigation of the plant led to the isolation of two ecdysone derivatives, two flavonol glycosides as well as the saponins pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside and 26-*O*-β-D-glucopyranosyl-(25*R*)-5-en-furost-3β,17α,22α,26-tetrol-3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside. On the basis of these results, methods for the qualitative analysis of these compounds in the mother tincture were developed. Furthermore, a HPLC-method for the quantification of the main steroidal saponin, pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, has been established revealing a concentration of about 1.3 mg/ml in a commercial mother tincture. **References:** 1. Nohara, T. et al. (1982) Chem Pharm Bull 30: 1851 – 6. 2. Weth A. (1997) Hagers Handbuch der Pharmazeutischen Praxis, Springer, Heidelberg.

P164 In vitro and *in vivo* antiproliferative activity of arucanolide isolated from *Calea pinnatifida*

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Arucanolide is a germacranolide, isolated from the dichloromethane leaves' crude extract. Germacranolides are frequently described as responsible for antiproliferative activities with apoptosis induction. Therefore, study of anticancer activity of *C. pinnatifida* extracts and substances became important. This compound was identified as involved with the antiproliferative activity by bio-guided assays evaluated in nine different tumor and one normal cell lines. Samples were tested in a concentration range from 0.25 to 250 µg/ml and activity was measured by

sulforrodamine B method and total growth inhibition was calculated by non linear regression. The most potent fraction showed high selectivity for melanoma (TGI_5: 1,04 µg/mL; TGI_6: 1,07 µg/mL) and kidney (TGI_5: 1,45 µg/mL; TGI_6: 1,32 µg/mL) cancer cell lines. The dichloromethane leaves' crude extract demonstrated *In vivo* activity when evaluated in solid and ascitic Erhlich tumor assay. Purification of this fraction by column chromatography afforded Arucanolide that was identified by comparison of the spectral data with literature [1].

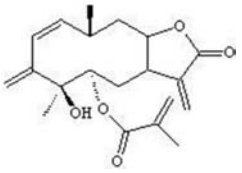

Fig. 1

References: 1. Ferreira, ZS et al. (1980) Phytochemistry 19(7): 1481 – 1484.

P165 Rare amino sugars in exopolysaccharides (EPS) from cyanobacteria of the genus *Synechocystis*

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Amino sugars are widely spread in natural polysaccharides, e. g. chitin in fungi, glycosaminoglycans in animals and lipopolysaccharides (LPS) in gram negative bacteria [1]. The most common amino sugars are N-acetyl-glucosamine and N-acetyl-galactosamine which also have been detected in cyanobacterial EPS [2]. The amino sugar N-acetyl-fucosamine (2-acetamido-2,6-dideoxy-D-galactose) has been found in the LPS of some gram negative bacteria. For the first time we identified a 2-N-acetyl-amino-2,6-dideoxy-hexose, propably N-acetyl-fucosamine, in EPS of *Synechocystis aquatilis* SAG 90.79. This EPS consists of only four sugars: 46% fucose, 37% arabinose, 12% amino hexose and 2% glucose independent on the conditions of cultivation. In contrast the EPS of *Synechocystis pevalekii* SAG 91.79 with more than seven sugars is more typical for cyanobacterial EPS [2]. It contains 24% mannose, 23% glucose, 12% fucose, 11% galactose, 9% xylose, 7% rhamnose and 4% of a newly found 2-amino-2-deoxy-pentose. A special feature of both EPS is the absence of uronic acids. They have an average sulphate content of about 20% and both can be separated into 4 to 5 fractions by ion exchange chromatography. The occurrence of amino deoxysugars accessorily points out the classification of cyanobacteria to the group of gram negative bacteria [3]. **References:** 1. Alban S. In: Hänsel R, Sticher O. Pharmakognosie – Phytopharmazie. Heidelberg: Springer Medizin Verlag; 2010:461 – 632. 2. De Philippis RS et al. (2001) J Appl Phycol 13:293 – 299. 3. Gupta R (1997) Antonie van Leeuwenhoek 72:49 – 61.

P166 Anti-arthritic activities of *Annona muricata* L. leaves extract on complete Freund's adjuvant (CFA) – induced arthritis in rats

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Annona muricata L. (Annonaceae) (AML) has been used as folkloric herbal medicine in many regions throughout the world in treating fever, cough, diarrhea, sedative, rash, ring worm and lactation for women afterbirth (1). The present study was carried out to investigate the anti-arthritic effects of AML ethanolic extract in complete Freund's adjuvant (CFA)-induced arthritis in rats. Edema in the left hind paw of rat was induced by the subcutaneous injection of 0.1 ml of heat-killed Mycobacterium tuberculosis in mineral oil (5 mg/ml). AML (3, 10, 30 and 100 mg/kg) or indomethacin (10 mg/kg) as positive control was administered orally once a day between days 1 and 14 post-CFA injection. Edema volume in each paw was measured by using a plethysmometer for 14 days. Levels of tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) in local tissue were measured using enzyme-linked immunosorbent assay (ELISA) on day 14 post-CFA injection. The result exhibited that AML at all doses (3, 10, 30 and 100 mg/kg) were significantly ($p < 0.05$) reduced the edema by 48.39, 66.67, 79.57 and 72.04% respectively. AML at higher doses 30 and 100 mg/kg significantly suppressed the local tissue TNF-α level by 42.81 and 51.82% and IL-1β level by 35.57 and 39.79% respectively on day 14 post-CFA injection. For TNF-α, the efficacies of the extract at doses 30 and 100 mg/kg were higher compared to the effect of indomethacin (51.82%). In conclusion, the findings suggest that AML present notable anti-arthritic activities that may be mediated by suppressing pro-inflammatory cytokines. **References:** 1. Perry, L.M., 1980. Medicinal plants of East and South East Asia, Cambridge, Massachusetts. Pp. 620.

P167 Hyoscyamine and scopolamine production of black henbane (*Hyoscyamus niger*) infected with *Pseudomonas putida* and *P. fluorescens* strains under water deficit stress

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Twenty plant growth promoting rhizobacteria (PGPR) strains belonging to *Pseudomonas putida* and *P. fluorescens* with different abilities for auxin production were screened based on their effects on vigor index of *Hyoscyamus niger* plants. Seedlings radicles and culture media were inoculated with these bacteria (10^9 CFU/ml) and grown under in vitro and growth room conditions. Thereafter, two strains namely PP-168 and PF-187 were selected, which had the highest values of VI. Subsequently, the effects of these two strains on Hyoscyamine (HYO) and Scopolamine (SCO) production in root and shoot tissues were investigated under three water deficit stress levels as 30, 60 and 90% water depletion of field capacity during the vegetative stage (45 to 90 days after planting) in pot experiment. Results indicated that the highest alkaloid content values in root (HYO: 0.268% DW; SCO: 0.122% DW) and shoot (HYO: 0.855% DW; SCO: 0.480% DW) were achieved in PF-187 treated plants grown under severe water stress conditions. By contrast, the maximum alkaloid yield in root (HYO: 1.927 $mg.plant^{-1}$; SCO: 0.835 $mg.plant^{-1}$) and shoot (HYO: 5.887 $mg.plant^{-1}$; SCO: 3.067 $mg.plant^{-1}$) were obtained in PP-168 treated plants under low water stress conditions (W1). Furthermore, the maximal total alkaloids yield (11.716 $mg.plant^{-1}$) in whole plant and 63% increase of shoot SCO yield were observed in the W1PP treatment compared with the respective uninoculated control. PGPR inoculation highly increased the roots and shoots dry matter and decreased severe negative effects of water stress on fine root growth, chlorophyll and relative water content.

P168 Stimulation of naphthoquinone production in *Impatiens balsamina* root cultures by methyl jasmonate

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Lawsone (1), lawsone methyl ether (2) and methylene-3,3′-bilawsone (3) are pharmacological active naphthoquinones (NQs) found in *Impatiens balsamina* L. (Balsaminaceae) [1,2]. *I. balsamina* root cultures have been established from the young leaf explants in liquid B5 medium supplied with 0.1 mg/l α-naphthalene acetic acid, 1.0 mg/l Kinetin, 2.0 mg/l and 6-benzyladenine [3]. However, these root cultures produced very low levels of (2). The main aims of this study were therefore to increase NQ production in *I. balsamina* root cultures using methyl jasmonate (MJ) as an elicitor.

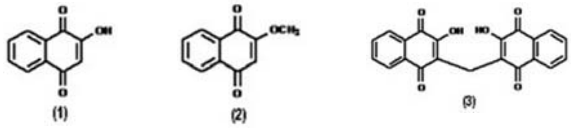

Fig. 1: Naphthoquinones found in *I. balsamina*

Treatment with MJ on day 21 of the root cultures was capable of increasing production of (1) and (2), but not (3). The content of (1) and (2) were increased by MJ treatment in a dose-dependent manner. However, treatment with MJ at 600 μM markedly diminished growth of the root cultures, as well as production of (1) and (2). MJ treatment at the concentration of 200 μM or higher resulted in the root cultures secreting NQs into the liquid media. An investigation of the optimum concentration of MJ, period of elicitor contact and age of the root cultures for elicitation revealed that the treatment of 21 day old root cultures with 300 μM MJ for 36 hr resulted in an increased production of (1), (2) and (3). The production levels were 10.0, 0.78 and 0.23 mg/g DW, which were 10.4-, 26.0- and 1.3-fold higher than the levels for the controls. **Acknowledgements:** Prince of Songkla University, Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0161/2548). **References:** 1. Yang, X. et al. (2001) Phytother. Res. 15:676 – 680. 2. Oku, H. et al. (2002) Biol. Pharm. Bull. 25:658 – 660. 3. Sakunphueak et al, (2010) Plant Cell Tiss. Organ Cult. In press.

P169 New jatrophanes from *Euphorbia bungei* Boiss.

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Spurges (*Euphorbia* species) have an important role in the Iranian traditional medicine as wart removers as well as anti-asthmatic- and anti-arthritic agents [1], and many of them are endemic. This, and the exuberant chemical profligacy of spurges in terms of the production of secondary metabolites, has provided a rationale to investigate the phytochemistry of *E. bungei* Boiss. This species contains a high concentration of fats and free fatty acid, but a diterpenoid fraction was eventually obtained by the combined use of liquid/liquid and solid/liquid partitions and lead acetate depigmentation. The major constituents of this fraction turned out to be jatrophane derivatives, as exemplified by the novel polyesters **1** and **2**, whose structures were elucidated by modern spectroscopic techniques.

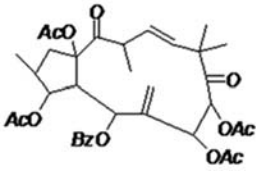

Fig. 1

References: 1. Zargari, A. (1993). Medicinal Plants, 5th ed., vol. 4. Tehran University Publication, Tehran.

P170 Isolation and biological evaluation of a triterpenoid from fruits of wild caraway (*Bunium persicum* Boiss.)

Shokoohinia Y[1], Taglialatela-Scafati O[2], Munoz E[3], Sajjadi S[1], Appendino G[4]
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Bunium persicum Boiss. is an Apiaceaous plant endemic to Iran plateau, and its fruits are used as spice because of their carminative, antispasmodic properties and to promote formation of milk in lactating women [1]. Anti-inflammatory [2], anti-oxidant [3], fungicide [4] and anti-histaminic [5] effect of the plant have been reported before, as well as studies on its volatile oil, that is rich in cyclic monoterpene aldehydes [6]. Surprising, few investigations have been carried out on the non-volatile constituents of this plant. We now report the isolation and bioactivity of the monoacetate of a pentacyclic triterpene diol (1) from this plant which showed moderately activity in the croton oil dermatitis assay representing a novel dietary phytochemical worth further biological investigation because of its broad consumption.

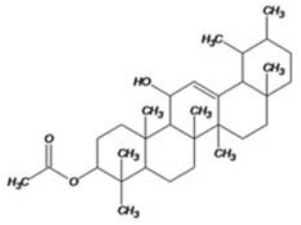

Fig. 1

References: 1. Ghasemi-Dehkordi, N. (2002) Iranian Pharmacopeae. Ministry of health publications, Tehran. 2. Khaidarov, K. K. et al (1991) Khimiko-Farmatsevticheskii Zhurnal. 25:73 – 5. 3. Shahsavari, N. et al (2008). Plant Foods Hum Nutr. 63:183 – 188. 4. Takayuki, S. et al (2007). J. Chem. Ecol. 33:2123 – 2132. 5. Boskabady, M.H. et al (2004). Int. J. Phytother. Phytopharmaco.11:411 – 5. 6. Foroumadi, A et al. (2002) J Ess. Oil Res.14:161 – 162.

P171 *Plantago altissima* L. as a potential natural antioxidant and anti-inflammatory agent

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Ancient use of plantains (genus *Plantago* L., Plantaginaceae) as herbal remedies is a consequence of their astringent, anti-toxic, antimicrobial, expectorant and diuretic properties. *P. altissima* L. is broadly distributed in Southern Europe and the Balkan Peninsula. As there are no data about biological activity of this species, some tests on activities of methanolic extracts have been undertaken. The extract has been characterized by LC-MS/MS [1] and several flavonoids as luteolin-7-*O*-glc and apigenin could be detected. The content of total phenolics (82.7 ± 3.7 mg gallic acid equiv/g of d. e.) and flavonoids (7.7 ± 0.2 mg quercetine equiv/g of d.e.) was determined by colorimetric techniques [1]. The radical scavenger capacity (RSC) was evaluated using various radicals and spectrophotometry [1] and the following IC_{50}-values were found: diphenylpicrylhydrazyl (10.7 ± 0.8 μg/ml), hydroxyl (312.5 ± 15.8 μg/ml), superoxide anion (27.2 ± 1. 7 μg/ml) and nitric oxide radical (1.3 ± 0.1 mg/ml). Inhibition of lipid peroxidation could be proven (IC_{50}= 177.7 ± 9.5 μg/ml). The results indicate comparable or higher extract activity than that of synthetic antioxidants as BHT or BHA (butylated hydroxytoluene/hydroxyanisol). Anti-inflammatory activity was examined by means of cyclooxygenase-1 (COX-1) and 12-lipooxygenase (12-LOX) inhibition and quantifying the COX-1 product 12-HHT (12-hydroxy-5,8,10-heptadecatrienoic acid) and 12-LOX product 12-HETE (12-hydroxy-5,8,10,14-eicosatetraenoate) by RP-HPLC-MS/MS [2]. Extracts inhibited both, COX-1 and 12-LOX (IC_{50}= 4.4 ± 0.3 and 3.6 ± 0.3 mg/mL, respectively). In this study, we report for the first time an antioxidant and anti-inflammatory activity of *P. altissima*, and accordingly consider this species as a promising source of natural antioxidant and anti-inflammatory agents. **Acknowledgements:** The Ministry of Science and Technological Development, Republic of Serbia (Grant No. 142036), supported this research work. **References:** 1. Beara I. et al. (2009) J Agric Food Chem 57: 9268 – 9273. 2. Beara I. et al. (2010) J Pharm Biomed Anal 52: 701 – 706.

P172 Detection and isolation by MS-coupled preparative HPLC of lysophosphatidylcholine derivatives from goji berries (*Lycium barbarum*)

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Goji (*Lycium barbarum*, Solanaceae) berries and juice are being sold as health food products and praised in advertisements and in the media for well-being and as an anti-aging remedy [1]. While the fruit itself is devoid of toxicity there have been increasing concerns about the quality of goji products with respect to pesticide contamination or possible

adulteration. As part of our investigations on goji, we detected in HPLC chromatograms of extracts a group of late-eluting compounds devoid of UV absorption. Their molecular weight could not be assigned to any known constituents of goji berries. An approach based on MS-coupled preparative HPLC was used for the isolation of these compounds. The experimental setup consisted of a HPLC-MS instrument equipped with an adjustable flow splitter and an additional pump delivering a make-up flow. Separations were performed on a semi-preparative RP-18 HPLC column (10 x 150 mm, i.d.). The compounds were identified as lysophopsphatidylcholine derivatives with fatty acid residues of variable length and degree of unsaturation by a combination of spectroscopic and chemical methods including ESI-MS, NMR, and GC analysis of the acyl residue after methanolysis. Interestingly, a mixture of phosphatidylcholine and lysophosphatidylcholine derivatives with related fatty acid composition has been detected in jojoba seeds [2]. On the other hand, such metabolites have not been reported hitherto in goji berries. These compounds may be useful as chromatographic markers for the analysis of goji products. At the same time they may give new hints with respect to the biological properties of goji berries and products. **References:** 1. Potterat O (2010) Planta Med. 76:7 – 19. 2. Leon F, Van Boven M, De Witte P, Busson R, Cokelaere M (2004) J. Agric. Food Chem. 52:1207 – 1211.

P173 Alkaloid content of nursery garden seedlings and natural seedlings of Norway spruce (*Picea abies*)

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Norway spruce (*Picea abies*) is an abundant and economically important plant species in northern Europe. Alongside of forestry, other ways of exploiting the wood are actively sought. Several potentially bioactive 2,6-disubstituted volatile piperidine alkaloids have been extracted from *P. abies* needles [1]. Since high intra-species variation seems to be typical for piperidine alkaloids in conifers [2], further investigations are needed. Spruce seedlings are traditionally planted in early summer but autumn planting is also taking place. The aim of this study was to compare the alkaloid contents of the nursery garden seedlings with the naturally regenerated, field grown seedlings over phenology (in spring and fall samples). Alkaloids from both needle and bark samples were extracted with solid phase partitioning methods and analyzed with GS-MS. Three alkaloid compounds, (+)-epidihydropinidine and two isomers of pinidine were detected in the samples. The field grown seedlings in both May and October had similar alkaloid yields than the nursery garden seedlings in May. In these samples (+)-epidihydropinidine was the main component in the needles and the only detected alkaloid in the bark. In September, the nursery garden seedlings contained 40% more alkaloids in the needles and 58% more in the bark than in May. Interestingly, also a different isomer of pinidine was detected in both needles and bark compared with that found in the natural seedling or nursery garden samples in May. These preliminary results suggest that the time of the year affects the total alkaloid amount and the quality of alkaloids in nursery garden seedlings. **References:** 1. Stermitz, FR et al. (1994) Phytochemistry 35:951 – 953. 2. Gerson EA, et al. (2009) Ann. Bot. 103:447 – 457.

P174 Development of whitening skin lotion from selected medicinal plants and its antityrosinase activity

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In Thailand, herbal cosmetics are becoming more popular especially for nourishing and whitening skin. In this study, *Artocarpus lakoocha* Roxb. (AL), *Glycyrrhiza glabra* Linn. (GG) and *Bombyx mori* Linn. (BM) were extracted and formulated as a herbal whitening skin lotion. Antityrosinase activity of each extract and of the lotion preparation were tested using the dopamine method [1]. Additionally, the stability study; tyrosinase inhibitory activity, TLC, colour and odour of the formulation kept at different temperatures (4 – 8, 25 – 28 and 45 °C) for 4 weeks were studied. The percentage of tyrosinase inhibition of AL, GG and BM at concentration of 1 mg/mL were found to be 87.88 ± 0.31 (IC_{50} = 50 µg/mL), 74.24 ± 0.62 (IC_{50}= 140 µg/mL) and 5.19 ± 0.61 (IC_{50} > 1000 µg/mL), respectively, while the activity of a reference standard kojic acid at the same concentration was 83.30 ± 0.51% (IC_{50}= 130 µg/mL). The whitening skin lotion containing 1.03% of AL, 0.42% of GG and 0.20%w/w of BM, exhibited antityrosinase activity at 98.60 ± 045%. During 4 weeks of storage at 4 °C, the antityrosinase activity, TLC, colour and odour of the lotion were not changed. Thus, the preparation containing AL, GG and BM which promoted high antityrosinase activity was appropriate as a herbal whitening skin lotion and should be stored at 4 °C for good stability. **Acknowledgements:** This study was granted by Thailand Research Fund for Industrial and Research Projects for Undergraduate Students (IRPUS) (No. I350A02003). **References:** 1. Kubo, I., Kinst-Hori, I. (1999) Planta Med 65: 19.9.

P175 Comparative GC/MS-profiling of triterpenes and stilbenes from three West African *Combretum* Species

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Combretum species are widely used medicinal plants in Africa and Asia for many medicinal purposes. Several phytochemical investigations of the genus focus mainly on pentacyclic triterpenoids [1,2] and various polyphenols like flavonoids and stilbenoids [3]. In spite of all the earlier work done, there is still lack of data on the chemistry of some of the West African species like *C. aculeatum, C. glutinosum* and *C. micranthum*. Herein, we present a preliminary comparative profiling of triterpenoids and stilbenoids from the dichloromethane fractions of leaf and stem bark of *C. aculeatum, C. glutinosum* and *C. micranthum* using GC-MS. The terpenoids and stilbenoids were identified by comparing their mass spectrum to those of ursolic acid and combretastatin A4, respectively.

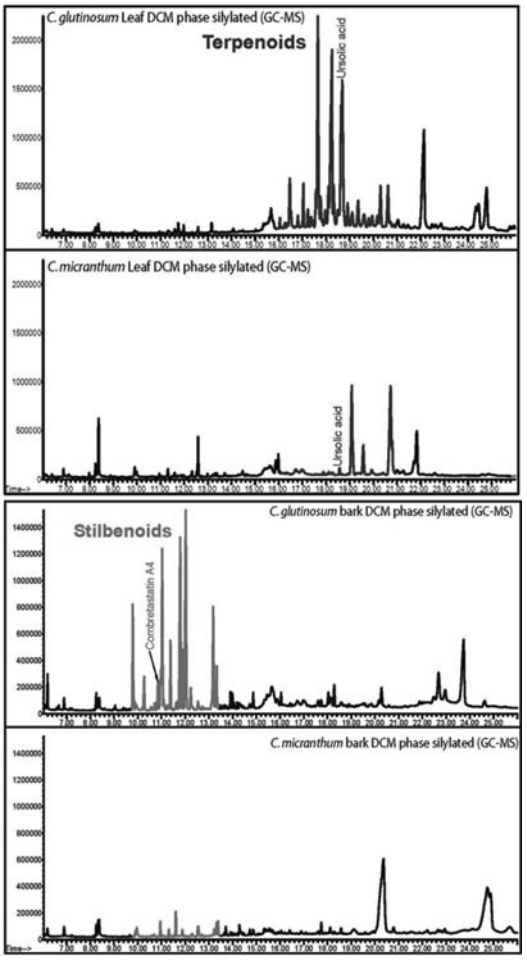

Fig. 1: GC/MS profil of triterpenes and stilbenes from *C. glutinosum* and *C. micranthum*.

Ursolic acid was identified in the leaf extracts of all the three species whereas combretastatin A4 was found in small amounts only in the bark extract of *C. glutinosum*. The chromatograms showed high variations between species and also between the two studied organs (leaf and bark). Triterpenes were mainly observed in the leaf extracts, while stilbenes were more concentrated in the bark extracts. The final results of this study will contribute to the selection of highly active species for medicinal uses and to their authentication. **References:** 1. Simon, G. (2003) Fitoterapia. 74: 339 – 344. 2. Litaudon, M. et al. (2009) J. Nat. Prod. 72: 1314 – 1320. 3. Pettit, G. R. et al. (1995) J. Med. Chem. 38: 1666 – 1672.

P176 Cytotoxic terpenoids from *Calocedrus macrolepis var. formosana*

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Calocetriol (**1**), diacetylcalocediol (**2**), abeoabietalignol (**3**), labdanecaryophyllic acid (**4**), and ferrugimenthenol (**5**) were isolated from the bark of *Calocedrus macrolepis* var. *formosana*. Compounds **1** and **2** are *seco*-abietane-type diterpenoids, and **3-5** with respective C_{30}, C_{35}, and C_{30} skeletons are novel meroterpenoids. Their structures were characterized by spectroscopic analysis. Of these compounds identified, **3-5** exhibited significant cytotoxic activities against human oral epidermoid carcinoma KB cells with IC_{50} values of 8.9 ± 0.1, 9.2 ± 0.4, and 9.0 ± 0.1 μM, respectively.

P177 New cystine knot peptides from a marine sponge *Asteropus* sp.

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Four novel peptides 1 – 4 were isolated from a marine sponge Asteropus sp., collected off the coast of Geoje Island, Korea. The primary structures of the peptides were determined by automated Edman degradation and corroborated by MALDI-TOF MS spectrometry. Six Cys residues in each peptide formed three intramolecular disulfide bonds and arranged in the pattern -C-C-CC-C-C-, which characterizes the cystine knot peptides similar to some conotoxins and spider toxins. The solution structures of the peptides by 1D and 2D NMR revealed the N-terminus of each peptide to be blocked by a pyroglutamic acid. Unlike the common cystine knot peptides, each peptide exhibited highly negative charge, which might contribute to different biological functions. Peptide 1 exhibited moderate suppressive effects on NO production at the concentration of 100 μM without significant cytotoxicity against the cells. In the neuraminidase inhibition assay, 1 showed mild activity with IC50 value of 181.69 μM.

Table 1: Cystine knot peptides from a sponge Asteropus sp.

Peptide	Sequence	Isoelectric point	Charge	Identity (%)a
ABU8 – 1	pEGCAFEGESCNVQFYPCCPGLGLTCIPGNPDGTCYYL	3.33	−4	100
ABU8 – 2	pEGCAFEGESCNVEFYPCCPGLGLTCIPGNPDGTCYYL	3.26	−5	97
ABU8 – 3	pEDCPGEGEQCDVEFNPCCPPLTCIPGDPYGICYII	3.09	−7	59
ABU8-a	pEGCAGPGEECIVGFYDCCPGYRCYPGDPGGICY	3.64	−4	60

[a] Sequence identity compared to ABU8 – 1

P178 The use of self-organizing maps in the study of phytotoxic activity of *Salvia* species (*Lamiaceae*)

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A screening study on the anti-germination activity of the fresh aerial part plant exudates of thirteen species of *Salvia* L.(*Lamiaceae*) against *Papaver rhoeas* L. and *Avena sativa* L. in Petri dish and pot experiments, evaluated on the basis of statistical indices both classical ones [1, 2] and proposed by our group has been carried out. All indices has shown that the greatest part of the *Salvia* exudates possess inhibitory activity on the two species germination and may be considered as promising mixtures of phytotoxins with a potential for development of natural product herbicides. Nevertheless, clear differences among the majority of the *Salvia* species are not highlighted. For this reason, a clustering algorithm based on Self-Organizing-Maps (SOMs) has been used to verify if this approach can single out small differences [3] that are not evident by classical methods. Mathematical elaborations have been applied before clustering to correctly feed the network. Raw data are passed to SOMs as matrix, so that if columns/rows contained too similar data, these columns/rows gave too few information to the process. A selection to these elements has been applied fixing some constraints about maximum and minimum of contained values, Variation Coefficient [4], and correlation between data columns. After elaboration, we clustered data for both target species and in both conditions getting four sets of results. In all these sets, the macroscopic evidences singled out by statistical indexes are present, but small difference can be qualitatively defined up to find 5 clusters in the *Salvia* species. **References:** 1. G. Chiapusio, A.M. Sanchez, M.J. Reigosa, L. Gonzalez, F. Pellissier (1997) J. Chem. Ecol. 23:2445 – 2453. 2. M.A. Ranal, D. Garcia De Santana (2006) Rev. Bras. Bot. 29:1 – 11. 3. M. Giacomini, C. Ruggiero, S. Bertone, L. Calegari (1997) IEEE TBME 44,12: 1185 – 1191. 4. M. Giacomini, J.L. McDermott, A.A. Giri, I. Martini, F.B. Lillo, O.E. Varnier (1998) J. Vir. Meth 73: 201 – 209.

P179 New bioactive compounds from *Derris malaccensis*, *Carissa carandas* and *Carissa spinarum*

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Derris malaccensis roots have been used locally as insecticide [1] whereas the stems of *Carissa carandas* and *C. spinarum* each have been used as bitter tonic [2]. In this study, these plants were subjected to chemical and biological investigations. Chromatographic separation of the extracts prepared from *Derris malaccensis* roots, *Carissa carandas* stems and *C. spinarum* stems led to the isolation of three new and eighteen known compounds. The structures of the isolates were determined on the basis of spectroscopic evidence (mainly NMR and MS). These compounds were then examined for their antioxidative property, antiherpetic potential and cytotoxicity. The structures of the new compounds were established as 6-oxo-dehydroelliptone (1), carandoside (2) and (6*S*,7*R*,8*R*)-7a-[(β-glucopyranosyl)oxy]lyoniresinol (3). Several of the isolated compounds showed interesting antioxidative activity, antiherpetic potential and cytotoxicity.

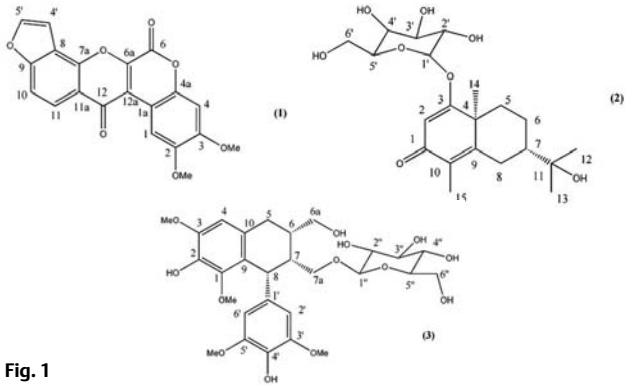

Fig. 1

Acknowledgements: The Thailand Research Fund for a 2005 Royal Golden Jubilee Scholarship, The 90th Anniversary Chulalongkorn University (Ratchadaphiseksomphot) Endowment Fund **References:** 1. Thasana, N. et al. (2001) Heterocycles 55:1121 – 1125. 2. Faculty of Pharmaceutical Sciences, Mahidol University (1995) Siam Medicinal Plants: National Wisdom. Amarin Printing and Publishing. Bangkok.

P180 Chemical constituents from the aerial parts of *Onosma erecta* (Boraginaceae)

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Onosma erecta is a Greek endemic plant belonging of Boraginaceae family. The methanolic extract of its aerial parts afforded after chromatographic separations simple hydroxylated compounds such as cis-3-(hydroxymethoxy)propenoic acid, 4-methyl-2-hydroxypentanone, both appearing for the first time as natural products, as well as tricarballylic acid along with the N-oxides of four pyrrolizidine alkaloids (PAs) (1 – 4). Structures 1, 3 and 4 reported in the present work as well as their free bases after reduction, are new natural products, while structure 2 has been determined before [1]. The chemical structures of all compounds have been determined by means of 1D and 2D NMR spectroscopy and ESI-MS.

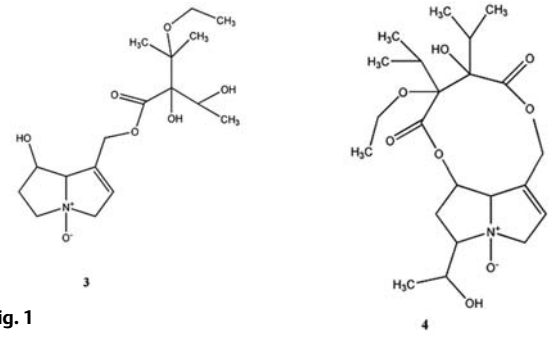

Fig. 1

References: 1. Logie, C. et al. (1994) Phytochemistry 37:43 – 109.

P181 Cytotoxic activity of hot water and ethanloic extracts of a poly-herbal preparation comprised of *Nigella sativa, Hemidesmus indicus* and *Smilax glabra*

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Recently we have reported that a decoction (hot water extract) comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizome) has a significant cytotoxic effect on human hepatoma cells (HepG2) and exerts protection against hepatocarcinogenic changes in rats [1 – 3]. The present study is a preliminary investigation to identify compounds responsible for the cytotoxic activity of this poly-herbal preparation. Hot water and ethanolic extracts of the poly-herbal preparation were tested for cytotoxicity on HepG2 cells using MTT and SRB assays. Concentrations of the extracts used varied between 300 µg/ml and 4800 µg/ml, and the cells were treated with each of these concentrations for 24, 48 and 72 h. HPLC profiles of thymoquinone (a known cytotocxic compound of *Nigella sativa*) and the hot water and ethanolic extracts were obtained by application of a reversed phase linear gradient of 80% water in methanol to 100% methanol. Both extracts showed a time and dose dependent cytotoxicity as measured by MTT and SRB assays. The hot water extract had marginally higher activity at lower concentrations (< 1200 µg/ml). HPLC profiles showed the presence of both polar and non-polar compounds in the two extracts. A higher aggregation of polar compounds was observed in the hot water extract. Thymoquinone was present in the ethanolic but not in the hot water extract. Thus, compounds other than thymoquinone appear to mediate the cytotoxicity of the hot water extract of this poly-herbal preparation. **Acknowledgements:** This work was funded by the National Science Foundation of Sri Lanka and grant from SIDA & SAREC. **References:** 1. Iddamaldeniya, S. et al. (2006) J Carcinog. 5: 6 – 11. 2. Thabrew, I. et al. (2005) Life sciences 77: 1319 – 1330. 3. SIddamaldeniya, S. et al. (2003) J Carcinog. 2: 6 – 12.

P182 Mutational breeding of *Centella asiatica* (L.) urban for medicinal purposes

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Centella asiatica (L.) Urban (Apiaceae) is one of medicinal plants widely used in Thailand. The plant contains active triterpenes, i.e. asiatic acid, madecassic acid, asiaticoside, and madecassoside which possess wound healing property. The Ministry of Public Health has recommended *C. asiatica* for wound healing[1]. Therefore, the demand of high quality C. asiatica raw material increased. The aim of this study was to improve lines of *C. asiatica* via polyploid induction for supplying to the herbal industry. *C. asiatica* terminal buds were soaked in colchicine concentration 0.025 – 0.400% for 12 – 36 h. After three passages of *in vitro* propagation by incubating the explants in liquid Murashige and Skoog (MS) medium with 4.45 µM thidiazuron (TDZ) for 15 days they have been transfered to semi-solid MS without growth regulator for 10 weeks. The surviving plants were investigated on ploidy level by flow cytometry and chromosome number. After that the selected control and polyploid plants were transplanted to soil under greenhouse conditions for 4 months. The plants were harvested and analyzed by HPLC. The optimum conditions for polyploidy induction were 0.025 – 0.100% colchicine for 12 – 24 h. We obtained 9 tetraploid and 56 mixoploid plants in M_1V_3 generation confirmed by flow cytometry and chromosome number. Tetraploid plants (M_1V_3) grew slower than mixoploid and control diploid plants and failed to grow *ex vitro*. The mixoploid plants (M_1V_3) demonstrated significantly higher phytomass and triterpenoid contents than those of the control diploid plant. The mixoploid plants (M_1V_7) showed genetic stability which were confirmed by flow cytometry. **Acknowledgements:** Mahidol University, TRF-Master Research Grants (MAG-WI515S098) **References:** 1. The Ministry of Public Health (2006) Essential Drug List of Herbal Medicinal Products. The agricultural co-operative federation of Thailand, Bangkok.

P183 The novel isoflavonoids inhibit RANKL-induced osteoclast formation

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Isoflavonoids are compounds characterised by structurally features which are similar to the mammalian estrogens and have received considerable attention for their preventive actions on bone loss. In the course of this study we synthesized novel isoflavone derivatives and examined the activities in bone cells. We found that the novel isoflavone derivatives markedly inhibited the receptor activator of nuclear factor kappa B ligand (RANKL) plus macrophage colony stimulating factor (M-CSF)-induced osteoclastic differentiation from bone marrow stromal cells and RAW264.7 macrophage cells. Isoflavone derivatives did not affect the cell proliferation and differentiation of human cultured osteoblasts. Our data suggest that the novel isoflavone derivatives inhibit osteoclastogenesis from bone marrow stromal cells and macrophage cells via attenuated of RANKL-induced p38, JNK and NF-*k*B activation.

P184 Camphoratins A-J, potent cytotoxic and anti-inflammatory triterpenoids from the fruiting body of *Taiwanofungus camphoratus*

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Ten new triterpenoids, camphoratins A-J (1 – 10), along with twelve known compounds were isolated from the fruiting body of *Taiwanofungus camphorates*. Their structures were established by spectroscopic analysis and chemical methods. Compound 10 is the first example of a naturally occurring ergosteroid with an unusual 14β proton configuration. Compounds 2 – 6 and 11 showed moderate to potent cytotoxicity with EC_{50} values ranging from 0.3 to 3 μM against KB and KB-VIN human cancer cell lines. Compounds 6, 10, 11, 14 – 16, 18 and 21 exhibited potent anti-inflammatory NO-production inhibition activity with IC_{50} values of less than 5 μM, which was more potent than the nonspecific NOS inhibitor N omega-nitro-L-arginine methyl ester (L-NAME). Therefore, camphoratin analogs represent a unique class of triterpenes with cytotoxic and anti-inflammatory dual-functions, and have a good potential to be developed into novel anticancer drugs or anticancer synergistic agents.

P185 Biosynthetic studies of the cyclocarbamate SB-253514

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Lipoprotein associated phospholipase A_2 ($LpPLA_2$) is responsible for hydrolysis of modified oxidized phospholipids from low density lipoprotein causing the release of pro-inflammatory lyso-phosphatidyl choline and oxidatively modified fatty acids. Inhibition of $LpPLA_2$ is therefore considered a novel therapeutic strategy in the treatment of diseases that have an inflammatory component such as atherosclerosis. One of these metabolites is SB-253514, a glycosylated lipopeptide produced by *Pseudomonas fluorescens* [1]. The structure consists of a 5,5-bicyclic carbamate moiety, connected via two carbons and one nitrogen atom to myristic acid, which is in turn glycosylated with rhamnose. During a genomic-driven screening for lipopeptide gene clusters, we recently came across the corresponding gene cluster of SB-253514 which involves unexpectedly a two modular NRPS gene cluster. Both, its unique structure and its powerful Lp-PLA_2-inhibition prompted our study of its biosynthesis. Of particular interest is hereby the modification of the putative involved second amino acid aside proline and the formation of the cyclocarbamate structure. This poster will describe our recent efforts to elucidate the biosynthesis using knockout strains as well as stable isotope feeding experiments.

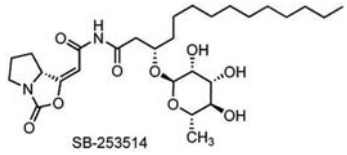

Fig. 1

Acknowledgements: Financial support by the German Research Foundation (DFG FOR 854) is gratefully acknowledged. **References:** 1. Pinto et al. (2000) Bioorg. Med. Chem. Letters 10:2015 – 2017.

P186 Comparative analysis of amino acid composition of some species of *Pulmonaria*

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Three species of Pulmonaria are prevailing in Eurasia, namely – P. officinalis L., P. obscura Dumort and P. mollis Wulf ex Hornem [1]. All of these are the sources of polysaccharides combined with vegetable proteins. This polysaccharide-protein complex has a pharmacological activity, especially as a chelating agent for Fe3+-ion than the antianemic activity of extracts made from these plants can be explained [2]. The aminoacid content in aerial parts of these plants have been defined by the aminoacid analyzer "Hitachi". The analyzer had been calibrated by using a composition of standard samples of amino acids. The sample solutions made from each plant were put into the column of the analyzer which was filled up with a sulphated copolymer (styrene with divinylbenzene). The elution of aminoacids has been done by buffer solutions with different pH (3,3 – 4,9). Aminoacids were detected by photometry after an effluent was reacted with ninhydrin. As a result was established the content of 10 replaceable aminoacids, such as: – alanine (7,1 – 7,3%), arginine (6,0 – 6,3%), asparagic acid (10,2 – 10,3%), gystidine (2,0 – 2,3%), glycine (6,2 – 6,5%), glutamic acid (12,4 – 13,1%), proline (4,9 – 6,1%), serine (5,0%), tyrosine (3,2 – 3,5%), cysteine (0,2 – 0,3%) and 7 irreplaceable aminoacids, such as: valine (6,3 – 6,5%), isoleucine (5,2 – 5,3%), leucine (10,6 – 10,8%), lysine (5,6 – 6,3%), metionine (1,2 – 1,3%), treonine (5,0%), phenylalanine (6,7 – 7,1%). The conclusion that in aerial parts of these plants contain 11 – 13% aminoacids. Qualitative and quantitative structure is not dependent on the species of plant. All three investigated species of Pulmonaria can be considered as a source of essential amino acids. **References:** 1. Tutin, T. et al. (1976) Flora Europeae vol. III. Cambridge Univ. Press, Cambridge. 2. Kruglov D. (2007) 3 rd Inter. Conf. "Basic Science for Medicine", SEP 28, Novosibirsk, RUSSIA.

P187 Phytochemical profile and *in vitro* activities of *Capsella bursa-pastoris* L. extracts

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Capsella bursa-pastoris (Cruciferae) leaves and roots have been used as an edible vegetable in some countries. Moreover, this species is applied in traditional medicine, as anti-bleeding, anticancer and wound-healing agents and to treat diabetes and fever. Phenolics, namely flavonoids, are well known bioactive compounds. Thus, the plant aerial parts were subjected to methanol (MeOH) and methanol:water ($MeOH/H_2O$) extractions and five flavonoids, including free and glycosylated derivatives, were identified and quantified by HPLC-DAD. The major compound found in both extracts was kaempferol-3-*O*-rutinoside. Cell free assays were used to evaluate the plant potentiality as radical scavenger, against 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), superoxide ($O_2^{\cdot-}$) and nitric oxide ($^{\cdot}NO$), as well as to determine their capacity for inhibition of both lipid peroxidation and acetylcholinesterase (AChE) activity. A dose-dependent response was observed in all assays and, in a general way, $MeOH/H_2O$ extract proved to be more efficient antioxidant and AChE inhibitor. **Acknowledgements:** Clara Grosso thanks the Fundação para

a Ciência e a Tecnologia for the Post-Doc fellowship (SFRH/BPD/63922/2009)

P188 Biotransformation of cardenolide-precursors by *Saccharomyces cerevisiae*

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Digitalis plants are the most important source for the extraction of cardenolides used in the treatment of cardiac insufficiency in humans. In order to study putative biosynthetic genes and enzymes we intend to establish a yeast system were glycosylated enzymes and P450 enzymes involved in cardenolide biosynthesis can be functionally expressed. For example, we were unable to express progesterone 5α-reductase, a glycoprotein, in *E. coli*. We also aim at the reconstruction of consecutive steps of the putative cardenolide pathway in yeast to produce commercially unavailable pregnanes from bulk precursors such as progesterone. We already cloned three genes encoding enzymes that are supposed to transform pregnanes during cardenolide formation: the Δ5 – 3β-hydroxysteroid dehydrogenase (3β-HSD, EC 1.1.1.51) from *Digitalis lanata*, which converts pregnenolone to isoprogesterone [1], the *Arabidopsis thaliana* Δ4,5-steroid 5β-reductase (At5β-StR, EC 1.1.1.145/1.3.1.23) which reduces progesterone to 5β-pregnane-3,20-dione [2] and an enzyme capable of hydroxylating pregnanes in position 21, the steroid-21-hydroxylase (Cyp21A1, EC 1.14.99.10) from *Mus musculus*. These genes were cloned into the Gateway® pYES-DEST52 vector system and then transformed into *Saccharomyces cerevisiae* strain BY4741a. Our data indicate, that the Cyp21A1 gene is functionally expressed in the recombinant yeast strain as it is capable of transforming progesterone into 21-hydroxyprogesterone. Product formation was demonstrated by GC-MS and TLC. Further studies are ongoing to show the functional expression of 3β-HSD and At5β-StR in yeast and to cotransform and coexpress these genes. **References:** 1. Herl, V. et al (2007) Planta Med 73(7): 704 – 10. 2. Herl, V. et al. (2009) Biochimie 91:517 – 525.

P189 Phenolics from *Phagnalon* inhibit oxidation of deoxyguanosine *in vitro*

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It is well known that oxidizing agents can modify the structure of different key biomolecules. Proteins and nucleic acids may suffer alterations which sometimes are detrimental for their function, or lead even to the rupture of the molecule. Specially, oxidative damage of nucleic acids is related to inflammation, cancer, and neurodegenerative diseases [1]. The present communication deals with the protective effect that some phenolic principles exert on the oxidation of the nucleoside, 2′-deoxyguanosine (dG), driven by either hypochlorite or activated human neutrophils. The hydroxycinnamate, 3,5-dicaffeoylquinic acid (DCA), the terpenic hydroquinone, 2-isoprenylhydroquinone-1-O-glucoside (IHG), and the flavonoid, chrysoeriol-7-O-glucoside (C7G) were isolated and identified by ourselves from *Phagnalon rupestre* [2] and *P. saxatile* (Asteraceae). Oxidation of dG to 8-hydroxy-dG and subsequent degradation products was performed with 0.9% NaOCl, 10% active chlorine, or with neutrophils stimulated with phorbol ester. Samples were analyzed by RP18- HPLC eluting with 10% MeOH, buffered with ammonium formate pH 5.0. The most active compound on NaOCl oxidation was IHG (74% inhibition at 0.1 mM), whereas C7G reached the highest efficacy in the leukocyte model (50% inhibition at 0.015 mM). **Acknowledgements:** Spanish Ministry of Science and Technology (SAF 2006 – 06726) and Generalitat Valenciana (GV 353/06). **References:** 1. Halliwell, B. (2007) Biochem. J. 401:1 – 11. 2. Góngora, L. et al. (2002) Planta Med. 68:561 – 564.

P190 New natural anthraquinones from cochineal (*Dactylopius coccus*)

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Cochineal is one of most well known organic red dyes. *Dactylopius coccus* Costa (Dactylopiidae) is a scale insect that is used as the source of the dye known as Mexican cochineal. Cochineal is today a natural food colorant (E120) and is also used in cosmetics and in pharmaceutical industry. Although cochineal has been used in art objects (textiles and paintings) for centuries, its exact chemical consistency is not well clarified [1] except for carminic acid which is the major component and kermesic and flavokermesic acids. Several minor components (typically less than 5% of the coloring material) remain unknown even though their presence is characteristic for the origin of cochineal. Chemical investigation of the methanol extract of the dried insects, after subsequent HPLC chromatographic separations, led to the isolation and structure elucidation of two new anthraquinones (**1** and **2**), along with the known compounds karminic acid, kermesic acid and flavokermesic acid. The structures of the new compounds were elucidated on the basis of their NMR and MS data. Interestingly, the new compounds were detected as minor constituents of historical art objects.

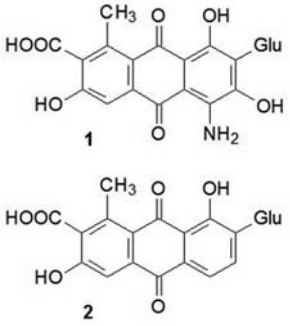

Fig. 1: New anthraquinones

Acknowledgements: The project was funded by the General Secreteriat for Research and Technology of Greece (Program PENED). **References:** 1. Peggie, D.A., et al (2008) Microchimica Acta 162: 371 – 380.

P191 Chemical constituents and their anti-inflammatory activities from *Korthalsella japonica*

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Korthalsella japonica is one kind of small parasitic subshrubs and mainly distributed in tropical Asia, Australia to Polynesia. The major components were flavanones and chromones, which were similar with Viscum coloratum. Among them, five new compounds, methyl-4-O-cinnamoyl-quinate, korthalin, korjaponin, 6′, 4″-dihydroxy-2′, 3″-dimethoxy- chalcone-4′-O-β-D-glucoside and viscolin 4′,4″-di-O-β-D-glucoside were isolated for the first time from the natural source. The isolated compounds were evaluated for their bioactivities. The results of anti-inflammatory experiments showed that viscolin has significant inhibitory activities in the release of superoxide anion and elastase in fMLP/CB-activated human neutrophils. Korthalin, 6′, 4″-dihydroxy-2′, 3″-dimethoxychalcone-4′-O-β-glucoside and viscolin 4′,4″-di-O-β-D-glucoside showed no or minor anti-inflammatory activities.

P192 Molecular characterization of polyketide synthases from *Ginkgo biloba*

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Ginkgolic acid (GA), a constituent in *Ginkgo biloba*, is regarded as a promising drug candidate because this compound inhibits protein SUMOylation [1]. GA is a salicylic acid derivative with a long carbon side chain, and is considered to be biosynthesized via polyketide pathway [2]. To obtain a cDNA for a polyketide synthase (PKS) involved in GA biosynthesis, we performed degenerate PCR and rapid amplification of cDNA ends reactions. Consequently, two cDNA clones encoding PKSs, named GbPKS1 and GbPKS2, were cloned and sequenced. The amino acid sequence of GbPKS1 showed > 90% identity with chalcone synthase (CHS), the ubiquitous plant PKS catalyzing the first committed step of flavonoid biosynthesis. Contrary, the amino acid identity between GbPKS2 and CHS was relatively low (~40%), and Thr197, a conserved residue in the CHS active site, was substituted into Gly in GbPKS2. The catalytic functions of GbPKSs were characterized with bacterially expressed recombinant enzymes. As expected, the recombinant GbPKS1 catalyzed chalcone production from 4-coumaroyl-CoA and three molecules of malonyl-CoA. On the other hand, GbPKS2 did not accept 4-coumaroyl-CoA as a starter substrate, but selected palmitoleoyl-CoA, the expected starter substrate for GA biosynthetic reaction, to produce triketide pyrone via two-step condensation with malonyl-CoA. GbPKS2 did not synthesize GA, but this enzyme is of interest because long-chain acyl-CoA utilizing PKSs have been rarely found to date. In addition, RT-PCR analysis demonstrated GbPKS2 is specifically expressed in fruit pulp tissue where GA is accumulated. **References:** 1. Fukuda, I. et al. (2009) Chem. Biol. 16:133 – 140. 2. Austin, MB. et al. (2003) Nat. Prod. Rep. 20:79 – 110.

P193 New furoquinoline derivative and anti-inflammatory constituents from *Zanthoxylum avicennae*

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Zanthoxylum avicennae (Lam.) DC (Rutaceae) is an evergreen shrub distributed in Vietnam, Philippines, southern China, and Taiwan [1]. A decoction of its stems is used as a stomach tonic and as a counter-poison to snake bite [2]. Previous studies of this plant have reported the isolation of flavonoids, alkaloids, coumarins, and terpenoids. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* anti-inflammatory activity, and *Z. avicennae* has been found to be one of the active species. The MeOH extract of *Z. avicennae* inhibited fMLP/CB-induced superoxide anion generation and elastase release in a concentration-dependent manner with IC_{50} values of 6.34 and 15.32 μg/mL, respectively. In our previous study, 8 new compounds and 18 known compounds have been isolated and identified from the stem wood of *Z. avicennae*. Further investigation of the EtOAc-soluble fraction has led to the isolation of a new furoquinoline derivative, zanthoavicenine (**1**) and 7 known compounds. The structure of the new compound **1** was determined through spectral analyses including extensive 2D NMR. This report describes the structural elucidation of the new compound **1** and the anti-inflammatory activities of the isolates. **References:** 1. Ho, T.C. (2007) Endemic Species Research 9: 29 – 52. 2. Perry, L.M. (1980) Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. The MIT Press: Cambridge, p. 370.

P194 Antiinflammatory profile of *Harpagophytum procumbens* crude extract and after its external metabolic activation in THP-1 monocytic cells *in vitro*

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Harpagophytum procumbens (Devil's Claw; DCE) has demonstrated anti-inflammatory effects in animal models and clinical efficacy in osteoarthritis. In the present investigations we used an in vitro model based on human THP-1 cells to evaluate the effect of crude DCE and after metabolic activation (DCEm) with S9 mix from Aroclor-induced rat livers on a panel of 12 inflammatory cytokine/chemokine using Multi-Analyte ELISArray Kits (SABiosciences, Frederick, USA) and the single cytokine ELISAs (TNF-α, IL-6, IL-8). Cytokine productions were induced in THP-1 cells by 25 μg/ml LPS and the effect of DCE dry extract (DER 1.5 – 3:1; 60% ethanol, Bioforce AG) at concentrations (0 – 250 μg/ml) was investigated after 24 h treatment. In addition, the effect of DCE after simulated liver metabolism by S9 mix was compared. As positive controls 10 – 7 M dexamethasone and 10 μg/ml cyclophosphamide were used. All concentrations of DCE exerted no cytotoxicity as measured by WST-1 assay (IC50 = 1.25 mg/ml). Both DCE extracts inhibited dose-dependently the secretion of TNF-α. The highest concentration (250 μg/ml) inhibited the release of TNF-α by about 80%. IC50 beeing 98 μg/ml and 47 μg/ml for DCE and DCEm. IL-8 and IL-6 secretion were moderately inhibited by about 25% at concentration of 50 – 100 μg/ml of DCE. The inhibitory effect on TNF- α release in THP-1 cells have been increased in the presence of metabolic activated DCEm as mirrored in their IC50 values. Our data suggest that proprietary DCE is an inhibitor of proinflammatory cytokines and that metabolic activation seems to play an important role in this effect.

P195 LC-SPE-NMR-MS analysis of *Strychnos usambarensis* fruits from Rwanda

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Strychnos usambarensis is a Rwandan tree of *Loganiaceae* family, whose roots and leaves had been widely investigated. They contain a huge number of indolomoterpenic tertiary alkaloids possessing antimalarial [1] and antitumoral properties [2]. Nevertheless, almost no studies had been carried on its fruits until now, except one which showed that demethoxycarbonyl-3,14-dihydro-gambirtannine was the major alkaloid in the apolar extract [3]. Alkaloids and glucoalkaloids have been successfully characterized in fruits of *Strychnos usambarensis*. Two distinct extracts (methanolic and ethyl acetate) were realized and studied by a hyphenated analytical method, combining high-performance liquid chromatography coupled with solid-phase extraction (SPE), mass spectrometry (HPLC – MS) and NMR spectroscopy. The HPLC gradient system consisted of acetonitrile and heptanesulfonic acid. The analytes were then accumulated by repetitive absorption on SPE cartridges. The trapped compounds were subsequently eluted with CD_3CN for acquisition of NMR data and analysed by electrospray ionization and time of flights mass spectrometry (ESI/Q – TOF/MS). This led to identification among others of palicoside in the methanolic extract, a glucoalkaloid never described in *Strychnos usambarensis* [4], and akagerine in the ethyl acetate extract, an alkaloid already known in *Strychnos usambarensis* roots [5,6].

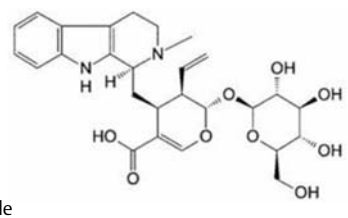

Fig. 1: Palicoside

Acknowledgements: This research was supported by the Belgian National Fund for Scientific Research (FNRS – grant N 3.4533.10). M.C. and M.F. are respectively Research Fellow and Senior Research Associate from the FNRS. **References:** 1. Frédérich, M. et al. (2004) Planta Med. 70(6):520 – 525. 2. Balde, E-H., S. et al. (2010) Int. J. Oncol. 36(4):961 –

965. 3. Angenot, L. et al. (1978), J. Pharm. Belg. 33(5):284 – 286. 4. Tits, M. et al. (1996) Planta Med. 62(1):73 – 74. 5. Angenot, L. et al. (1975) Tetrahedron Lett. 16(16):1357 – 1358. 6. Brandt, V. et al. (2001) Phytochemistry 57(5):653 – 659.

P196 Isohexenylnaphthazarins from *Lithospermum canescens* (Michx.) Lehm. and *Arnebia euchroma* (Royle) Jonst. *in vitro* culture

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The alkannins, shikonins and related isohexenylnaphthazarin compounds are natural lipophilic red pigments. They occur in many species that belong to the Boraginaceae family. Because of the importance of the shikonin-related compounds for pharmaceutical and cosmetic applications, the species from boraginaceous, among them *Lithospermum* and *Arnebia* genus, are investigated using biotechnology approaches. Our investigation aimed at isolating and elucidating structures of pigments of shikonin type and another compounds from hairy roots of *Lithospermum canescens* and *Arnebia euchroma* callus and cell suspension culture. Four alkannines: isobutylalkannin, β,β-dimethylacrylalkannin, acetylalkannin and β-hydroxyisovalerylalkannin, together with ACS and shikonofuran C and shikonofuran D were isolated and determined from the n-hexane extract of *L. canescens* transgenic roots by modern spectral means (MS, NMR), while several phytosterols, esterified acids as well as hydrocarbons were characterised by GC-MS. This is the first detailed report on chemical composition of transgenic roots from species of *Lithospermum* genus. The phytochemical investigation of n-hexane extract from callus and cell suspension culture of *A. euchroma* with respect to pigment compounds resulted in the isolation of the following alkannin derivatives: deoxyalkannin, alkannin, acetylalkannin, isobutyrylalkannin, β-hydroxyisovalerylalkannin, α-methylbutyrylalkannin, propionylalkannin, teracrylalkannin. Their structures were determined by MS and NMR methods. All mentioned pigments except deoxyalkannin were isolated for the first time from *Arnebia in vitro* cultures. Isobutyrylalkannin and propionylalkannin are reported in the present work as novel metabolites within the *Arnebia* genus. **Acknowledgements:** This study has been financially supported by a Polish Ministry of Science and Higher Education project 3499/B/P01/2007/33.

P197 New alkylresorcinols from the bulbs of *Urginea indica* L. collected in Nigeria

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Alkylresorcinols constitute a category of lipophilic polyphenols found in specific plant families. In this work, several alkylresorcinols presenting the substitution pattern of structures 1 and 2, were isolated from the dichloromethane extract of the air-dried bulbs of *Urginea indica* L. (Liliaceae) collected in Nigeria. Compounds of structure **1** with n = 17, 19 and 21 as well as compounds of structure **2** with n = 12, 14 and 17 are new. In addition, several other known compounds were identified: polyunsaturated fatty acids and squalene. The structures of the new compounds were elucidated on the basis of their NMR and MS data. The exact number of homologues in each series **1** and **2** and the exact length of the side chain was found using GC-MS analysis. The new compounds of structure **2** are lower homologues of cichoriols previously reported in Cichorium spinosum [1].

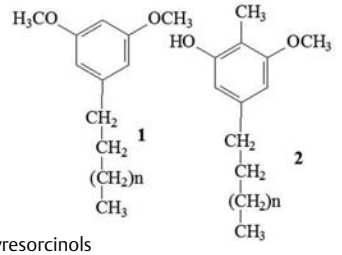

Fig. 1: New alkyresorcinols

References: 1. Melliou, E. et al. (2003) J. Agric. Food Chem. 128:1620 – 1625.

P198 *In vitro* protection against hydrogen peroxide-induced oxidative stress and cell death in ARPE-19 cells by Curcumin

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Curcumin, the main active phenolic compound present in turmeric, possesses a wide range of pharmacological properties including antioxidant activities [1]. Although the exact etiology of age-related macular degeneration (AMD), the leading cause of blindness in the elderly, is not known, the retinal pigment epithelium (RPE) is considered a primary target via oxidative cell damage [2]. The purpose of this study is to determine if curcumin protects RPE cells against H_2O_2-induced oxidative stress and cell death. Cultured RPE cells (ARPE-19) were pretreated with curcumin (25 – 100 μM) for 1 h before washing and incubation with H_2O_2 (100 – 600 μM) for 24 h. The percentage of viable cells was determined using trypan blue dye exclusion. Nuclear chromatin condensation, a hallmark of apoptosis, was determined using Hoechst 33342 staining and then visualized by fluorescence microscopy. The results demonstrated that treatment of ARPE-19 with H_2O_2 resulted in dose-dependent cell death and an increase in the number of cells containing condensed nuclei in a time-dependent manner. Pretreating cells with 100 μM curcumin for 1 h prior exposure to 600 μM H_2O_2 notably attenuated cell death and decreased the percentage of cells containing condensed nuclei. These results indicate that *in vitro*, curcumin provides substantial protection against H2O2-induced cell death and nuclear condensation in ARPE-19 cells and may therefore, be useful as a potential agent in the treatment of disorders associated with oxidative stress-induced cell damage such as AMD. **Acknowledgements:** This work was supported partially by research grants from Suranaree University of Technology and Mahidol University. **References:** 1. Sharma, OP. (1976) Biochem. Pharmacol. 25:1811 – 1812. 2. Winkler, BS. et al. (1999) Mol. Vis. 5:32.

P199 Cannabinoid receptor activity of polyacetylenes and polyenes from *Echinacea pallida*

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Echinacea products are among the best-selling herbal preparations in the western world, mainly used for the treatment of upper respiratory tract infections and the common cold. The fractionation of a supercritical CO_2-extract of *Echinacea pallida* (Nutt.) Nutt. roots led to the isolation of seven polyacetylenes and polyenes. The structures were determined by UV, NMR and MS in comparison with data from literature [1], [2]. The aim of our recent study was to investigate the potential cannabinoid receptor activity of the isolated compounds on human CB_1R and CB_2R and compare those results with prior studies [3]. So far only alkamides have been shown to bind to the human CB_2R with high affinity [4], [5]. As cannabinoid receptors couple to G_i/G_0 proteins, the activity of the intrinsic GTPase can be measured using the radioactively labelled GTP derivative [γ-^{32}P]GTP. The amount of ^{32}Pi release allows a conclusion to be drawn about the pharmacological behaviour of the tested compound [6]. Dose-response curves focusing on CB_2R activity were drawn for (8Z)-pentadeca-8,11-diene-2-one and (8Z)-pentadeca-8-ene-2-one, resulting in logEC_{50} values in the micromolar range (−5.66 ± 0.27 and −5.02 ± 0.26, respectively) and a stimulation of GTPase activity of 10%± 6 and 14%± 2, respectively as compared to basal GTPase activation. This is in contrast to some Echinacea alkamides that stimulated GTPase activity up to 136 ± 14%. Observing no significant activity at either receptor, these findings support the hypothesis that polyacetylenes and polyenes do not affect the immune system via the cannabinergic system [4]. **References:** 1. Pellati, F. et al. (2006), Phytochemistry, 67(13):

1359 – 1364. 2. Bauer R. et al. (1988), Planta Med., 54(5): 426 – 430. 3. Egger, M. et al. (2008), Chemistry, 14(35): 10978 – 10984. 4. Woelkart, K., Bauer R. (2007), Planta Med., 73(7): 615 – 623. 5. Raduner, S. et al. (2006), J. Biol. Chem., 281(20): 14192 – 206. 6. Wenzel-Seifert, K. et al. (1999), J. Biol. Chem., 274(47): 33259 – 33266.

P200 The effect of Huang Lian Jie Du Tang and its single herbal components on NO production in RAW 264.7 macrophages, cell viability and proliferation

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The Chinese herbal mixture Huang Lian Jie Du Tang is used in traditional Chinese medicine to clear heat and relieve toxicity. According to Western medical terminology it is used against eczema and skin diseases, chronic inflammatory intestinal diseases such as Morbus Crohn or Colitis ulcerosa, as well as against psychiatric disorders like schizophrenia and depression. Recent studies on the mixture showed that it is influencing LTB4 formation and NO production [1]. The aim of this study was to determine the inhibitory activity of the decoctions of the mixture and of the single herbs on NO production in RAW 264.7 macrophages as well as their influence on cell viability and proliferation using an XTT assay. The results of the mixture were compared with the activity of the single herbs. In addition, the decoctions were fractionated using liquid-liquid extraction with n-heptane, dichloromethane, ethyl acetate and n-butanol, and the gained fractions were also tested. The study showed that only the dichloromethane fraction of the decoction of Radix Scutellariae was able to inhibit NO production (Fig.1). The XTT assay (performed with CCRF-CEM leukemia cells) demonstrated no or only little influence of the decoctions and fractions on cell proliferation and cell viability. Further investigations are in progress to prove whether the inhibition of NO production is due to baicalein which is a major compound of Radix Scutellariae, or whether also other constituents contribute to the effect.

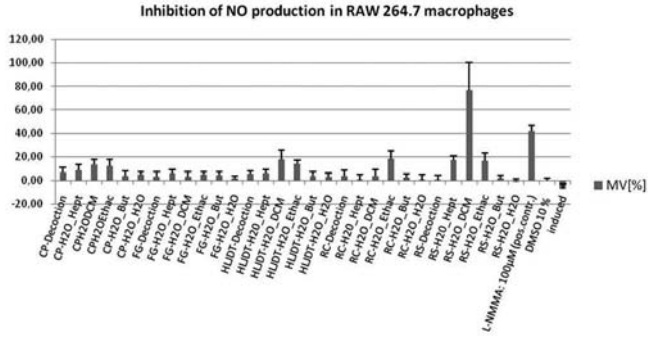

Fig. 1: Inhibition of NO production in RAW 264.7 macrophages

Acknowledgements: The investigations were financially supported by the Austrian Federal Ministries of Health and of Science and Research within the research project "TCM and Age Related Diseases". **References:** 1. Zheng, H. (2009) J. Pharm. Pharmacol. 61:1699 – 1707.

P201 Isolation of iridoid glucosides from Euphrasiae herba using droplet counter-current chromatography

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Euphrasia officinalis is a hemiparasite belonging to the family of Orobanchaceae. It grows unattached or attached to various host plants in grasslands of Eurasia. Traditionally but not evidence based used for various eye sufferings it is also known as eyebright (Augentrost). Typical active constituents beside phenolics such as phenylethanoids, lignans, phenolic acids and flavonoids are iridoids like aucubin, catalpol, euphroside, melampyroside, boschnaloside and others. Interestingly, the transfer of iridoids from the host plant to Euphrasia species was described, too [1]. For isolation of iridoid glucosides from Euphrasiae herba a practicable droplet counter-current chromatography method was developed according to iridoid isolations from *Premna japonica* [2]. On the basis of TLC pre-experiments the solvent system $CHCl_3$:MeOH:H_2O:n-PrOH 9:12:8:1 was used in descending mode. The iridoid enriched sample was gained from a methanolic extract by separation against apolar solvents and by a column chromatography with Al_2O_3. The structures of isolated iridoids were established by spectroscopic evidence. Thus the results obtained provide possible marker compounds useful for future pharmacopoeial analytics as well as for pharmacological investigations on eye diseases. **Acknowledgements:** Leipzig University, Tobias Liebold **References:** 1. Rasmussen, L.S. et al. (2006) Biochem. Syst. Ecol. 34: 763 – 765. 2. Otsuka, H. et al. (1991) Phytochemistry 30: 1917 – 1920.

P202 Phytochemical study of the seed shell of the *Myrica gale* by HPLC-SPE-NMR

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Myrica gale L. (Myricaseae), commonly known as sweet gale and bog myrthle [1], is a shrub widely distributed at high latitudes in the northern hemisphere [2]. It is known that the fruits of *M. gale* were used as a predominant beer additive before the utilization of hops (*Humulus lupulus* L.) in the tenth century [1].

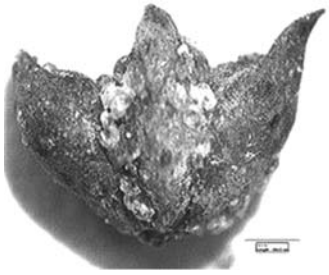

Fig. 1

HPLC-SPE-NMR is a hyphenated technology developed in the 1990th for separation of components and elucidation the structures from a mixture. We applied HPLC-SPE-NMR and LC-MS to elucidate the structures of 13 phenols, inculding seven dihydrochalcones, four flavonols and a chalcone, from 10 mg extract of *M. gale* seed shell. Seven compounds were isolated from *M. gale* for the first time, and the rare *C*-methyl dihydrochalcones may serve as the chemotaxonomic markers for the *Myrica* genus. The occurrence in the seed shell sugguests involvement of the compounds in the protection against pathogenic microorganisms or herbivores. **Acknowledgements:** Authors acknowledge the Max Planck Society (MPG) and the International Max Planck Research School (IMPRS) for the financial support. **References:** 1. Behre, KE (1999) Veg. Hist. Archaeobot. 8: 35 – 48. 2. Skene, KR. et al. (2000) J. Ecol. 88: 1079 – 1094.

P203 Analysis and stability of the constituents of *Curcuma longa* and *Harpagophytum procumbens* tinctures by HPLC-DAD and HPLC-ESI-MS

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In continuation of our studies on the constituents and stability of herbal drug preparations [1,2], the present work reports the qualitative and quantitative profiles of the constituents of tinctures of *Curcuma longa* ground rhizomes and *Harpagophytum procumbens* radices and their chemical stability. The tinctures were prepared according to the European Pharmacopoeia XI [3]. The ratio between the dry plant material and the final tincture was 1:5. Batches (about 10 ml) of each tincture were subjected to accelerated thermostability testing at 40 °C. during a 6-month period. Two methods based on liquid chromatography with diode array detection (HPLC/DAD) coupled to an electrospray ionization (ESI) interface were developed for the determination of constituents in both the tinctures. The constituents were identified by UV and MS spectral data and further confirmed by comparison with reference data or authentic samples. Isolations and NMR analyzes were carried out in order to confirm the presence of artifacts where necessary. Constituents responsible for the pharmacological activity (iridoids expressed as harpagoside, ver-

bascoside and curcuminoids) were considered for the quantitative analysis. The developed assays were simple and effective and permitted the quality control of both tinctures. The methods were validated for linearity, limits of detection and quantification, precision, including time precision, and accuracy. All validation criteria were fulfilled. Concerning the stability studies, results showed that *H. procumbens* tincture remained stable under the conditions applied, whereas curcuminoids in *C. longa* tincture were reduced to 30% of their initial amount in the accelerated thermostability test. **References:** 1. Bilia, AR et al. (2001) Chromatographia 53: 210 – 15. 2. Bilia, AR et al. (2007) J. Pharm. Biom. Anal. 44: 70 – 78. 3. Eudra/Q/92/021: 'Note for Guidance on stability testing of new active substances and medicinal products'.

P204 Furanocoumarins from root of *Peucedanum ruthenicum* (Apiaceae)

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Peucedanum ruthenicum M. Bieb. (Apiaceae) is one of the *Peucedanum* complexes. This complex has some problem in systematic classification. *P. ruthenicum* aerial parts and root were collected from Mazandaran Provinces in the north of Iran on October and deposited with (Salimian 39) Voucher No. at private herbarium of Dr. Hossain Akhani in Dept. of Plant Sciences, Faculty of Sciencses, University of Tehran, Tehran, Iran. For possibility of its comparison to other genus and species in Pencedanum complex, different compounds from *P. ruthenicum* extract were isolated with chromatographic methods. Coumarins, phenolic acids, terpenoids, flavonoids, fatty acids, normal alkans and one carbohydrate were isolated and identified and identified with different spectroscopic methods such as NMR, Mass, UV and IR. But the coumarins which isolated and purified from root extract were reported. They are included: peucedanin 1, oxypeucedanin hydrate 2, xanthotoxin 3, psoralen 4, isopimpinellin 5, bergapten 6, 7-methoxy coumarin 7 and three new coumarins as 12-methoxy furanocoumarin 8; 5-prenyl, 12-methoxy furanocoumarin 9 and 5-prenyl furanocoumarin, 12 (3, 3-dimethyl) acrylate 10.

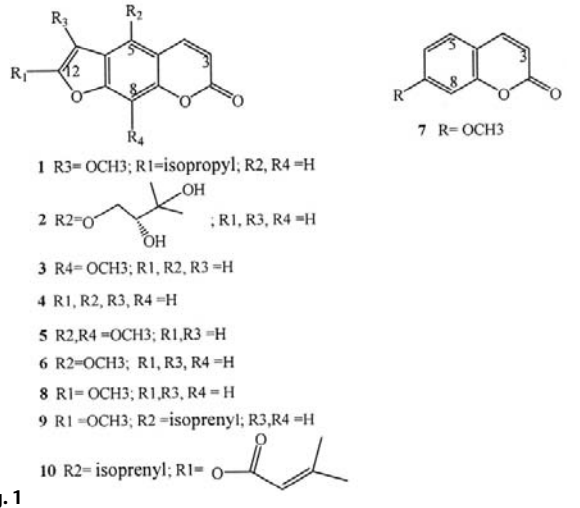

Fig. 1

P205 Topical anti-inflammatory activity of ointments with 1% of crude water extracts of rhizoma and herb *Potentilla reptans* L. in croton oil model of mouse ear inflammation

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This study tested the ability of an ointment with extract of *Potentilla reptans* L. to inhibit mouse ear inflammation in response to topical application of croton oil. The European Five-Finger Grass is astringent and has wound healing effect due to the tannin content (1,2). The examined material was picked in autumn in the surroundings of Sarajevo, dried in thin layer and pulverized immediately before extraction. Water extract (1: 2) made by percolation was drayed under nitrogen at 40 °C. Extractions were made from rhizoma and herb separately. Ointment basis was made from paraffin (soft and hard). Dray water extracts were incorporated in ointment basis. Ointments were applied to one ear of Swiss mice (n = 8) 2.5 mg/ear 30 min after croton oil administration in acetone (10 µg/ear) (2). For comparison, 2 other groups were treated with croton oil and after 30 minutes with 1) the ointment basis alone, 2) Hydrocortisone 1% ointment (2,5 mg/ear). The other ear of the same animal was used as control one. After treatment, ears were observed for three days, and appearance changes were photo documented. Similar pharmacological reaction was found for booth ointments with extract of rhizome and extract of herb of Potentilla reptans, whose pharmacological reaction was similar to Hydrocortisone ointment. Ointments with water extracts of the tested *P. reptans* have significantly reduced inflammation in time for 30 – 50% in relation to control ear. **References:** 1. Hansel R, Keller K, Rimpler H, Schneider G (Hrsg.), Hagers, Handbuch der Pharmazeutischen Praxis, 5. Aufl., Bde 4 – 6, (Drogen), Springer Verlag Berlin, Heidelberg, New York, 1992 – 1994. 2. De Natale, A., Pollio, A., 2007. Plant species in the folk medicine of Montecorvino Rovella (inland Campania, Italy). Journal of Ethnopharmacology 109, 295 – 303. 3. Williamso E.M., Okpako D.T. Evans F.J. (1996) Selection, Preparation and Pharmacological Evaluation of Plant Material in: Pharmacological Methods in Phytotherapy Research; John Wiley Sons p. 131 – 153.

P206 Sesquiterpene lactones influence IL-6 secretion of keratinocytes and thereby modulate CD 54 expression of fibroblasts

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Sesquiterpene lactones (STL) are known as the allergy causing principle in Asteraceae species. It is known that low molecular weight haptens, in addition to the capability to form full allergens, must elicit pro-inflammatory signals to trigger an immune reaction in the skin. Keratinocytes are the first cell population that encounters a hapten and are therefore believed to secrete such signals [1]. In an effort to identify the hitherto unknown "danger-signals" in the case of STL, we found elevated levels of interleukin-6 (IL-6, detected by ELISA) in cell culture supernatants of keratinocytes (HaCaT cell line) treated with STL (helenalin, alantolactone, costunolide, dehydrocostuslactone) or the strong sensitizer dinitrochlorobenzene (DNCB). The STL parthenolide, however, displayed a contrary effect, i. e. a decrease of IL-6 concentration below control level. Subsequently, we investigated the influence of keratinocyte supernatants on the expression of the adhesion molecule CD 54 (ICAM-1) on normal human dermal fibroblasts (NHDF). CD 54 enables fibroblasts to bind T-Lymphocytes via LFA-1 and should play a role in T-cell mediated skin reactions. Supernatants of HaCaT treated with STL, again with the exception of parthenolide, caused an increase of CD 54 expression on NHDF. Consistently, we could show that addition of anti-IL-6-antibodies counteracted the CD 54 up-regulation. However, NHDF treated directly with IL-6 did not show a CD 54 up-regulation. We conclude that an increase in IL-6 caused by STL is involved in the observed modulation of CD 54-expression but not its sole cause. Search for further signalling factors contributing to these effects besides IL-6, including a PIQOR-Microarray, is in progress. **Acknowledgements:** Financial support from brial allergen GmbH, Greven, is gratefully acknowledged. **References:** 1. Gober, M., Gaspari A. (2008) Curr. Dir. Autoimmun. 10: 1 – 26.

P207 Natural sesquiterpene lactones as inhibitiors of leukaemia-associated transcription factor c-Myb

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The transcription factor c-Myb is essential for immature hematopoietic cell proliferation. It has been discovered that changes in the c-myb gene are responsible for development of some kinds of acute lymphatic leukaemia. Inhibition of this target might be of interest as a novel therapeutic strategy [1]. Up to present, however, no inhibitor is known that might be used in therapy. Our main interest is directed towards sesqui-

terpene lactones (STLs). Such compounds show a wide variety of biological activities and some are known to inhibit transcription factors, e.g. NF-κB. We have recently discovered that some STLs inhibit transcription of a c-Myb regulated gene. Especially the helenanolide Mexicanin-I (structure as depicted) showed high potency as a c-Myb inhibitor [2]. Based on a bioassay that reflects a direct connection between fluorescence and c-Myb activity, we found that Mexicanin-I at concentrations of 1, 3 and 10 μM inhibits the c-Myb induced fluorescence activity to 66, 42 and 16%, respectively, compared to an untreated control. It has been verified by MTT-assay, that these results are not solely based on cytotoxic effects. A selection of further STLs of different structural types was shown to cause effects of varying magnitude in this assay. Furthermore, several crude extracts of Asteraceae were tested and the active compounds obtained by bioactivity-guided isolation. Based on the chemical structures of the investigated compounds, it is possible to draw first conclusions about structure-activity-relationships.

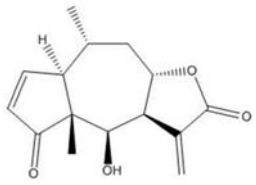

Fig. 1: Mexicanin-I

Acknowledgements: Financial Support from the José Carreras Leukämie-Stiftung is most gratefully acknowledged. **References:** 1. Ramsay, R.G. et al. (2008), Nature Rev. Cancer 8: 523 – 534. 2. Klempnauer K.-H. et al. in preparation.

P208

Isolation of two triterpenes from *Lythrum salicaria* L. and the effect of betulinic acid on the proliferation of osteoblastic MC3T3-E1 cells

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Purple loosestrife – *Lythrum salicaria* L. (Lythraceae) is well known as a medicinal plant, which grows commonly in damp places of moderate climate in Asia, Europe and North America. Its pharmacological activity is mostly due to its phenolic constituents, mainly tannins [1]. It is used for its beneficial health effects against diarrhea, chronic intestinal catarrh, hemorrhoids, eczema, varicose veins and bleeding of the gums [2]. The powdered herbal parts of *Lythrum salicaria* L. (Lythraceae) were extracted with 80% methanol. The concentrated methanol extracts were suspended in water and partitioned with ethyl acetate and then n-butanol, sequentially. The ethyl acetate extract was applied to silica gel column chromatography and afforded one sterol and two triterpene compounds. Their chemical structures were determined by mass and NMR spectroscopy as β-sitosterol (1), betulinic acid (2), and 3β-hydroxy-20(29)-lupen-28-oic acid methyl ester (3). These two triterpene compounds were isolated for the first time from *Lythrum salicaria* L. The effect of betulinic acid (2) on the proliferation of osteoblastic MC3T3-E1 cells was examined by checking the cell viability. Betulinic acid (2) showed a tendency of increasing the growth of osteoblastic MC3T3-E1 cells. **References:** 1. Suhad, S. et al. (2009) FARMACIA 57:192 – 200. 2. Mantle D. et al. (2000) J. Ethnopharmacol. 72: 47 – 51.

P209

Complement system inhibitor constituents of the dichloromethane extract from leaves of *Acalypha guatemalensis*

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Acalypha guatemalensis Pax & Hoffm. (Euphorbiaceae), a plant native from Guatemala and Honduras, is used in folk medicine to treat protozoal infections, gastrointestinal and venereal diseases, rheumatism, cancer and for several other purposes [1]. In a previous work, the dichloromethane extract from dried leaves showed an inhibitory effect on classical pathway of the complement system by in vitro assays [2]. The aim of the present investigation is the characterization of its active fractions and/or constituents. Powdered dried leaves were submitted to extraction with increasing polarity solvents: hexane and dichloromethane. The active extract was submitted to a bioassay-guided fractionation through silica gel CC eluted with a gradient of CH2Cl2:MeOH (99:1 to 60:40), affording twelve fractions. Their activity on classical complement pathway was determined in human serum by hemolytic assay, using quercetin as positive control [3,4]. The active fractions: AGD-II, AGD-V, AGD-VIII and AGD-XII (IC50 = 5.83, 2.39, 4.15 and 4.73 μg/mL, respectively), were successively fractionated through silica gel and SephadexR LH-20 CC, giving mixtures of free sterols and their acetates. Their structures were elucidated by standard spectroscopic techniques and by comparison with literature data. Phytosterols, particularly sitosterol, stigmasterol and campesterol, have been previously described as potent inhibitors of the complement system [5 – 7], which plays an important role in host defence, inflammation or allergic reactions. **Acknowledgements:** S. Sciotto to Fondazione Antonio Imbesi, Universitá degli Studi di Messina, Messina, Italy. **References:** 1. Cáceres, A. (2009) Vademecum Nacional de Plantas Medicinales. Editorial Universitaria, Universidad de San Carlos de Guatemala. Guatemala. 2. Risco, E. et al. (2002) 50th Annual Congress of the Society for Medicinal Plant Research, Barcelona, Spain. 3. Klerx JP. et al. (1983) J Immunol Methods 63:215 – 220. 4. Huang et al. (1998) J Nat Prod 61:757 – 761. 5. Yamada, H. et al. (1987) Chem Pharm Bull 35:4851 – 4855. 6. Oh, SR. et al. (1998) Planta Med 64:456 – 458. 7. Cerqueira, F. et al. (2003) Planta Med 69:174 – 176.

P210

Microbial contamination of commercial samples of Matricariae flos from Romania

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Our research was focused on the assessment of pharmaceutical quality of 9 commercial samples (C1-C9) of Chamomillae flos marketed by Romanian pharmacies and one control sample (C0) exclusively produced by Sidroga for the pharmaceutical network. For a complete image of the quality we analyzed the samples in accordance to the requirements of the 5th European Pharmacopoeia [1] (in force at the time the products were marketed) including the microbial contamination. The contaminants are presentted in the table below.

Table 1: Microbial strains found in the investigated samples of Matricariae flos

	Samples									
Microbial strains	**C0**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Escherichia coli	–	–	+	–	–	–	–	–	–	–
Moulds	–	Aspergillus sp.	–	Aspergillus niger, Rhyzopsnigricans, Mucor mucedo, M. racemosus, Penicilium sp.	–	–	Penicilium sp.	–	–	–
Yeasts	+	+	+	+	+	+	+	+	+	+
TMC (CFU/g)	2000	1500	5000	2000	5800	6300	2800	3000	9800	1200

All in all, TMC (total microbial count) values for all samples were within the imposed limits, but compared to actual European Pharmacopoeia [2] 80% of the chamomile samples would not comply regarding the TYMC (total yeast and mould count). **References:** 1. European Pharmacopoeia, 5th ed., EDQM, Strasbourg. 2. European Pharmacopoeia, 6th ed., EDQM, Strasbourg.

P211 Synthesis and biological evaluation of berberine derivatives

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Berberine **1**, a quaternary isoquinoline alkaloid, has a long history of use in traditional Indian and Chinese medicine. It is found in the root, rhizome and stem bark of many plant species such as *Coptis japonica* Makino, *Berberis vulgaris* L. and *Hydrastis canadensis* L. In various pharmacological studies, berberine has shown weak, but unspecific antibacterial, antitumoral, anti-inflammatory, antileishmanial and antiphotooxidative effects [1 – 5]. In this study, berberine and several natural and synthetic derivatives, both quaternary and basic, were tested for specific efficacy against bacteria and fungi, human leukemic HL-60 cells, and for inhibition of chlorophyll-sensitized photooxidation of linoleic acid. All compounds were weakly or not toxic against HL-60 cells. In the agar diffusion test, derivatives with a hydroxy group in position 9 (protoberberines of general formula, **2**) were found to have the largest effect against the fungal species tested (*Aspergillus niger*, *Hyphopichia burtonii*), while berberine exhibited only marginal activity. The protoberberines perhaps inhibit 24-sterolmethyltransferase (24-SMT) [6].

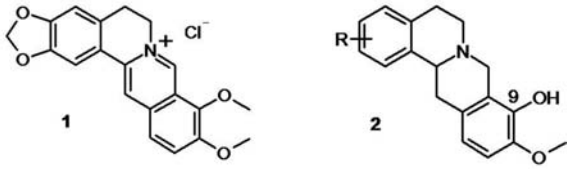

Fig. 1: Berberine **1** and protoberberine of general formula **2**

Of all compounds, berberine showed the highest antiphotooxidative activity (61%). It was equipotent with the standard lipophilic antiphotooxidant ascorbyl palmitate, but distinguished by its water solubility. This is of interest in the conservation of food products. **References:** 1. Iwasa, K. et al. (1996) Eur. J. Med. Chem. 31:469 – 478. 2. Kettmann, V. et al. (2004) Pharmazie 59:548 – 551. 3. Kuo, CL. et al. (2004) Cancer Lett. 203(2):127 – 137. 4. Vennerstrom, JL. et al. (1990) Antimicrob. Agents Chemother. 34:918 – 921. 5. Kim, JP. et al. (2000) J. Agric. Food Chem. 48:1058 – 1063. 6. Park, K-S. et al. (1999) J. Antimicrob. Chemother. 43:667 – 674.

P212 Free radical scavenging activity and phenolic contents of hydroalcoholic extracts from basidoma and mycelial mass of *Agaricus brasiliensis*

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Agaricus brasiliensis (*A. blazei*), a medicinal mushroom originary from Brazil, is largely studied due to the antitumoral and immunomudalatory activities of its β-glucans. Little is known about its phenolic content and antioxidant properties. The objective of this study was to compare antioxidant activity and phenolic content of hydroalcoholic extracts from the basidioma and the mycelial mass of *A. brasiliensis*. The antioxidant activity was evaluated using DPPH (1,1-dyphenyl-2-picryl-hydrazine). Total phenolics were quantified by the Lowry reagent and the main phenolics found in the hydroalcoholic extracts were identified by HPLC. The total phenolic contents were 19.49, 43.12 and 54.40 microg/ml, respectively, for the basidoma, young mycelial mass and old mycelial mass. The extract concentrations yielding 50% free scavenging activity measured using DPPH were 6.0, 10.5 and 12.0 mg/ml, respectively for the basidoma, young mycelial mass and old mycelial mass. Analysis by HPLC showed that gallic acid, syringic acid, benzoic acid and pyrogallol were the main phenolic compounds in all hydroalcoholic extracts. Syringic acid was the main phenolic compound in the basidioma extract while pyrogallol was the main phenolic compound in the young and old mycelial mass extracts. The data obtained show that the antioxidant activity of *A. brasiliensis* is associated with its phenolic content and both basidoma and mycelial mass are useful sources of antioxidant compounds. **Acknowledgements:** Financial support: CNPq, Fundo Paraná and Fundação Araucária.

P213 Phytochemical investigation and microscopic analysis of aerial parts of *Otostegia persica*

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Otostegia persica (Burm.) Boiss. (Labiatae) is one of the four endemic species of plants which grows in Iran. In traditional medicine, aerial parts of the plant are used for treatment of diabetes, rheumatism and oral infections [1]. The aerial parts of *Otostegia persica* (collected in May 2008 from Sistan & Baluchestan Provinces, Iran) were extracted with petroleum ether (PE), chloroform (CH), ethyl acetate (EA), butanol (BU) and methanol (ME). A new isoflavonoid (6,4′,5′-trihydroxy-3′-methoxy isoflavone 7-O-(β-D-glucopyranosyl-(1 – 4)-α-L-rhamnopyranoside) with orange color was isolated and identified from the ME fraction. The total phenolic content of all fractions was measured with the Folin-Ciocalteau method [2], with some modifications. The BU and EA fractions showed the highest amount of phenol content (288.1 and 271.9 mg of gallic acid/100 g fraction), respectively. The total glycosylated flavonoid level was measured by the DAB 10 standard method with some modifications, and apigenin was used as standard. The results showed thet the ME and BU fractions contained 72.66 and 68% flavones, respectively. *Otostegia persica* powder had a pale yellowish-green color with little odor and bitter taste. The diagnostic characters are: (a) the covering trichomes (unicellular and multicellular) with wide base and some times warted walls. (b) The epidermis showing diacytic stomata, big cicatrix, spiral, double helix and annular thickening vessels; some paranchymatous cells had beaded or sinuous walls. (c) The cluster crystals of calcium oxalate and (d) the epidermal cells of the stigmas which were extended to form long, finger-like papillaes. **References:** 1. Tofighi, Z., Alipour, F., Yassa, N., et al. (2009). IJEOT.3:pp.45 – 48. 2. Pourmorad, F., Hosseinimehr, S.J., et al. (2006). Afr J Biotechnol. 5(11):pp.1142 – 1145.

P214 Helicascolide C, a new lactone from an Indonesian marine algicolous fungus

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From the agar-producing red alga *Gracilaria* sp., collected from the coast of South Sulawesi, Indonesia, an endophytic fungal strain KT32 was isolated. The fungus was then mass cultivated to produce external secondary metabolites. The ethyl acetate extracts of the culture broth exhibited antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio anguillarum* and *Aeromonas salmonicida*. The active compounds were determined using bioassay-guided fractionation. Separation by chromatography on silica gel column yielded an antimicrobially active fraction. Further purification of the compounds was done through semi-preparative HPLC. The new natural product helicascolide C was obtained as colorless crystals. Thin plates (5 µm) were measured at 100 K with a Saturn92 CCD detector mounted on a Micromax007 rotating anode X-ray source (Rigaku). The collected data (0.85 Å resolution, space group P212121, a = 5.718(8), b = 9.789(10), c = 20.33(10)) revealed a new chemical entity similar to known helicascolides. Previously the compounds helicascolides A and B were found from the marine fungus *Helicascus kanaloanus* [1]. The structure of the new compound reveals a keto group replacing an alcohol group. Additional characterization was done on the basis of NMR spectroscopic data and mass spectrometric analyses. **References:** 1. Poch, G.K., Gloer, J.B. (1989) J. Nat. Prod. 52 (2):257 – 260.

P215 Benzopyranone and cytotoxic constituents from the root of *Antidesma japonicum*

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Antidesma japonicum Sieb. & Zucc. (Euphorbiaceae) is an evergreen shrub distributed in the northern and central parts of Taiwan, Japan, the Ryukyus, and southern China [1]. The chemical constituents and biological activities of this species have never been studied. In a screening program of Formosan plants, the MeOH extract of the root of this plant showed significant cytotoxic activity and was partitioned into EtOAc, *n*-BuOH and H_2O-soluble layers. Investigation of the active EtOAc-soluble layer led to the isolation of a new benzopyranone, antidesmanone A, along with 17 known compounds, including a benzopyranone: 5,7-dihydroxy-2-heneicosyl-chromone; 2 phenylalkanoids: corylifolin, vanillic acid; 1 coumarin: 3-(1,1-dimethylallyl)-scopoletin; 2 lignans: (−)-syringaresinol, (−)-(2*R*,3*R*)-1,4-*O*-diferuloylsecoisolariciresinol; 2 alkaloids: antidesmone, *N*-*trans*-feruloyltyramine; 3 flavans: epicatechin, catechin, gallocatechin; 2 triterpenoids: β-amyrin, betulinic acid, and 4 steroids: β-sitosterol, stigmasterol, β-sitosteryl 3-*O*-β-D-glucopyranoside, stigmasteryl 3-*O*-β-D-glucopyranoside. (−)-(2*R*,3*R*)-1,4-*O*-Diferuloylsecoisolariciresinol showed cytotoxicity against four colon cancer cell lines, HT-29, CT-26, Colo 205 and HCT-25, with IC_{50} values of 7.02, 9.62, 10.11 and 12.25 μM, respectively.

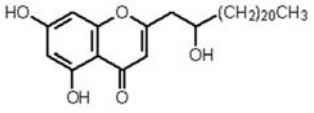

Fig. 1: Antidesmanone A

Acknowledgements: This work was kindly supported by a grant (NSC 97 – 2320-B-039 – 025) from the National Science Council of the Republic of China. **References:** 1. Hsieh, C.F., Chaw, S.M., Wang, J.C. (1996) Euphorbiaceae in Flora of Taiwan, 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan: Vol. 3: 414 – 504.

P216 Quantitative determination of undeclared theobromine and theophylline in energy drinks by reversed-phase HPLC

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Caffeine, theophylline and theobromine are the most important naturally occurring methylxanthines. Although there is great similarity between theophylline and caffeine in their pharmacological and toxicological properties, it appears that theophylline has stronger toxic effects than caffeine and is certainly more toxic than theobromine [1]. This work has been aimed to assess theobromine and theophylline content in 13 different energy drink samples commercially available from the local market. Also, the purpose of this work was to adapt and use the HPLC method proposed by Sharma et al. for the determination of theobromine and theophylline in energy drinks [2]. HPLC was performed with a gradient mobile phase composed of acetonitrile and 0.1% orthophosphoric acid (w/v) in water, and peaks were detected at 210 nm. Degassed and diluted samples were analysed on Lichrospher 100 RP18e column (250 X 4.0 mm, 5 μm), at 30^0C and 1.0 $mLmin^{-1}$ flow rate. The theobromine and theophylline content in energy drinks varies according to the type of the brand and goes up to 74.99 and 1.85 μg/mL, respectively. The used HPLC method is simple, sensitive and accurate and can be applied to all kinds of energy drinks for fast routine analysis of undeclared theobromine and theophylline content. **References:** 1. Babu K. M., Church R. J., Lewander W. (2008) Clin. Pediatr. Emerg. Med. 9: 35 – 42. 2. Sharma V. et al. (2005) Journal of Food Composition and Analysis 18(6):583 – 594.

P217 Phytochemical analysis of *Striga hermonthica* by DART-MS and HPLC-MS

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A parasitic plant, *Striga hermonthica* (Del.) Benth., is one of the biggest biological constraints to cereal production in sub-Saharan Africa and western Kenya. It commonly infects major crops such as sorghum, maize, millet, and rice, lowering grain yield. For this reason, reseach on *S. hermonthica* has mainly focused on controling this weed, but little is known about the constituents of this plant. Thus, DART-MS and LC-ESI-MS were used in order to identify secondary metabolites in *S. hermonthica*. Direct analysis in real time (DART) is a new ionization technique which is conducted in the open air, allowing for the rapid and direct analysis of various types of samples (solid, lipid and gases) with no previous sample preparation. From the dry powder analysed by DART-MS and the methanol extract analysed by LC-ESI-MS, the flavonoids apigenin (*m/z* 271), luteolin (*m/z* 287) and chrysoeriol (*m/z* 301) and coumaric acid (*m/z* 165) were first identified. β-sitosterol (*m/z* 415) and the alkaloid venoterpine (*m/z* 150), which already have been reported in this plant, also were detected. These results are expected to serve as basic information for further phytochemical studies on *S. hermonthica* and to discriminate among different chemotypes as well as *Striga* spp.

P218 New purification method for vitamin A derived aging pigments

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A2E, vitamin A-derived aging pigment, implicated in the pathogenesis of age-related macular disease (AMD), is one of the major compounds that accumulate as lipofuscin pigments in retinal pigment epithelial (RPE) cells with age and in some retinal disorders. Here we report a new purification method for A2E and *iso*-A2E, a double bond isomer of A2E, from a reaction mixture of two all-*trans*-retinal molecules and one ethanolamine molecule by using cation exchange resin. When the mixture complexed with the resin, it was serially eluted with 80% methanol containing sodium hydroxide (pH12), 100% methanol and 100% methanol with 0.1% trifluoroacetic acid (TFA), most of A2E and *iso*-A2E were eluted with 100% methanol solution containing 0.1% TFA. All-*trans*-retinal was mostly eluted with 80% methanol containing sodium hydroxide (NaOH). Identities and recoveries of the isolated compounds were determined by HPLC analysis. This single method for the purification for vitamin A-derived aging pigments will provide an efficient scale up protocol for the preparation of A2E and *iso*-A2E.

P219 Lignans and flavonoids as apoptosis inducers

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Apoptosis is an important mode of "programmed" cell death, which involves the genetically determined elimination of cells. As a vital component of normal cell turnover, inappropriate apoptosis is a factor in many human diseases including cancer, where unwanted cells are unable to be eliminated by the body. Many anticancer drugs today act by way of apoptosis induction [1]. In our ongoing search for bioactive molecules from medicinal plants [2], fifteen phenolic compounds, including lignans and flavonoids were assayed for their apoptosis induction activity on (HuH-7) human liver cancer cells. The lignans, including six dibenzylbutanes and one butirolactone were isolated previously from the dichloromethane extract of *Pycnanthus angolensis* or obtained by derivatization [3,4]. Further studies on the ethyl acetate extract of this species have led to the isolation of seven isoflavones and one flavanone. Their structures were identified based on spectroscopic methods including 2D NMR experiments. Methodology for apoptosis detection included cell viability assays, nuclear morphology evaluation, and general cas-

pase-3-like activity assessments. The compounds tested at a concentration of 20 µM showed varying degrees of apoptosis induction activity after 24 h exposure. Caspase activity assays confirmed these results. **Acknowledgements:** This study was supported by a fellowship from FCT, Portugal (reference number SFRH/BPD/30492/2006). **References:** 1. Elmore S. (2007) Tox. Path. 35:495 – 498. 2. Mansoor T.A. et al. (2009) Bioorg. Med. Chem. Lett. 19:4255 – 4258. 3. Abrantes M. et al. (2008) Planta Med. 74: 1408 – 1412. 4. Duarte N. Et al. (2010) Planta Med. DOI: 10.1055/s-0029 – 1240892.

P220 Skin lightening effect of *Dimocarpus longan* arils extract and its active principle

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The fruit of *Dimocarpus longan* (Sapindaceae) is a sweet fruit which is distributed in China and South Asia. It has been traditionally used as tonic and for the treatment of amnesia and insomnia [1]. Dried arils of *D. longan* were extracted by sonication with 50% ethanol. The extract was subjected to a bioactivity guided isolation process focusing on skin lightening activity. The extract of *D. longan* showed potent inhibitory activity on tyrosinase and melanin formation in melan-a cells. It also revealed higher skin lightening activity than hydroquinine, which was the positive control in in-vivo tests. An activity guided isolation of single compounds from the 50% ethanol extract using RP-HPLC was performed to identify the active principle in the extract. Final purification was performed with preparative-TLC (reversed phase). DART-TOF-MS revealed a protonated ion peak at *m/z* 127, and the structure was elucidated by ^{1}H- and ^{13}C-NMR studies. From all the spectroscopic data, the compound was identified as 5-hydroxymethyl-2-furfural (5-HMF). 5-HMF showed significant skin lightening effects in vitro. From the results, it is possible that *D. longan* extract and 5-HMF may be developed as a functional skin lightening cosmetic. **References:** 1. Jiyoung, R. et al. (2003) Arch. Pham. Res. 26(2):138 – 142.

P221 Inhibitory effects of prenyl flavonoids isolated from *Artocarpus communis* on mushroom tyrosinase and melanin production in cultured melanoma cells

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Eighteen prenylated flavones, six new compounds (**1**-**6**) and twelve known compounds (**7**-**18**), were isolated from the heartwood and cortex of *Artocarpus communis*. In the tyrosinase inhibition assay, norartocarpetin (**8**), artogomezianone (**9**), cudraflavone A (**15**), and artonin M (**18**) exhibited strong inhibitory activities in concentration-dependent manner, while artocommunol CG (**3**), artocommunol CI (**5**), artocarpin (**7**), and cyclocommunol (**10**) showed moderate inhibitory activities on mushroom tyrosinase. The inhibition kinetics, analyzed by Lineweaver-Burk plots, indicated for **8** and **9** to be a competitive inhibitor, and for **15** and **18** to be a mixed-type inhibitor. Compounds **7**, **9**, **15**, and **18** also inhibited melanin production in B16 melanoma cells. These results suggest that some prenylflavones from *A. communis* may serve as candidates for skin- lightening agents.

P222 New labdane-type diterpenes and antitubercular constituents from *Hedychium coronarium*

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Hedychium coronarium Koenig (Zingiberaceae) is a perennial rhizomatous herb, distributed in India, Malaysia, Vietnam, southern China, and Taiwan [1]. Various labdane-type diterpenes are widely distributed in plants of the genus *Hedychium*. Many of these compounds exhibit cytotoxic, anti-inflammatory, and hepatoprotective activities and show inhibitory effects on the increase in vascular permeability, NO production, and iNOS induction. Two new labdane-type diterpenes, hedychicoronarin A (**1**) and hedychicoronarin B (**2**), and ten known compounds (**3**-**12**) have been isolated and identified from the rhizome of *Hedychium coronarium*. The structures of new compounds **1** and **2** were determined through spectral analyses including extensive 2D NMR data. Among the isolated compounds, (+)-coronarin A (**3**) and coronarin D methyl ether (**4**) exhibited antitubercular activities with MICs = 80 and 50 µg/mL, respectively, against *Mycobacterium tuberculosis* $H_{37}Rv$ *in vitro*. The structural elucidation of new compounds **1** and **2** and the antitubercular activities of the isolates will be discussed in this symposium. **References:** 1. Wang, J.C. et al. (2000) Zingiberaceae in Flora of Taiwan, 2nd edition. Taipei: Editorial Committee of the Flora of Taiwan; Vol. 5: 707 – 724.

P223 Phenolic fingerprint of non-fermented and fermented aqueous extracts from *Hamamelis virginiana*

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Hamamelis virginiana L. (witch hazel) is known for its high level of tannins. Because of anti-inflammatory and wound recovery aiding effects, preparations from *Hamamelis* are widely used for treatment of dermatological disorders [1]. Preparations described in the German homoeopathic pharmacopoeia represent fermented aqueous extracts. Because research on fermented plant extracts has only been scarcely performed detailed information on the chemical composition of these preparations is required [2, 3]. In the present investigation, aqueous extracts of fresh *Hamamelis virginiana* leaves were studied by RP/HPLC-ESI/MS. The following characteristic phenolic compound classes could be identified: Cinnamic acid derivates, proanthocyanidins, flavonol glycosides and gallotannins. Contrary to previous literature [4, 5] oligomeric gallotannins made up the main part in the non-fermented extract, including galloylhexoses consisting of six up to ten gallic acid units. After six months of fermentation virtually no oligomeric gallotannins could be detected any more, whereas the gallic acid fraction increased in the same time range. Additionally, reduction of the flavonol glycoside and proanthocyanidin content was observed. Notably the cinnamic acid derivates were rather stable during the examination period. Comparing non-fermented and fermented aqueous *H. virginiana* leaf extracts, considerable differences in the phenolic compound pattern were observed. These findings corroborate the apprehension that data on the chemical composition of *Hamamelis* leaves cannot be transferred to aqueous fermented preparations derived therefrom [2]. Further research is underway to obtain continuative insights into pathways and kinetics of phenolic compound conversion in *Hamamelis virginiana* extracts. **References:** 1. Laux P., Oschmann R. (1993) Z. Phytother. 14:155 – 166. 2. Biber A. et al. (2009) Pharmeur. Sci Notes 1:1 – 4. 3. Millet A. et al. (2009) J. Pharm. Biomed. Anal. 49:1166 – 1171. 4. Wang G. et al. (2003) J. Pharm. Biomed. Anal. 33:539 – 544. 5. Schulz H. et al. (1988) J. Chromatogr. 442: 353 – 361.

P224 Formation of tetrahydroanthracenes in tissue cultures of *Cassia bicapsularis* L.

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A callus culture of *Cassia bicapsularis* L. (Fabaceae) was established on Murashige-Skoog medium supplemented with 1.0 mg/L 2,4-D and 0.1 mg/L kinetin [1] and grown in the dark. It produced two new yellow fluorescent compounds which are tetrahydroanthracenes. These compounds were absent from in vitro plants. Similarly, our previous studies of the active constituents showed that intact plants cultivated in Egypt produced flavonoids and anthraquinones only. The structures of the new compounds from the callus culture were established on the basis of spectral and chemical evidence in comparison with literature data [2].

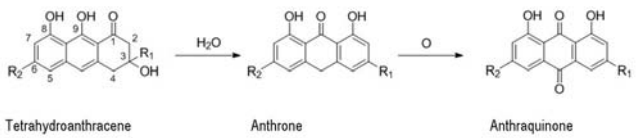

Fig. 1: Tetrahydroanthracene, Anthrone, Anthraquinone

The yellow fluorescent compounds showed typical features of 2 H,4 – 3,8,9-trihydroxy-tetrahydroanthracene, which can lose water and isomerize to the corresponding anthrones. These latter quite unstable compounds are oxidized to anthraquinones in the presence of O2 [2]. **References:** 1. Nazif N, Rady M, Seif El-Nasr M (2000) Fitoterapia 71:34 – 40. 2. Monache G et al. (1991) Phytochemistry 30:1849 – 1854.

P225 Polyphenols from *Cymbopogon citratus* inhibit iNOS expression and NO production – a promising source of new anti-inflammatory drugs

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Inflammation is known to be associated with several pathologies, and due to the side effects of the current anti-inflammatory drugs there is an urgent need to find safer compounds. The inhibition of production of the inflammatory mediator nitric oxide (NO) has been used as a strategy to develop new anti-inflammatory drugs. *Cymbopogon citratus* (Cy) is used in traditional medicine to treat inflammation, diabetes and other health problems, but little is known about its mechanism of action. In a previous report, we demonstrated that Cy has strong antioxidant activity related to its polyphenolic content [1]. In the present study we evaluated the anti-inflammatory action of polyphenolic fractions (PFs) from a lipid- and essential oil-free infusion of Cc leaves, analyzing the NO production, the NO synthase (iNOS) expression and mitogen activated protein kinases (MAPKs) activation, in the mouse macrophage cell line Raw264.7. We observed that PFs inhibited the NO production and iNOS expression induced by the strong inflammatory stimulus lipopolysaccharide, but did not affect the LPS-mediated MAPKs activation. The acid phenolics and tannins were the principal polyphenols responsible for the in vitro Cy anti-inflammatory activity. In conclusion, the polyphenols isolated from *Cymbopogon citratus* have anti-inflammatory activity and these compounds can be a promising natural source of new anti-inflammatory drugs. **Acknowledgements:** FCT and POFC/FEDER for financial support. Research supported by FCT PhD fellowships SFRH/BD/41283/2007 and SFRH/BD/46281/2008 and the project FCOMP-01 – 0124-FEDER-011096 (ref FCT PTDC/SAU-FCF/105429/2008). **References:** 1. Figueirinha, A. et al. (2008) Food Chem 110: 718 – 728.

P226 Essential oil of *Centaurea pannonica* (Heufel) Simonkai and antioxidant activity of the methanol extract

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During the last few decades, an intensive search of the safety of synthetic food additives has been carried out and many of them have been found to possess toxic activity [1]. Therefore the general trend is to replace them in food processing by natural antioxidants. Essential oils or plant extracts are proposed as alternative sources for the preservation of food [2]. *Centaurea pannonica*, is an erect perennial growing wild plant belonging to the Asteraceae family. Its essential oil was isolated from air-dried aerial parts by hydrodistillation and analyzed by GC and GC/MS [3]. Forty five compounds were identified, representing 82.2% of total oil, with 9-octadecanoic acid (34.0%), caryophyllene oxide (8.0%) and spathulenol (6.0%) being the main compounds. The methanol extract of *C. pannonica* was investigated for its antioxidant potential by using five different assays, including: total antioxidant capacity, free radical scavenging (DPPH), inhibitory activity toward lipid peroxidation, Fe^{3+}-reducing power and Fe^{2+}-chelating ability. The content of total phenolics in the methanol extract was determined according to the Folin-Ciocalteu procedure [4] and calculated as 105.96 mg ± 9.22 GA/g dry extract. Moreover a spectrometric method with aluminum chloride [5] was used for the determination of total flavonoids, which was estimated to be 111.36 ± 10.03 mg rutin (RU)/g dry extract. The results show a significant antioxidant activity of the investigated extract compared to standard antioxidant compounds, such as butylated hydrohytoluene (BHT), gallic acid (GA), ascorbic acid (AA) and α-tocopherol.

Table 1: Antioxidant activity of *C. pannonica* methanol extract

	Total antioxidant capacity (mg AA/g dry extract)	IC_{50} (μg/ml)		
		DPPH scavenging activity	Inhibitory activity toward lipid peroxidation	Metal chelating activity
C. pannonica	214.26 ± 12.91	53.05 ± 2.52	7.10 ± 0.05	(1.62 ± 0.09) x 10^3
BHT	-	15.61 ± 1.31	1.00 ± 0.03	-
GA	-	3.79 ± 0.21	255.43 ± 15.01	-
AA	-	6.05 ± 0. 25	> 1000	-
α-tocopherol	-	-	0.48 ± 0.02	-

Acknowledgements: Greek foundation of Scholarships (IKY) and Ministry of Science, Republic of Serbia (project No. 142025). **References:** 1. Akoh, C.C. and Min, D.B. (1998) Food Lipids: Chemistry, nutrition, and biotechnology. Marcel Dekker. New York. pp. 423 – 448. 2. Pokorný, J. (1991) Trends Food Sci. Tech. 2:223 – 227. 3. Lazari, D. et. al. (2000) Flavour Fragr. J. 15:7 – 11. 4. Pharmacopoeia Jugoslavica 4th ed. (1984) National Institute for Health Protection: Belgrade. 5. Brighente, I.M.C. et.al. (2007) Pharm. Biol. 45: 156 – 161.

P227 Antioxidant activity and redox properties of flavonoids from *Limonium narbonense*

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Limonium spp are perennial plants that are mainly distributed near coasts and in salt marshes, and also on saline and alkaline soils in continental interiors. Some *Limonium* species are used in folk medicine for the treatment of rheumatism and menstrual disorders. *L. narbonense* Mill. grows wildly along seashore areas subject to periodical floods such as the Venice lagoon. In the course of our studies we found that the methanolic extracts from roots and aerial parts of *L. narbonense* Mill., collected near Venice, showed antioxidant activity as has been observed by DPPH· assay. The EC_{50} determined by the DPPH· assay were 11.8 and 23.1 μg/mL for aerial parts and roots, respectively. Phytochemical analysis carried out on methanol extracts led to the identification of several polyphenols including: myricetin glycosides, kaempferol and gallic acid. These and other chemical constituents are reported for the first time for this species. Furthermore, the antioxidant activity of the main isolated flavonoids was examined using the DPPH· test. The knowledge of their redox properties can help to understand the antioxidant activity of the flavonoids isolated from *L. narbonense*. To this aim, we investigated by cyclic voltammetry the electrochemical properties of myricetin and myricetrin, which were present in higher concentration and, for comparison, also quercetin, rutin and kaempferol. The first oxidation process resulted a quasi-reversible electron transfer, allowing the estimation of its standard potential E°. A good correlation between EC_{50} and E° has been found, which leads to a possible criteria for predicting the antioxidant ability of a such class of compounds.

P228 Enhanced skin permeation of verbascoside-cyclodextrin complex loaded into liposomes

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The biological properties of *Lippia alba* extracts are related to the presence of some polar components endowed with strong antioxidant, anti-inflammatory and antibacterial activities [1,2]. Verbascoside which is the main constituent in *L. alba* extracts could be a good candidate for topical applications. Two conditions are required: it should permeate through the stratum corneum and reach the deeper cutaneous layers, without significant leakage into the systemic route. The main aim of this work was to enhance the in vitro percutaneous penetration of verbascoside, by complexing it with β-Cd (1:1 M by colyophilization) and formulating it into liposomes. Liposomes were prepared by the ultrasonication technique and were formulated in a 3% PHMC gel. Skin permeation was evaluated across excised pig skin using Franz cells and kinetic studies were performed for 8 hours. Then, skin was adequately treated and divided in stratum corneum, epidermis and dermis. Verbascoside was extracted from each section and dosed with HPLC. Both formulations didn't show any passage through "systemic circulation" during 8 hours. The association of liposomal formulation and β-Cd, compared to the only liposomal formulation used, showed an increase of permeation of verbascoside through the stratum corneum to the epidermal sites. In both formulations diffusion to dermis is very limited, so systemic absorption after in vivo administration could be excluded, in accordance with the absence of permeation in receptor compartment of Franz cells during 8 hours. **References:** 1. Timóteo, P et al. (2008) Nat. Prod. Commun. 3: 2017 – 2020. 2. Fu, G. et al. (2008) Curr. Med. Chem. 15: 2592 – 2613.

P229 Isolation of isoflavonoids from *Amphimas pterocarpoides* and structure elucidation using LC-HRMS^n and NMR techniques

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In an ongoing project directed toward the discovery of novel phytoestrogens, fifteen isoflavonoids have been traced from the methanolic extract of the stem back of *Amphimas pterocarpoides* (Leguminosae – Papilionoideae) using an ultra high performance liquid chromatography-photodiode array (UHPLC-PDA) system, hyphenated with a hybrid, high resolution Ion trap-Orbital trap (LTQ-Orbitrap Discovery) mass spectrometer equipped with an electrospray ionization (ESI) probe in negative and positive mode. The proposed structures of these isoflavonoids were determined by analysing the chemical information of each peak such as retention time (Rt), λmax, m/z value of the quasi-molecular ion, m/z value of the MS/MS fragment ions, along with the molecular formulas. Furthermore, the high accurate mass spectra together with the isotope peak information allowed the prediction of possible molecular formulas. The UHPLC-ESI-MS guided fractionation of that methanolic extract, followed by various chromatographic techniques including CC, MPLC, prep-TLC and prep-HPLC resulted in the isolation of eight known isoflavonoids, two isoflavonoid glycosides as well as one novel isoflavone (7-methyl dihydrotectorigenin).

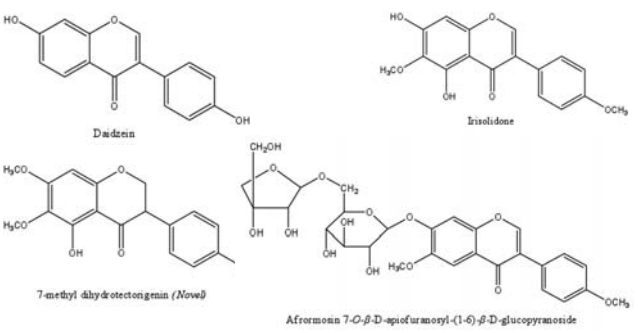

Fig. 1: Selected isolated isoflavonoids from *Amphimas pterocarpoides*

The structure elucidation of the purified compounds was performed via 1 and 2D NMR techniques. The NMR data were in accordance with the HRMS and MS/MS data verifying the proposed chemical structures.

P230 Characterisation of arabinogalactan from larch

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Larch arabinogalactan (LAG) is a polysaccharide obtained from the wood of larch tree (Larix species). LAG is approved by the U.S. Food and Drug Administration (FDA) as a source of dietary fiber [1], but also has potential therapeutic benefits as an immune stimulating agent [2]. Our research tends to structural analysis of LAG as the main component of FiberAid™. Elementary analysis of LAG has shown that carbon and hydrogen atoms can be found in a 1: 2 molar ratio. No nitrogen or sulphur atoms could be detected, which indicates that LAG is not attached to a protein moiety. Quantification of neutral sugars by acetylation pointed out a 1: 5.8 ratio of arabinose (Ara) to galactose (Gal) as main monosaccharides. Determination of uronic acids by specific reduction with deuterium-labelling ($NaBD_4$) revealed small amounts of glucuronic acid (GlcA). Linkage type analysis by methylation showed that the main components are 1,3,6-Gal(p) and 1,6-Gal(p), as well as there being minor amounts of 1,3-Gal(p), 1,3-Ara(f) and terminal Ara(f), Ara(p), Gal(p) and GlcA(p). We were able to prove this by ^{13}C-NMR spectroscopy data, which led to the following structural proposal:

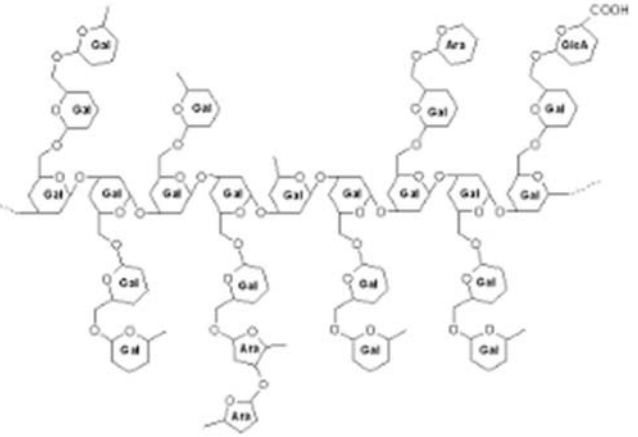

Fig. 1: Structural proposal for LAG

Acknowledgements: We thank Lonza Ltd., Visp (CH) for financial support of this work. **References:** 1. Robinson R et al. (2001) J Am Coll Nutr 20: 179 – 285. 2. D'Adamo P (1996) J Naturopath Med 4: 32 – 39.

P231 Pro-apoptotic effects of lowbush blueberry proanthocyanidins in human colon carcinoma SW480 and SW620 cells

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Proanthocyanidins (Pcy) are oligomeric flavan-3-ols with strong anticancer properties described in different models [1]. We have previously demonstrated in vitro as well as in a rat model of colon carcinogenesis, the potential of apple Pcy in colorectal cancer chemoprevention [2 – 4]. We have performed by microcapillary cytometry a high content screening assay on the ability of Pcy-rich extracts to induce apoptosis in metastatic human colon cancer cell line (SW620). Here, we focused our interest on lowbush blueberry (LB) (*Vaccinium angustifolium* Aiton, Ericaceae) and demonstrated that LB Pcy were more potent to induce apoptosis than apple Pcy, our internal standard, both on SW620 and SW480 cell lines (see table).

Table 1: Comparison of the pro-apoptotic activities of apple and LB Pcy-rich extracts

	% apoptosis		
Sample (75 µg/ml)	SW480 cells	SW620 cells	% flavan-3-ols
Apple Pcy-rich extract	55.3 ± 9.4	64 ± 8.9	150.6 ± 5.7
LB Pcy-rich extract	92.3 ± 6.1	94.9 ± 1.3	101.7 ± 1.2

No synergy was observed between LB Pcy and TNF-α, and no effect of LB Pcy on surface death receptors expression in both cell lines was found. So, the hypothesis of a death receptor clustering was verified by immunocytochemistry. In order to highlight the chemopreventive potential of LB Pcy in CCR, the intracellular mechanisms of action, as well as the *in vivo* efficacy of an LB standardized Pcy-extract, are being currently studied. **References:** 1. Nandakumar V et al., Cancer Letters (2008); 269: 378 – 387. 2. Gossé F et al., Carcinogenesis (2005); 26(7): 1291 – 1295. 3. Seiler N et al., Anticancer Res. (2006); 26: 3381 – 3386. 4. Maldonado-Celis ME et al., Cell. Mol. Life Sci. (2008); 65(9): 1425 – 1434.

P232 *Cynoglossum columnae* Ten. – chemical profiling through biotechnological and phytochemical approach

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Cynoglossum columnae Ten. (Boraginaceae) is an annual species of Mediterranean region. Plants of this family are investigated for naphthoquinone red pigments, found in the underground parts, which are bioactive constituents known as: wound healing, anti-inflammatory, antimicrobial, antitumor agents [1]. Boraginaceae family is also known for its content of hepatotoxic pyrrolizidine alkaloids (PAs) [2]. Three groups of *in vitro* root cultures were obtained and investigated: the natural roots cut off from seedlings, natural roots regenerated from shoots in the following passages and hairy roots obtained as a result of transformation with *Agrobacterium rhizogenes* – ATCC 15834 strain. The roots were cultured in various liquid media (ex.: MS, B5, LS, DCR) and tested for their growth and production of naphthoquinones. The best results gave DCR medium [3] with twice reduced amount of all components and full sugar value. Preliminary phytochemical analysis was performed using RP-HPLC DAD method and showed the presence of six naphtoquinone derivatives in natural roots and their post culture media (DCR/2); none in the transformed root cultures was observed. Phytochemical investigation in plants cultivated in nature was made and six PAs have been isolated and determined by modern spectroscopic methods as rinderine, 3′-acetylrinderine and echinatine in the form of both their N-oxides as well as their bases after reduction. Sixteen more, known PAs, were also identified after reduction as echimumiline, heliosupine, heliotridine, echinatine, rinderine, retronecine, integerrimine and triangularicine type derivatives. **References:** 1. Papageorgiou V. P. et al. (1999) Angew. Chem. Int. Ed. 38 (3): 270 – 300. 2. Chojkier M. (2003) J Hepatol 39: 437 – 446. 3. Gupta P. K., Durzan D. J. (1985) Plant Cell Rep. 4:177 – 179.

P233 HPLC – DAD analysis of galanthamine in *Galanthus elwesii* Hook.

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Galanthamine, an important alkaloid found in Amaryllidaceae species, is a long acting, selective, reversible and competitive acetylcholinesterase inhibitor. It is used for the treatment of mild and moderate cases of Alzheimer's disease [1]. Although the total synthesis of this alkaloid has been reported [2,3], the production of this alkaloid from Amaryllidaceae plants, is still considered important. Therefore, various methods have been described concerning the quantification of this compound in Amaryllidaceae species [4 – 6]. In this study, aerial parts and bulbs of *Galanthus elwesii* Hook., collected from three different localities in Southern Turkey, were quantitatively analyzed for their content of galanthamine, by using High Performance Liquid Chromatography (HPLC). The chromatographic separation was performed using an isocratic system with a mobile phase of trifluoroacetic acid-water-acetonitrile (0.01:90:10) applied at a flow rate 1 mL min^{-1} using diode array detector [4]. The contents of galanthamine in aerial parts and bulbs of *G. elwesii* collected from Cimi village (Antalya) were 0.35 and 0.04%, respectively. The aerial parts of *G. elwesii* collected from Ibradi (Antalya) was found to contain 0.29% galanthamine, whereas the bulbs contained 0.10% of this alkaloid. Galanthamine was not detected in samples of *G. elwesii* growing in Kayrak village (Mersin). **Acknowledgements:** This study was financially supported by Ege University Research Fund (09/ECZ/037). B. Sarikaya was a recipient of TUBITAK research fellowship. **References:** 1. Heinrich, M., Teoh, H.L. (2004) J Ethnopharm 92:147 – 162. 2. Tanimoto, H. et al. (2007) Tetrahedron Lett 48:6267 – 6270. 3. Reddy, J.M. et al. (2008) Synthetic Commun 38:2138 – 2149. 4. Mustafa, N.R. et al. (2003) J Liq Chromatogr R T 26:3217 – 3233. 5. Abou-Donia, A.H. et al. (2008) Phytochem Anal 19:353 – 358. 6. Bastos, J.K. et al. (1996) J Nat Prod 59:638 – 640.

P234 Pressurized steam as valuable extraction option to produce TCM decoction

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Decoction is one of the major forms of preparing drugs in traditional Chinese medicine (TCM). The traditional procedure consists of two boiling steps of the drug in a suitable vessel [1]. However, other machines such as pressure cookers are coming into discussion to prepare decoctions, e.g. for small-scale industry. The focus of this study was on the preparation of decoctions in pressurized steam, especially on the resulting extraction yields and the time needed for preparation. These findings were compared to conventional decoctions prepared in a simple vessel on a hotplate, or with an electric brewing pot. The experiment was carried out with the TCM drug aged tangerine peel (Chenpi, Citri reticulatae pericarpium), which contained hesperidin in the amount of 89.1 mg/g (= 100%) in the herbal drug. Quantification was performed by HPLC analysis [2]. The results of the pressurized steam process (small autoclave, 1 h-1 h 40 min, at 120 °C) showed a yield of hesperidin in the range of 6.2 – 9.7%. By contrast, a conventional decoction (1 h soaking, and 40+20 min decoction) yielded only 3.4 – 3.9% of hesperidin, and a decoction in a brewing pot lasting 2 h, showed similarly low yields of hesperidin, namely 3.0 – 3.3%. The present study showed that preparations of aged tangerine peel, Chenpi, extracted inpressurized steam contained a more than twofold amount of hesperidin, compared to decoctions obtained by a conventional TCM procedure. Whether these findings could be generalized to other substances than hesperidin, or other drugs, will be the subject of further investigation. **Acknowledgements:** We would like to thank Lian Chinaherb, Switzerland, for the supply of herbal drug material as well as SWISSMEDIC, Swiss Agency for Therapeutic Products, Pharmacopoeia division, for the financial support. **References:** 1. EDQM (2010): Preparation of drugs for traditional Chinese

medicines, In: Pharmeuropa 22.2 2. EDQM (2007): Mandarin epicarp and mesocarp (draft monograph), In: Pharmeuropa 19.1.

P235 7-Chloro-6-desoxy-harpagide, a major iridoid glucoside from *Leonurus cardiaca* L. (Ph. Eur.)

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Continuing our search for possible cardioactive single constituents of primary and refined antiarrhythmic extracts of *Leonurus cardiaca* [1] we report herewith the isolation of a chlorinated major iridoid glucoside besides the known ajugol, ajugoside and galiridoside [2, 3]. The structure of 7-chloro-6-desoxy-harpagide was determined by ESI-MS and 1 d/2 d $^{1}H/^{13}C$ NMR spectroscopical experiments. This unusual chlorinated iridoid has been found only once, namely in the related Lamiaceae *Physostegia virginiana* [4] with the not very comprehensible name 'stegioside I'. Thus this compound may prove useful as analytical marker substance as well as possible pharmacologically active compound, as for example shown for the anxiolytic activity of an iridoid enriched preparation from *L. cardiaca* [5].

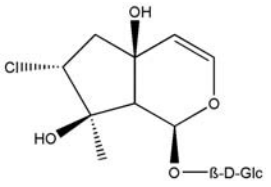

Fig. 1

Acknowledgements: Universität Leipzig Sabine Strahler, Ramona Oehme **References:** *1. Ritter, M. et al. (2010) Planta Med, 76, 572 – 582. 2. Weinges, K. et al. (1973) Liebigs Ann Chem 4, 566 – 572. 3. Guiso, M. et al. (1974) Gazz Chim Ital 104, 25 – 33. 4. Nass, R., Rimpler, H. (1996) Phytochemistry 41, 489 – 496. 5. Makarov, V. et al. (2006) Eksp Klin Farmakol, 69, 23 – 25.*

P236 Influence of Ispaghula seed husk polysaccharides on the gene expression of normal human skin keratinocytes (NHK)

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An acidic xyloglucan isolated from Ispaghula seed husks (IP) increased the proliferation of human keratinocytes[1]. Since the underlying mechanism remained unclear a microarray study was performed to elucidate the influenced signal pathways. Cells were incubated with 10 µg/ml IP for six hours before mRNA isolation and transcription. The cDNA was labeled with Cy3 (untreated) and Cy5 (treated cells). After hybridization to the PIQORTM Skin Microarray the fluorescent label incorporation rate was calculated. It was noticed that 49% of the spotted genes were not expressed neither in treated nor untreated NHK. Compared to the untreated NHK cells incubated with IP exhibited no difference in expression of 27% genes but 15% were slightly and 9% were significantly regulated, whereby mostly a down regulation occurred. The most influence on the expression belongs to genes of extra cellular matrix (ECM) and cytokine signaling followed by transcription factors and parts of the cell metabolism. Minor control on gene expression was observed in regard of proliferation, inflammation, cell cycle, differentiation cytoskeleton and DNA repair related genes. Whereas in the most cases a down regulation occurred effected genes of the cytoskeleton were mostly up-regulated and in case of proliferation related genes the number of down regulated genes equates the number of up-regulated genes. In conclusion the gene expression pattern is consistent with the results observed in preliminary work[1] but it poses the question how the multiple signal pathways work together. **References:** 1. Deters AM., Schröder KR., T. Smiatek T, Hensel A., 2005, Ispaghula (Plantago ovata) seed husk polysaccharides promote proliferation of human epithelial cells (skin keratinocytes and fibroblasts) via enhanced growth factor receptors and energy production. Planta medica, 71: 33 – 39.

P237 Potential antioxidant and anti-inflammatory properties in *Teucrium salviastrum* Schreb.

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Teucrium salviastrum Schreb. (Lamiaceae) is an endemic species from central and northern highlands of Portugal occurring usually in areas above 1.000 m [1]. *Teucrium* spp. have been widely used as medicinal herbs and the biological activities of some species were already studied [2,3]. Here, for the first time, the phenolic composition of *T. salviastrum* extracts was determined, and their antioxidant and anti-inflammatory activities were assessed *in vitro*. Aerial parts of the plant were pulverized and extracted successively with dichloromethane, ethanol and 50% aqueous ethanol. Total phenols were determined by the Folin-Ciocalteu colorimetric method. Extracts were screened for their antioxidant activity using the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method. The anti-inflammatory activity was assessed by the Griess method, evaluating the nitric oxide (NO) production, in the macrophage cell line Raw264.7 stimulated with lipopolysaccharide (LPS). HPLC-PDA-ESI/tandem MS was performed to obtain the phenolic profiles of the extracts. The DPPH method revealed that the ethanolic and hydro-alcoholic extracts are good free radical-scavengers. Additionally, these extracts inhibited LPS-induced macrophage NO production, a well known marker of the inflammatory processes. Relevant results were obtained for the ethanolic and hydro-alcoholic extracts, which showed, respectively, 0.175 and 0.095 g of total phenols expressed in gallic acid equivalent/1 g dry extract, which were, for most part, constituted by phenolic acid derivatives and a luteolin derivative. These results suggest that both ethanolic and hydro-alcoholic extracts of *T. salviastrum* have antioxidant and anti-inflammatory activities which could be related to the phenolic compounds and that the species has potential medicinal properties. **Acknowledgements:** To QREN, Mais Centro Eixo 4 – Protecção e Valorização Ambiental for financial support and LEM/UC integrated in RNEM of Portugal for the HPLC/MS analyses. **References:** 1. Cavaleiro, C. et al. (2002) Flavour Fragr. J. 17: 287 – 291. 2. Sharififar, F. et al. (2009) Food Chem. 112: 885 – 888. 3. Abdollahi, M. et al. (2003) Pharm. Res. 48: 31 – 35.

P238 Diterpenquinones derivatives of the roots of *Horminum pyrenaicum*

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Horminum pyrenaicum L. is the only representative of the monophyletic genus *Horminum* (Lamiaceae) and occurs in the south Alps as well as the Pyrenees. Previous phytochemical studies [1] revealed the presence of horminone and 7-O-acetylhorminone in the aerial plant parts as well as agastaquinone, 3-deoxyagastaquinone and coleon U 12-methylether in the sub aerial plant parts. Reinvestigation of the DCM extract of the root material carried out by means of silica gel and Sephadex LH 20 column chromatography as well as semi-preparative HPLC and LC-SPE-NMR enabled the identification of four abietane-diterpenquinone derivatives, two nor-abietane diterpenquinones and two abeo 18 (4→3) abietane diterpenequinones. The compounds were identified by mass spectrometry and 1- and 2-D NMR as horminone, 7-O-acetylhorminone, inuroyleanol and its 15,16-dehydro-derivative, representing a new natural product. Additionally, the nor-abietanes agastaquinone and 3-deoxyagastaquinone and the *abeo* 18 (4→3) abietanes agastol and its 15,16-dehydro-derivative were identified.

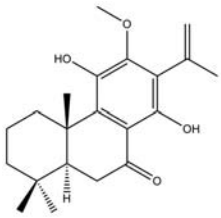

Fig. 1: 15,16-Dehydroinuroyleanol

References: 1. Arnold, U. (2003) Ph.D. thesis at the University of Innsbruck, 154 pp.

P239 Ecdysteroids reverse resistance of human *mdr1* gene transfected mouse lymphoma cells

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Multidrug resistance is a major health problem that affects cancer therapy. The use of compounds as adjuvants, with the potential to improve the effect of existing chemotherapeutics that have fallen by the wayside due to MDR, is a new approach for the therapy of MDR cancer. Ecdysteroids are steroidal hormones of the invertebrates. They are also frequently found in plants, where they are held to play an important defensive role against non-adapted herbivores. Ten ecdysteroids were tested for their cytotoxicity against L5178 mouse T-cell lymphoma cells transfected with pHa MDR1/A retrovirus (MDR) and its parental cell line (non MDR). The activity of the compounds as modulators of the efflux of rhodamine123 by the human ABCB1 pump (commonly known as P-gp) of the MDR cells was determined by flow cytometry. The compounds were also tested for their combination activity in presence of doxorubicin, a known substrate of the ABCB1 pump. It was observed that these of non-cytotoxic compounds showed modulation of the efflux activity by the ABCB1 pump. In combination with doxorubicin, it was observed a synergistic effect by the ecdysteroids and doxorubicin, with a decrease of the IC50 of doxorubicin. In the presence of 18 μM of the 20-hydroxyecdysone 2,3,20,22-diacetonide, the IC50 of doxorubicin was reduced by ten times. Considering that ecdysteroids are of extremely low toxicity in vertebrates, including humans, and act as mild anabolics and adaptogenes, our findings may lead to the discovery of a potent adjuvant with a highly beneficial side-effect profile in the chemotherapy of MDR cancer. **Acknowledgements:** This work was supported by Hungarian Research Fund (OTKA K72771) grant. The author thanks Dr. Imre Ocsovszki for the flow cytometry measurements.

P240 Diterpenoids in transformed root culture of *Salvia austriaca* Jacq.

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Salvia austriaca Jacquin (Austrian sage), is medicinal herbaceous plant native of high altitudes across Russia and eastern Europe [1]. It has been described that the roots of this species produce abietane diterpenoids [2]. In this work we established of *S. austriaca* hairy root culture by infection of aseptic shoots with *Agrobacterium rhizogenes* strain A4. Transformation of the roots was confirmed by PCR method by detection of rolB and rolC genes of Agrobacterium in the root cells [3]. The roots were maintained in half-strength hormone-free B5 liquid medium [4]. The dried and powdered hairy roots were extracted 3 times with n-hexane using an ultrasonic bath. The filtrated and evaporated extract was purified by column chromatography on Sephadex LH-20 and eluting with acetone to obtain a fraction rich in abietane diterpenoids. Further separation of the constituents of this fraction by preparative thin layer chromatography (TLC) led to isolation of taxodione, 6-deoxy-taxodione, 15-deoxy-fuerstione and a few other diterpenoids. **References:** 1. Clebsch B., Barner CD (2003) The New Book of Salvias. Timber Press. Portland, Oregon. 2. Nagy, G. et. al. (1999) Phytochemistry 52:1105 – 1109. 3. Hosokawa K. et. al. (1997) Plant. Cell. Tiss. Org. Cult. 51:137 – 140. 4. Gamborg O.L. et. al. (1968) Mill Exp. Cell. Res. 50:151 – 158.

P241 Estrogenic activities of flavonoids in Thai medicinal plant *Dalbergia parviflora*

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In a search for new phytoestrogens from Thai medicinal plants, an extract of the heartwood of *Dalbergia parviflora* (Leguminosae) was investigated, and thirteen new compounds, khrinones A, B, C, D, E, isodarparvinol A, B, dalparvin, dalparvin A, B, dalparvinol C, neokhrinol A and (3*S*)-sativanone, along with 55 known compounds, have been isolated and characterized.[1] Isolates were evaluated for their cell proliferation stimulatory activity against the MCF-7 and T47D human breast cancer cell lines, and isoflavones such as genistein, biochanin A, tectorigenin, and 2′-methoxyformononetin stimulated the proliferation of both cells, and concentrations of lower than 1 μM of these compounds showed equivalent activity to 10 pM of estradiol (E2). The new isoflavanone also showed activity against both cell types, although it was less active than that of the corresponding isoflavone (2′-methoxy-formononetin). On the other hand, none of the isolates showed any significant effects on human breast cancer BT20 cell proliferation, and these results indicated that the stimulative activity of these compounds was not general to any cell proliferations. We also examined the steroidogenesis activity of some flavonoids using H295R human adrenocortical carcinoma cells. One ompound showed 200 folds of E2 production compared to genistein with LC-MS analysis whereas its activity was 1/200 to that of genistein in E-screen assay. These results show us that some constituents are able to act as estrogenic substances by modulating various cytochrome P450 enzymes involved in steroidogenesis. **References:** 1. Umehara, K. et al. (2009) J. Nat. Prod. 72:2163 – 2168, (2008) Phytochemistry 69:546 – 552.

P242 *In vitro* antiproliferative evaluation of onopordopicrin isolated from leaves of *Arctium lappa* L.

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Because of the need for new anti-cancer drugs, many compounds have been evaluated for treatment of the disease [1]. We assessed the crude extract (CE), ethyl acetate fraction (EAF), and onopordopicrin compound isolated from leaves of *Arctium lappa* L. CE was obtained by 50% hydroalcohol extraction (Ultra-Turrax), and was partitioned with ethyl acetate to obtain the EAF. EAF was subjected to sequential column chromatography with Merck ® silica gel (0.063 – 0.200 mm), and Merck ® silica gel (0.040 – 0.063 mm), thus obtaining onopordopicrin, which was identified by spectroscopic methods (NMR, MS) and by comparison with the literature. The *in vitro* antiproliferative test was performed on human tumor cell line Caco-2. The activities of the fractions and compound against Caco-2 cells were determined by sulforhodamine assay, as described by Skehan et al. [2]. The cells were seeded in 96-well tissue plates at a density of 8×10^5 cells/mL for 24 h in the CO_2 incubator. The compounds were dissolved in dimethyl sulfoxide and added to the medium at various concentrations. After incubation for 48 h, the cell monolayers were fixed with trichloroacetic acid and stained for 30 min with 0.4% sulforhodamine B. The protein-bound dye was extracted with 10 mM unbuffered Tris base for determination of optical density in a computer-interfaced, microtiter plate reader (Power Wave XS, BIO-TEK®). The results of CC_{50} in Caco-2 cells were 347.6 ± 26.9 (RSD%= 7.7) 24.7 ± 3.0 (RSD%= 12.2), and 19.8 ± 3.7 (RSD%= 18.5) μg/mL for EC, EAF, and onopordopicrin, respectively. These results showed that onopordopicrin may be a promising anti-cancer drug. **Acknowledgements:** *CAPES, CNPq, INCT_if.* **References:** 1. Aggarwal BB. et al. (2008) Planta Med. 74: 1560 – 69. 2. Skehan P. et al. (1990) J. Natl. Cancer Inst. 82: 1107 – 12.

P243 Antioxidant homoisoflavonoids from *Chionodoxa forbesii* Baker

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Chionodoxa is a genus within the Hyacinthaceae, which species are high in homoisoflavonoids (HFs) [1]. Antibacterial, anti-mutagenic and anti-inflammatory effects have been described for some HFs, especially for those from Eucomis and Scilla [2]. A detailed examination of HFs from Chionodoxa forbesii Baker (syn. Ch. gigantea Whittall) is missing in literature, thus the aim of this work was to determine its compounds. A mixture of substances yielded by extraction with ethanol and ether was separated by column chromatography on silica gel and polyamide. The compounds were identified using FT-IR, ESI-MS and ^{1}H- NMR and ^{13}C-NMR. Thereby, HFs of the 3-benzyl-4-chromone-, 3-benzyl-3-hydroxy-4-chromanone and the scillascillin-type were determined. The pattern of substances was found to be in strong relation to this of Ch. luciliae Boiss. [3]. For measuring the antioxidant potentials the ABTS radical decolourization assay following the method described by Re et al. [4] and the DPPH free radical scavenging method were used. Ascorbic acid and trolox served as references. Scillascillin-type HFs showed low activity, whereas 3-benzyl-4-chromone-type HF had a high potential. Cytotoxic effects of HFs on human cells were tested using trypan blue vital stain on HEK-293 cells. It was found, that the tested HFs do not show any adverse cytotoxic effects. **References:** 1. Heller, W., Tamm, C. (1981) Fortschr. Chem. Org. Naturst. Vol. 40. Springer. Wien. 2. Du Toit, K. et al. (2007) South African J. Bot. 73: 236 – 241. 3. Corsaro, M.M. et al. (1992) Phytochemistry 31: 1395 – 1397. 4. Re, R. et al. (1999) Free Radical Biol. Med. 26: 1231 – 1237.

P244 Monodesmosidic cholestane glycosides from *Ornithogalum dubium* Houtt. and their inhibitory activity on proliferation of various mammalian cell lines

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Ornithogalum dubium Houttuyn (Hyacinthaceae) is a bulbous species which is part of the natural flora of South Africa. Because of its lovely raceme cylindrical flowers O. dubium is planted as an indoor plant in many regions. Its phytochemistry and pharmacological effects have not been analyzed in very detail so far [1]. The aim of our work was to determine saponine compounds of O. dubium and their potential inhibitory effects on the proliferation of human cell lines. For this purpose, substances were isolated from fresh bulbs via extraction with organic solvents followed by column chromatography on silica gel and Sephadex® LH-20. Structures were established using FT-IR, ESI-MS and 1D- and 2D-NMR. Acid-catalyzed hydrolysis was used to yield the aglycones and to determine the sugar moieties. Monodesmosidic cholestane glycosides with different sugar moieties, based on five aglycones, were found. Most of these substances were isolated before by Kuroda et al. from the bulbs of O. saundersie Baker [2]. Furthermore, two substances, which aglycones differ from the known ones, were found. We tested these compounds on their inhibitory activity on cell proliferation. Therefore human embryonic kidney cells (HEK-293), human fibroblast cells (WS-1) and human promyelocytic leukemia cells (HL-60) were investigated while using a MTT- and a neutral red assay. **References:** 1. Pohl, T.S. et al. (2000) Curr. Org. Chem. 4: 1287 – 1324. 2. Kuroda, M. et al. (1993) Tetrahedron Lett. 34: 6073 – 6076. Kuroda, M. et al. (1995) Chem. Pharm. Bull. 43: 1257 – 1259. Kuroda, M. et al. (1996) Tetrahedron Lett. 37: 1245 – 1248.

P245 Isolation and identification of antispirochetal labdane-type manoyloxides from *Cistus creticus* L. by novel TLC-extractor/MS and GC/MS

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Just recently we reported on the growth inhibiting activity of volatile oil from *Cistus creticus* L. against *Borrelia burgdorferi* s.s. *in vitro*, determined by bioassay guided procedure [1]. In order to elucidate the definite active principles of *C. creticus* this work describes the isolation and identification of four volatile labdane-type diterpenes of the manoyloxide group. This includes the extraction of TLC spots coupled to an electrospray mass spectrometer using the novel TLC extractor method by H. Luftmann [2]. Thus manoyloxide, 13-epi-manoyloxide, 3-acetoxy-manoyloxide and 3-hydroxy-manoyloxide could be identified, confirmed by 1 d/2 d-^{1}H/^{13}C-NMR analyses. In preparative scale these diterpenes were isolated by combined silica gel 60 and RP-18 silica gel CC and confirmed by GC/EI-MS (cp. [1]), which appears more suitable than ESI-MS due to lipophilic manoyloxides. **Acknowledgements:** Leipzig University, Lothar Hennig **References:** 1. Hutschenreuther A et al. (2010) Pharmazie 65: 290 – 295. 2. Luftmann H (2004) Anal Bioanal Chem 378: 964 – 968.

P246 Phytochemical investigation of *Alstonia marquisensis*, an endemic plant from French Polynesia

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The genus *Alstonia* (Apocynaceae) is a pantropical plant, well-known to be a rich source of original secondary metabolites including bioactive ones. Their leaves and bark had been reported from different countries to have traditional medicine uses to treat various ailments. We report herein a first phytochemical investigation of *Alstonia marquisensis* which is an endemic species grown in Marquesas archipelago. Since only a very limited amount of plant material (leaves and bark) was available, HPLC-SPE-NMR together with HPLC-MS was used for structure elucidation. From the leaves, phenolics compounds were found as major components: narcissin, 3-caffeoylquinic acid and 5-feruloylquinic acid as well as their respective cis-isomers. The examined bark material was investigated for the occurrence of alkaloid compounds. The identified structures belong to the akuammicine type such as alstovine [1] and vincanidine [2]. **References:** 1. Legseir, B., Phytochemistry, Vol.25, No.7, pp. 1735 – 1738, 1986. 2. Yagudaev, M.R., Khimiya Prirodnykh Soedinenii, No. 2, pp. 210 – 212, 1983.

P247 Taiwanin A inhibits MCF-7 cancer cell activity through induction of oxidative stress, upregulation of DNA damage checkpoint kinases, and activation of p53 and FasL/Fas signaling pathways

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This study investigates the anti-MCF-7 breast cancer cell effects and the underlying pharmacological activity and mechanism of taiwanin A, a major lignan isolated from Taiwania cryptomerioides. Our results show that taiwanin A time-dependently induced reactive oxygen species level and DNA damage in MCF-7 cells, which were likely activated ATM and Chk. Taiwanin A could also up-regulate p53, phosphorylated p53, p21Cip1, and p27Kip1 and down-regulate the G2/M checkpoint Cdk1-cyclin A/B, leading to induction of G2/M cell-cycle arrest in MCF-7 cells. Blockade of p53 gene expression by siRNA further demonstrated that the cell-cycle arrest induced by taiwanin A was p53-dependent. The FasL/Fas-mediated apoptotic signaling cascade was involved in taiwanin A-induced apoptosis via activation of caspases-10 and -7 (but not caspase-8), and proteolytic cleavage of PARP. In contrast, mitochondria-initiated apoptotic pathway was not involved. This is the first report to delineate novel mechanism of the action of taiwanin A against MCF-7 cells, suggesting this lignan may have value for development as an anti-breast cancer agent.

P248

In vitro antioxidant activity and tannin content of *Echium italicum* L.

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Echium italicum L., Pale Viper's-bugloss, Italian Viper's bugloss (Boraginaceae) is a perennial, shrub-like plant, inhabiting thermophilic, sandy grounds of the submediteranian area [1]. Its leaves are used as seasoning; apiarists use the plant to make uniquely flavoured honey [2]; flowers are used as an "anti-stress", tranquilizer, and energizer drink, fighting common cold and bronchitis [3]. It is also being used for snake bites and as an aphrodisiac [4]. Roots of *E. italicum* has long been used for its strong anti-inflammatory and wound healing effects [5]. In previous investigations, total phenolic and flavonoid content of this plant were reported, as well as reducing power and chelating ability [6]. In order to complete the information on its antioxidant properties, four assays were carried out using methanolic extract of aerial parts of the plant: total antioxidant capacity, DPPH free radical scavenging, the inhibitory activity toward lipid peroxidation, hydroxyl radical scavenging activity. Results are given table below as mean ± SD. Contents of condensed tannins and gallotannins were found to be 21.49 and 28.85 mg gallic acid/g, respectively. Total antioxidant capacity was 112,92 μg ascorbic acid/g.

Table 1: Antioxidant activity of methanolic extract of *E. italicum* L.

	IC_{50} (μg/mL)		
	DPPH free radical scavenging	Inhibitory activity toward lipid peroxidation	Hydroxyl radical scavenging activity
E. italicum L.	164,33 ± 1.05	102,96 ± 0.96	150,02 ± 1.53
Ascorbic acid	6.05 ± 0. 25	> 1000	160,55
BHT	15.61 ± 1.31	1.00 ± 0.03	33,92
a-Tocopherol	–	0.48 ± 0.02	–

Acknowledgements: Ministry of Science, Republic of Serbia (project No. 142025) **References:** 1. Josifovic, M. (1974) Flora Sr. Srbije. SANU. Beograd. 2. 9. Wright, C.A. (2001) Mediterranean vegetables. Harvard Common Press. Boston. 3. Moallem SA, Niapour M (2008) Journ. Etnopharmacol.117(1): 108 – 114. 4. Al-Qur S (2008) Journ Nat Prod 1: 10 – 26. 5. Albreht A et al. (2009) Journal of Chromatography A, 1216: 3156 – 3162. 6. Niciforovic, N. et al. (2009) Planta Medica Abstracts 75 (9):1055.

P249

Development and validation of an analytical method for the determination of derivatives of ortho-hydroxycinnamic acid of *Echinodorus grandiflorus*

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Echinodorus grandiflorus (Cham. & Schltdl.) Micheli, a member of the family Alismataceae, is a native semi-aquatic plant widely distributed in Brazil, particularly in tropical regions of the country, and is popularly known as "chapéu-de-couro" [1]. This plant is used in folk medicine as an anti-inflammatory and diuretic [2]. The chemical profile of *E. grandiflorus* consists basically of terpenoids, sesquiterpenes, and phenolic compounds [3 – 5]. A UV-Vis spectrophotometric method was developed and validated for the quantification of derivatives of ortho-hydroxycinnamic acid in leaves of *E. grandiflorus*. The method was validated by regulation RE 899/2003 of the National Health Surveillance Agency, Brazil, and the ICH guidelines. The response of this method was linear in concentrations ranging from 0.25 – 0.90 μg mL^{-1} with a linear-regression correlation coefficient of 0.9972. The content of derivatives of ortho-hydroxycinnamic acid was successfully determined, with satisfactory reproducibility and recovery. The recovery was 107.56%. This method fulfilled all the validation parameters and was rapid, feasible, and low-cost. Thus, it can be applied by dispensing pharmacies and small laboratories for routine quality control analysis of *E. grandiflorus*. **Acknowledgements:** ANVISA (Agência Nacional de Vigilância Sanitária, Brazil) and Brazilian Pharmacopoeia. **References:** 1. Lorenzi, H. (2000) Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. Instituto Plantarum de Estudos da Flora. Nova Odessa. 2. Lorenzi, H., Matos, F.J.A. (2002) Plantas medicinais no Brasil: nativas e exóticas. Instituto Plantarum de Estudos da Flora, Nova Odessa. 3. Manns, D., Hartmann, R. (1993) Planta Medica, 59:465 – 466. 4. Pimenta, D.S. et al. (2006) Anais da Academia Brasileira de Ciências 78:623 – 628. 5. Schnitzler, M. et al. (2007) Brazilian Journal of Pharmacognosy 17:149 – 154.

P250

Estrogenic properties of prenylated isoflavones in U2OS human osteosarcoma cells: structure-activity relationships

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In a continuation study aiming to discover novel phytoestrogens, we recently reported the isolation and characterization of novel and known isoflavonoids from *Erythrina poeppigiana* (Walp.) O.F. Cook. (Fabaceae), among which eight appear to be structurally related to genistein with isoprenyl and/or 7-methoxy substitution. In addition, significant binding affinities for the two isotypes of the estrogen receptor ERα and ERβ were demonstrated [1]. These isoflavones derivatives have been investigated for their estrogenic properties in receptor subtype specific reporter gene assay, with a particular focus on their estrogen receptor alpha and beta (ERα and ERβ) selectivity, and their structure-activity relationships using a bone-derived human osteosarcoma cell line (U2OS cells) stably expressing ERα or transiently expressing ERβ. According to our results, an isoprenyl substitution at position 3? together with a 7-methoxy substitution on the genistein skeleton is associated with a statiscally significant activation of the ERα- and ERβ-dependent reporter gene expression in U2OS cells starting from 0.1 nM; while for genistein itself a statistically significant activation was observable at 1 nM. On the other hand, a double prenylation at position 8 and 3? is associated with an almost complete loss of function on the reporter gene activation in U2OS-ERα, but in ERβ expressing system, the effectiveness remains on a statistically significant level, demonstrating an "exclusive ERβ-selectivity" in U2OS human osteosarcoma cells. **References:** 1. Djiogue, S. et al. (2009) J. Nat. Prod. 72 (9): 1603 – 1607.

P251

Involvement of calcium and cAMP in flavonoid production by cell cultures of *Hypericum androsaemum* L.

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Hypericum androsaemum L. is an herbaceous plant widely distributed throughout Europe which has been used in traditional medicine on account of the diuretic and hepatoprotective properties of its leaves [1]. These biological effects have been ascribed to the different flavonoids and phenolic acids known to be present in this species. Cell suspension cultures established from hypocotyl-derived callus of *H. androsaemum* were reported [2] to accumulate low amounts of flavonoids, with the highest levels being found during the stationary phase (day 14). More recently [3], it was shown that treatment of 11-day-old cultures for 72 h with 15 mM $CaCl_2$ or 5 μM calcium ionophore A23187 increased considerably the accumulation of flavonoids and the activity of phenylalanine ammonia-lyase (PAL), which is the first committed enzyme in the phenylpropanoid pathway. In similar experiments, the addition of 20 μM forskolin (an activator of adenylyl cyclase) or 100 μM dibutyryl cAMP (db-cAMP, a membrane-permeable analogue of cAMP) also enhanced the flavonoid levels recorded on day 14, but only the latter treatment caused a significant increase in PAL activity. Moreover, the stimulatory effects of db-cAMP were prevented or markedly inhibited by pretreatment of cells with the calcium channel blocker verapamil (100 μM). Taken together, these results suggest that both calcium and cAMP are involved in flavonoid metabolism of *H. androsaemum* cell cultures. **Acknowledgements:** FCT and POCTI/FEDER for financial support **References:** 1. Novais, M. et al. (2004) J. Ethnopharmacol. 93: 183 –

195. 2. Paranhos, A. (2006) Planta Med. 72: 1060 – 1061. 3. Paranhos, A. (2007) Planta Med. 73: 1017.

P252 Antioxidant activities and polyphenolic contents of three selected *Micromeria* species growing in Croatia

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The genus *Micromeria* (Lamiaceae) is represented by nine species in Croatia, three of which (*M. croatica* (Pers.) Schott, *M. dalmatica* Benth. and *M. pseudocroatica* Šilic) are endemic [1]. *Micromeria* species are perennial herbs or dwarf shrubs growing mostly in the Mediterranean region. Some of them are traditionally used against heart disorders, headache, colds, wounds and skin infections, as well as condiments [2]. In the present paper, antioxidant activities of *M. croatica*, *M. juliana* and *M. thymifolia* were evaluated using five *in vitro* antioxidant assays, in comparison with plant polyphenolic constituents and reference antioxidants. All studied plant extracts exhibited considerable DPPH and hydroxyl free radical scavenging activity, reducing power, iron-chelating ability and total antioxidant capacity in the order: *M. croatica* > *M. juliana* > *M. thymifolia*. Among the tested plants, the ethanolic extract of *M. croatica* was found to be the most effective DPPH radical scavenger (IC_{50} 4.7 μg/ml), even stronger than BHT (IC_{50} 6.5 μg/ml). The highest activity toward hydroxyl free radicals was recorded for the same extract (IC_{50} 249.65 μg/ml). Additionally, it also showed the strongest reducing power (IC_{50} 9.64 μg/ml) and iron-chelating ability (IC_{50} 227.47 μg/ml). In phosphomolybdenum assay, *M. croatica* displayed twofold lower total antioxidant capacity than ascorbic acid. Total polyphenol (9.69 – 13.66%), phenolic acid (5.26 – 6.84%), flavonoid (0.01 – 0.09%) and tannin contents (3.07 – 6.48%) in dried plant samples were determined spectrophotometrically. A good correlation between antioxidant activities and contents of phenolic acids and tannins was established, indicating their responsibility for antioxidant capabilities of *Micromeria* species. **References:** 1. Lovašen-Eberhardt (2000) Nat. Croat. 9:19 – 20. 2. Duru, ME. et al. (2004) J. Ethnopharmacol. 94:43 – 48.

P253 Antiproliferative activity of the extracts and compounds of *Centaurea arenaria* on human tumour cell lines

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As part of our ongoing research on the antiproliferative compounds from Hungarian species of the Asteraceae family, extracts of several *Centaurea* species native to Middle and Eastern Europe have been tested previously against human tumour cell lines (cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF7) and skin epidermoid carcinoma (A431)), using the MTT assay. [1]. High cell proliferation inhibitory activity was recorded for n-hexane, chloroform and aqueous methanol extracts prepared from the whole plant of *Centaurea arenaria* M.B. ex Willd. The chloroform extract displayed the highest activity (higher than 85% at 10 μg/ml concentration), and was therefore subjected to a bioassay-guided multistep separation procedure. Flavonoids (eupatilin, eupatorin, 3′-methyleupatorin, apigenin and isokaempferid), lignans (arctigenin, arctiin, matairesinol and (±)-syringaresinol), the sesquiterpene cnicin, serotonin conjugates (moschamine and *cis*-moschamine), β-amyrin and β-sitosterin-β-D-glycopyranoside were obtained for the first time from this species. The structure elucidations were performed by means of UV, MS and NMR spectroscopy. The isolated compounds were evaluated for their tumour cell growth inhibitory activities on abovementioned cells, and it was found that flavone, sesquiterpene and lignan-type compounds and a serotonin conjugate exerted pronounced concentration-dependent effects. The highest activities were demonstrated by the lignans arctigenin (IC_{50} 0.73 – 4.47 μM), arctiin (IC_{50} 1.80 – 19.53 μM) and matairesinol (IC_{50} 7.51 – 36.23 μM). **Acknowledgements:** Financial support from the Hungarian Scientific Research Fund (grant OTKA K72771) and National Development Agency (grant TÁMOP 4.2.2 – 08/1) is gratefully acknowledged. The authors thank Dr. Tamás Rédei (Hungarian Academy of Sciences, Institute of Ecology and Botany, Vácrátót, Hungary) for the identification of the plant material. **References:** 1. Réthy, B. et al. (2007) Phytother Res 21:1200 – 1208.

P254 Guaianolides from *Crepis dioscoridis* L.

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The genus *Crepis* L. is well known for its abundance in sesquiterpene lactones, the majority of which belong to the costunolide type guaianolides [1]. Due to the pharmacological and chemosystematic interest of these compounds, we investigated the chemical profile of C. *dioscoridis* L. collected in W. Greece. Air-dried and macerated aerial parts were extracted with a mixture of cyclohexane, diethyl ether and methanol (1:1:1). The extract was concentrated *in vacuo* and fractioned by V.L.C. followed by repeated CC on silicagel using mixtures of DM:MeOH of increasing polarity. The fractions were monitored by TLC and 1 H-NMR. 8-Epigrosheimin and 11βH-11,13-dihydrointegrifolin [2] were yielded by RP18-HPLC (MeOH:H2O 3:2), while three costus type and two hieracin type glucosides were isolated by RP18-HPLC (MeOH:H2O 9:11). The structures of the isolated compounds were elucidated by high-field NMR spectroscopy (1 H-NMR, 1 H-1 H COSY, NOESY, HSQC and HMBC). **Acknowledgements:** The authors wish to thank Assistant Prof. Th. Constantinidis (Lab. of Systematic Botany, Faculty of Biology) for the identification of the plant material **References:** 1. Zidorn C. (2008) Phytochemistry 69:2270 – 96. 2. Kisiel W. et al. (2000) Phytochemistry. 54:763 – 6.

P255 New tigliane diterpenes from *Euphorbia grandicornis*

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Phorbol esters are specific acylated polyhydroxy $\Delta^{1,6}$-tigliane diterpenes, which occur in plants of Euphorbiaceae and Thymelaeaceae family. Phorbol derivatives are promising candidates of drug development for HIV therapy, because they reactivate HIV-1 latency by protein kinase C dependent NF-κB activation, and avoid the new infection of CD4+ cells by down-regulating the expression of the HIV 1 receptors [1,2]. Recently we reported the isolation and structure determination of three phorbol analogues from *Euphorbia grandicornis* Goebel, a succulent cactiform South African plant whose phytochemical investigation has not been reported previously [3]. In continuation of our investigations we report now on the isolation and structural characterization of further six diterpene polyesters. The chloroform-soluble phase of a methanol extract of the fresh aerial parts of *E. grandicornis* was fractionated by column chromatography on polyamide, then by vacuum liquid chromatography on silica gel. Selected fractions from these separations were further purified by preparative TLC and HPLC to yield six pure compounds, including three new natural products. The structure elucidation was performed by means of HRESIMS and advanced two-dimensional NMR methods, including ^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, NOESY, HSQC, and HMBC experiments. The three known compounds were identified as 12 deoxyphorbol mono- and diesters, acylated with acetic, isobutyric and 2-methylbutyric acids. All of the three new compounds are 12-deoxy-16-hydroxyphorbols; one of them was found to have an unusual parent alcohol with 5-ene-7-ol function. **Acknowledgements:** This work was supported by Hungarian Scientific Research Fund (OTKA) (PD 78145) **References:** 1. Marquez, N. et al. (2008) Biochem. Pharmacol. 75:1370 – 1380. 2. Avila, L. et al. (2010) Phytochemistry 71:243 – 248. 3. Rédei, D. et al. (2009) Planta Med. 75:989.

P256 Growth and market trends for herbal products in the United States

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The market for herbal dietary supplements in the United States in 2009 has exceeded US$5 billion according to recent market data.1 Despite the

economic downturn around the world in 2009 and decreases in sales of most classes of consumer goods, sales of herbal dietary supplements increased over 14% and almost 5%, respectively, in both mainstream stores (e.g., drugstores, grocery stores, and other mainstream retail outlets) and in health food stores, with sales in all channels of trade increasing 4.8%. Sales for herbs with a significant amount of clinical research – e.g., as those sold and licensed as phytomedicines in the EU – continued to be listed in the rankings of the "top 20" in sales, demonstrating the continued correlation between clinical research and market success. Sales for herbs with known immunomodulating properties increased significantly, probably due to consumer concerns over the spread of the H1N1 virus. Market experience in the United States often portends future trends in other countries. This presentation will also focus on specific popular herbs and possible reasons for their market success. **References:** 1. Cavaliere, C. et al. (2010) HerbalGram. 86:62 – 65.

P257 Analysis of tartary buckwheat (*Fagopyrum tataricum*) aroma compounds with GC-MS

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Tartary buckwheat herb is an important functional food. Its grains have high nutritional value because of favourable amino acid composition, minerals, dietary fibre and a higher amount of flavonoids (rutin) than common buckwheat (Fagopyrum esculentum), but it is rarely consumed because of its bitter taste [1,2]. Aroma of tartary buckwheat significantly differs from that of common buckwheat in which salicylaldehyde was proven as the most characteristic component [3]. However, volatile aroma components of tartary buckwheat were not investigated so far. The aim of this work was identification and quantification of individual compounds responsible for aroma of tartary buckwheat. Volatiles from freshly ground buckwheat grains were extracted with distillation in Likens-Nickerson apparatus. The extracts were analysed by gas chromatography and mass spectrometry (GC-MS) with electron ionisation (EI). Compounds were identified on the basis of their EI spectra, and by comparison of their retention times with reference compounds. The compounds with significant contribution to the aroma (odour activity value, OAV > 10) are mainly those with oxygen-containing functional groups like aldehydes ((*E*,*E*)-2,4-hexadienal, nonanal, (*E*)-2-nonenal, (*E*)-2-undecenal, phenylacetaldehyde), phenols (2-methoxy-4-vinylphenol), furan derivatives (2-pentylfuran) and sulphur compounds (isopropyl disulphide, 2-acetylthiazole), but no salicylaldehyde was found in the extracts. **References:** 1. Fabjan, N. et al. (2003) J. Agric. Food Chem., 51:6452 – 6455. 2. Chia-Ling, L. et al.(2008) J. Agric. Food Chem., 56:173 – 178. 3. Janeš, D., Kreft, S. (2008). Food Chem. (109):293 – 298.

P258 New dihydropyrone derivates and further antitumour compounds from *Conyza canadensis*

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Canadian horseweed [*Conyza canadensis* (L.) Cronq.], a cosmopolitan plant of Asteraceae, has been used traditionally to treat diarrhea, dysentery and arthritis. Its decoction was also applied for cancerous diseases in North America [1,2]. As a part of our comprehensive screening programme for Hungarian Asteraceae species [3,4], the *in vitro* antitumour effects of lipophilic and aqueous extracts made from the aerial parts and the root of Conyza canadensis were tested by the MTT assay. The *n*-hexane extract of the root inhibited markedly the growth of three human tumour cell lines (HeLa, MCF-7 and A-431), whilst the chloroform extract of the root showed moderate antiproliferative activity. Here we report the activity-guided isolation and structure elucidation of compounds responsible for antitumour effects of the horseweed root. For the fractionation of the n-hexane and chloroform extract different chromatographic methods were used. Two new natural compounds, *E*-conyzapyrone and *Z*-conyzapyrone having an unusual C-10 dihydropyrone structure were isolated through antiproliferative assay guidance, together with a rare C-18 fatty acid (9,10,12-trihydroxy-10*E*-octadecenoic acid), and apigenin from the chloroform extract. From the *n*-hexane extract the C-10 acetylene derivates 4*Z*,8*Z*-matricarialactone and 4*E*,8*Z*-matricarialactone, triterpenes (friedelin, epifriedelanol, taraxerol, simiarenol) and sterols (stigmasterol, sitosterol, spinasterol) were obtained. The structure elucidation was carried out by extensive NMR and MS studies. Pharmacological analysis of the isolated compounds revealed that acetylene-type compounds have the most significant cell growth inhibitory potency with IC_{50} 1.10 – 4.74 µg/ml. *E*-conyzapyrone and *Z*-conyzapyrone exerted moderate antiproliferative activity with IC_{50} 7.83 – 17.05 and 6.98 – 12.05 µg/ml, respectively. **Acknowledgements:** Our investigation was supported by the Hungarian Scientific Research Fund (OTKA 72771). **References:** 1. Grünwald, J., Brendler, T., Jänicke, C. (Eds.) (2000) PDR for Herbal Medicines. Thomson. 2. Hartwell, J. (1968) J. Nat. Prod. 31: 71 – 170. 3. Réthy, B. et al. (2007) Phytother. Res. 21: 1200 – 08. 4. Csupor-Löffler, B. et al. (2009) Phytother. Res. 23: 1109 – 15.

P259 Isolation and structure elucidation of four new pentacyclic diterpene polyesters from *Euphorbia falcata* L.

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In our continuing search for biologically active new compounds from Hungarian Euphorbiaceae species, the chemical constituents of *Euphorbia falcata* L. were investigated. *E. falcata* an annual herb, widely distributed in garbage places, crops, and fallow lands in Hungary. This plant has not been investigated previously. From the methanol extract of *E. falcata* four new pentacyclic euphoppin-type diterpene polyesters have been isolated. We report herein the isolation and structure determination of these compounds. The whole plants were extracted with MeOH at room temperature and, after concentration, the extract was partitioned between $CHCl_3$ and H_2O. The organic phase was fractionated by column chromatography on polyamide, then by vacuum liquid chromatography on silica gel. Selected fractions from these separations were further purified by RPC and preparative TLC, to yield pure compounds **1-4**. The structure elucidation was performed by extensive spectroscopic analysis, including 1D and 2D NMR (^1H-^1H COSY, HSQC, and HMBC) and HRESIMS experiments. The stereochemistry of the compounds was studied by NOESY measurements. The isolated compounds were identified as di-, tetra-, penta- and hexaester derivatives of a polyfunctional euphoppin-related diterpene alcohol, acylated with acetic, propanoic, isobutanoic, n-hexanoic and benzoic acids. The 5 – 7-6 – 3 fused carbon skeleton indicates that compounds **1-4** biogenetically may be formed from a lathyrane precursor. These type of diterpenes occur rarely, and were only isolated previously from *E. aleppica* and *E. decipiens* [1,2,3]. **Acknowledgements:** This work was supported by Hungarian Scientific Research Foundation (OTKA PD 78145). Vasas A acknowledges the János Bolyai scolarship of the Hungarian Academy of Sciences. **References:** 1. Yang, L. et al. (1995) J. Nat. Prod. 58:1883 – 1888. 2. Öksüz, S. et al. (1996) Phytochemistry 42:473 – 478. 3. Ahmad, VU. et al. (1998) Phytochemistry 48:1217 – 1220.

P260 Antioxidant activity of *Pseudolysimachion spicatum* (L.) Opiz (Scrophulariaceae)

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The purpose of this study was to investigate radical scavenging properties and antioxidant activity of leaf, stem, and flower of *Pseudolysimachion spicatum* (L.) Opiz (=*Veronica spicata* L.). *P. spicatum* is a perennial herb with erect or ascending stem up to 60 cm high and blue flowers arranged in dense, many flowered terminal raceme up to 30 cm long. *P. spicatum* was collected in Velebit Mountain and methanolic extracts of leaf, stem, and flowers were prepared by ultrasonication. Content of total phenols was determined by using Folin-Ciocalteu reagent, content of flavonols using aluminium chloride and content of phenolic acids with nitrite-molybdate reagent. Antioxidant activity was established using following methods: radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical, reducing power, chelating activity and β-carotene-linoleic acid assay. The extracts were rich in polyphenols

(211 – 831 mg/g), phenolic acids (2 – 27 mg/g) and flavonoids (16 – 37 mg/g). In all the analyses the extracts were found to possess marked antioxidant activities. The activity of extracts in β-carotene-linoleic acid assay was significantly higher than the activity of butylated hydroxyanisole (BHA) (ANOVA, $p < 0.05$). In most of the assays, activity of stem and leaf extracts was somewhat less pronounced than the activity of flower extract. However, leaf extract demonstrated the strongest reducing properties. DPPH radical scavenging activity correlated well with content of total phenols ($r^2 = 0.89$, $p < 0.0001$). That suggests that phenolic compounds play an important role in antiradical activity of investigated plant organs.

P261 Antioxidant capacity, phenol and flavonoid contents in *Eryngium amethystinum* L. (Apiaceae)

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The role of reactive oxygen species in many pathological conditions such as cardiovascular diseases or diabetes has been in the focus of research interests in the past decade. Eryngium amethystinum L. (= E. glomeratum Lam.) is a Mediterranean herb, light blue to purple in color. Its young shoots and roots are used as vegetables. Quantity of phenolic substances (total phenol and flavonoid) was determined in methanolic extracts of leaves, stems and flowers of E. amethystinum. In order to estimate reactivity of the extracts with free radicals, their reducing effects, as well as their ability to chelate Fe2+ ions and inhibit lipid peroxidation, antioxidant activity was established using following methods: radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical, reducing power, chelating activity and?-carotene-linoleic acid assay, respectively. Antiradical activity of the extracts in reaction with DPPH free radical was rather modest in comparison with butylated hidroxianisol (BHA). On the other hand, reducing power and chelating activity of the extracts were comparable to the activities of positive controls, BHA and quercetin, respectively. The activity of the extracts was most prominent in reaction with?-carotene and linoleic acid. In that assay, there was no difference in activity of stem, flower and BHA, while the activity of leaf was significantly higher ($p < 0.01$). The antioxidant activity of the extracts in most of the performed assays correlated well with the quantity of phenols ($0.74 < r2 < 0.99$, $p < 0.05$) and flavonoids ($0.57 < r2 < 0.90$, $p < 0.05$). The results of this study indicate that E. amethystinum might be a beneficial source of dietary antioxidants.

P262 Obtaining of anthocyanins from red cabbage using different solvents mixtures and treatments

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Secondary metabolites from plants exhibit health-promoting properties and their use as pharmaceuticals, which is of current interest in food industry. Increase concern on the safety of synthetic colorants in food has led to more attention on natural colorants as food additives as alternative. In our studies, red cabbage which has high concentration of anthocyanins, was treated with different solvent mixtures of ethanol and also with different temperature for extraction and anthocyanin conttent measured by spectrophotometry. 50% ethanol resulted in highest concentration of anthocyanin extraction. Interestingly, hot water extraction also resulted in high concentration. Storage temperature, time and treatments like refrigeration, freezing and freeze drying of samples for a period of 4 weeks were also evaluated. The anthocyanin content was reduced considerably in the refrigerated samples when compared to the freezed and freeze-dried samples. This shows the effect of time and temperature as treatment parameters which affect the availability of plant secondary metabolites. **Key words:** anthocyanin, red cabbage, solvent, refrigeration, freezing and freeze-drying.

P263 Comparison of antioxidant activity of bark of seven species of genera *Frangula* Mill. and *Rhamnus* L. (Rhamnaceae)

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Plants of genus *Rhamnus* L. and *Frangula* Mill. are frequently used in phytomedicine, mainly for their purgative properties [1]. The purpose of this study was to compare radical scavenging and antioxidant activity of bark of two *Frangula* Mill. (*F. alnus* L., *F. rupestris* (Scop.) Schur) and five *Rhamnus* (*R. alaternus* L., *R. cathartica* L., *R. intermedia* Steud. et Hohst., *R. orbiculata* Bornm., *R. saxatilis* Jacq.) species which grow in Croatia. Methanolic extracts of barks were prepared by ultrasonication. Content of total phenols were determined by using Folin Ciocalteu reagent. Antioxidant activity was investigated using following methods: radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical, reducing power, chelating activity and β-carotene-linoleic acid assay. The greatest content of phenolic substances was found in extract of *F. alnus* (63 mg/g) while the phenolic content in the other extracts was almost twice lower (between 38 and 23 mg/g). The extracts demonstrated notable antioxidant activity in all the assays. The strongest chelator of Fe^{2+} ions was the extract of *R. orbiculata*. The extract of *F. alnus* was the most active in all the remaining assays. The activity of *F. alnus* extract in β-carotene-linoleic acid assay was significantly higher than the activity of butylated hydroxyanisole (BHA) (ANOVA, $p < 0.05$). Antioxidant activity in all the tests correlated well with content of total phenols (r^2 was between 0.46 and 0.79, $p < 0.001$). This suggests that phenolic compounds are main antioxidant substances in bark of investigated *Frangula* and *Rhamnus* species. **References:** 1. Hänsel R., Sticher Ö. (2007) Pharmakognosie-Phytopharmacie. Springer Medizin Verlag, Heidelberg.

P264 Protein content of 5 algae from Persian gulf and Arabian sea

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Marine macro algae, due to their richness in minerals, polysaccharides, vitamins and proteins; can be a suitable source of healthy food. Some of them have been traditionally used, in some special countries, as food items and for medicinal and industrial properties [1]. Determination of their nutrient compounds and findings of bioactive molecules can expand their dietary market as supplements and functional food. The present study was aimed at evaluation of protein content of some algae belong to Persian Gulf and Arabian Sea. Freeze dried samples of 5 species of algae were used for protein extraction. Then the extracted protein was precipitated by TCA. For quantification of proteins, Lowry method was used. The test was repeated for three times [2]. Results and the names of algae are shown in Table 1. The algae with high protein content can be used as supplements or protein alternatives in dietary food.

Table 1: Algae from Persian Gulf and Arabian Sea and their protein content

Cladophropsis sp.	Colpomenia sinousa	Cystoceria myrica	Laurencia papillosa	Iyengaria stellata	algae
1.88	0.77	Not detected by this method	1.16	Protein content (gr/ 100 gr of dry weight)	2.27

References: 1. Dawczynski, Ch. et al. (2007) food chemistry 103: 891 – 899. 2. Barbarino, E. et al. (2005) journal of applied phycology 17: 447 – 460.

P265 Chemical composition and antioxidant activity of *Allium flavum* L. extracts

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Members of the genus *Allium* have been used and cultured for thousands of years for their spiritual meaning, medicinal properties, pungency and characteristic taste. Only two species of genus *Allium* (*A. sativum* L. and *A. cepa* L.) are well researched, while data on the chemical composition and biological activities of other species, including *Allium flavum* L. are very rare. In the present study we investigated chemical composition and antioxidative properties of methanolic extracts of *Allium flavum* L. (subgen. *Allium*/sect. *Codonoprasum*) from five locations in Serbia. Phytochemical profile was determined by measuring total phenolic, total flavonoid and total anthocyanin content and by HPLC-DAD-MS/MS analysis of methanolic extracts of whole plants and headspace GC/MS analysis of volatile compounds of fresh bulbs. Flavor intensity associated with sulfur compounds was determined by measuring alliinase activity. The antioxidant activity was evaluated by measuring their 2,2-diphenyl-1-pycrylhydrazile (DPPH) radicals scavenging capacity, ferric reducing ability (FRAP) and effect on lipid peroxidation (LP). High contents of total phenolics (5.5 – 14.0 mg gallic acid equivalents/g of dry extract) and total flavonoids (1.9 – 5.0 mg quercetin equivalents/g of dry extract) were found, while anthocianin content was very low (11 – 33 μg cyanidin-3-glucoside equivalents/g of dry extract). Allinase activity was found to be extremely high (28 – 48 mg pyruvic acid/g of dry extract), while amount of volatile compounds was negligibly small. Chemical profile of methanolic extracts of *A. flavum* from different locations was independent of environmental conditions, only small differences in quantitative composition were found. Dominant flavonoid is dihexosyl quercetin. Investigated extracts exhibited high antioxidant activity.

P266 Application of an HPLC method for analysis of the extract from *Paullinia cupana* var. *sorbilis* (Mart.) Ducke

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Paullinia cupana var. *sorbilis* (Mart.) Ducke (Sapindaceae), popularly known as "Guaraná", is a tropical South American plant found in northern Brazil. Guaraná is widely used in medicine, cosmetics, and industry due to its versatile biological activities, and is popularly used as a stimulant of the central nervous system (CNS) in cases of intellectual and physical stress, and as an antidiarrheic, diuretic, and antineuralgic agent [1,2]. It has shown antioxidant, anti-amnesia, and antidepressive effects in animal models [3 – 7]. These activities are mainly attributed to the presence of polyphenols, which explains the interest in quantifying these constituents in guaraná preparations, as well as in validating analytical methodologies. Caffeine, epicatechin, catechin, and procyanidins B1, B2, B3, B4, A2, and C1 have been isolated and identified from its semipurified extract [1]. High-performance liquid chromatography (HPLC) methods have been used to quantify isolated polyphenols or these compounds in complex biological matrices, such as herbal raw materials and extractive preparations. To separate and identify some substances present in the extract, we developed an RP-HPLC method. The chromatograms were obtained from various gradient elution systems, in order to establish the ideal conditions for the analysis of guaraná extract, using 0.05% TFA methanol: acetonitrile (25:75, v/v) and 0.05% TFA water as the mobile phase. Gradient reversed-phase chromatography was performed using a stainless-steel column (250 x 4.6 mm i.d., 4 μm) and detection at 210 nm. The results demonstrate the efficiency of separation using the proposed method. **Acknowledgements:** *CAPES, CNPq, INCT_if.* **References:** 1. Henman, A R (1982) J. Ethnopharmacol. 6: 311 – 338. 2. Yamaguti-sasaki, E et al. (2007). Molecules 12: 1950 – 1963. 3. Audi, EA, Mello, JCP (2000) Fundação Universidade Estadual de Maringá, Cl. Int. A61P 25/24; A61K 35/78. BR #PI00066389. 28/11/2000. 4. Otobone, FJ et al. (2005) Braz. Arch. Biol. Techn. 48:723 – 728. 5. Otobone, FJ et al (2007) Phitoter. Res. 21:531 – 535. 6. Espinola, EB et al. (1997) J. Ethnopharmacol. 55:223 – 229. 7. Mattei, R et al. (1998) J. Ethnopharmacol. 60:111 – 116.

P267 Semisynthesis and pharmacological investigation of lipo-alkaloids prepared from aconitine by transesterification with eicosanoic acid analogues

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Previously we reported the semisynthesis of 9 aconitine-derived lipo-alkaloids transesterified with fatty acids differing in the number of carbon atoms and double bonds in their chains [1]. When these compounds were tested for COX-1, COX-2 and LTB_4 formation inhibitory activities it was found that 14-benzoylaconine-8-O-eicosapentaenoate exhibited notable activities through all three in vitro anti-inflammatory test systems (inhibition (%): COX-1: 54.51, COX-2: 66.07, LTB_4: 45.96, at 50 μM concentration). Although no data exist on the natural occurrence of aconitine derived lipo-alkaloids containing eicosanoic acid analogues at C-8, the prospective of further charting the possible structure-activity relationships of lipo-alkaloids is reinforced by papers dealing with the importance and natural roles of different unsaturated eicosanoic acid derivatives in the process of inflammation [2]. The present paper reports the semisynthesis of 7 eicosanoate analogues of aconitine-derived lipo-alkaloids, prepared according to the modified method of Bai et al [3]. In the reactions, aconitine was transesterified by eicosanoic, 11-eicosenoic, 8,11,14-eicosatrienoic, 11,14,17-eicosatrienoic, 8,11,14,17-eicosatetraenoic and 5,8,11,14,17-eicosapentaenoic acids resulting the corresponding 14-benzoylaconin-8-O-esters and pyroaconitine. The reaction mixtures were purified of necessity by gelfiltration, preparative TLC and centrifugal planar chromatography. Purity of the obtained lipo-alkaloids was proved with the aid of NMR spectroscopy. The COX-1, COX-2 and LTB_4 formation inhibitory activities of this eicosanoate lipo-alkaloid series were also investigated. It was observed that the 14-benzoylaconine-8-O-eicosapentaenoate has the highest activities (inhibition (%): COX-1: 82.84, COX-2: 33.67, at 50 μM concentration) between the eicosanoate analogues. **References:** 1. Borcsa, B. et al. (2009) Planta Med. 75: 910. 2. Rouzer, C. et al. (2009) J. Lipid Res. 50: S29 – 34. 3. Bai, Y. et al. (1994) J. Nat. Prod. 57: 963 – 970.

P268 *Ligusticum mutellina* (L.) Crantz: a pharmacognostic overview

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Mountain lovage represents the plant *Ligusticum mutellina* (L.) Crantz or *Meum mutellina* (L.) Gaert, Apiaceae. It naturally grows in alpine areas, and is known as an antitumoral ethnobotanic remedy. Also, the roots are used in the production of a type of Schnapps in Bayern, Germany [1]. The present study consists of an analysis of some pharmacognostic parameters describing the aerial part of the plant, harvested from mountains in northern Serbia. Polyphenolic compounds were determined in the methanolic extract performing HPLC MS analysis. Microelements and heavy metals were quantified through the SAA technique [2,3]. The antimicrobial activity of an ethanolic extract was estimated and an evaluation of the antiproliferative potential was done by the phytobiologic test on *Lepidium sativum*. Effects on the angiogenic process were determined by performing the chick chorioallantoic membrane assay [4]. 6 polyphenolic structures were revealed in the nonhydrolyzed sample; rutoside was found in high concentration (79.23ug/ml). In the hydrolyzed sample 4 polypheolic compounds were evidenced with quercetin being the most concentrated compound (13.83ug/ml). The vegetal product is in conformity with the official quality parameters concerning the content of heavy metals. The ethanolic extract proved to be active on the selected bacterial cultures. The aqueous extracts in concentrations between 0,35% and 6% expressed an inhibitory effect of 70 – 89%. The

CAM assay showed possible antiangiogenic activity, dependent on the concentration of the extractive solution. The results obtained indicate an antimicrobial and a possible antiproliferative effect of different extracts of *Ligusticum mutellina* (L.) Crantz. **References:** 1. Peev, C. et al. (2007) Chem. Nat. Comp. 43(3): 259 – 262. 2. Peev, C. et al.(2006) Tim. Med. J. 56(2): 233 – 36. 3. Peev, C. (2007) Mugurii foliari, materii prime in gemoterapie. Mirton. Timisoara. 29 – 42. 4. Feflea, S.et al. (2009) Rev. Med. Farm.55(3), 346 – 349.

P269 The antioxidant activity of wild rose fruit

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The vegetal products used in the pharmaceutical and food area from the wild rose (*Rosa canina*, L. Rosaceae) are the fruits and the seeds, Cynosbati fructus and Cynosbati semen. The fruits contain vitamins (C, B, PP, K), carotenoids, pectins, tannins, carbohydrates, while the seeds contain fats, oil and vitamin F [1,2]. The present study aims to analyze two types of 6% extractive solutions (hydroalchoolic maceration and hydro – infusion) of dried and 6 months – frozen wild rose fruits, by determining the content of: vitamin C (titrimetric analysis), total polyphenols (Folin Ciocâlteu method), total carotenoids (spectrophotometrically) as well as by establishing the antioxidant activity (DPPH method and ORAC method) [3,4]. The vegetal products were harvested in October 2009 from the hills in the Danube river area, Moldova Noua, Romania. Dried wild rose fruits showed the highest concentration of vitamin C (154.20 mg/100 g). The content of total polyphenols was similar for both dried and frozen samples. The antioxidant activity of the extractive solutions was not significantly influenced by the type of extraction or extractive solvent, but higher values were obtained in the case of frozen wild rose fruits (71,27 Eq Trolox/1 ml comparing to the extractive solutions of dry fruits 41,03 Eq Trolox/1 ml by ORAC method). **Acknowledgements:** Bilateral Project No. 198/2009 **References:** 1. Molay, K.R. et al. (2010) Int. J. Food Sc. Nutr. 61:109 – 124. 2. Kilicgun, H. et al.(2009) Pharmacogn. Res. 6:417 – 420. 3. Peev, C. (2007) Mugurii foliari, materii prime in gemoterapie. Mirton. Timisoara. 26 – 35. 4. Szajdek, A. et al. (2008) Plant Foods Human Nutr. 63:147 – 156.

P270 Antioxidant activity and composition of essential oils of four cultivated aromatic plants in North-West Greece

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There is a remarkable development of interest in medicinal and aromatic plants worldwide and their cultivation may contribute to the amelioration of the local agricultural economies. In the present study, the essential oil composition of four cultivated aromatic plants in North-West Greece has been investigated by capillary GC-MS with an HP-5 column and with an EI detector: Ocimum basilicum, Salvia officinalis, Origanum vulgare subsp. hirtum and Thymus vulgaris. The major compounds found in essential oils were (a) for O. basilicum: linalool (55.2%) and α-bergamotene (5.9%), (b) for S. officinalis: α-pinene (6.8%), eucalyptol (17.5%), α-thujone (30.5%), β-thujone (6.0%), camphor (12.7%) and α-caryophyllene (6.2%), (c) for O. vulgare subsp. hirtum: p-cymene (11.7%), γ-terpinene (14.5%) and carvacrol (51.9%) and (d) for T. vulgaris: p-cymene (29.6%), γ-terpinene (16.3%) and thymol (26.8%). Moreover, the essential oils were tested for their free radical scavenging activity using the following in vitro assays: i) interaction with the free stable radical of DPPH (1,1-diphenyl-2-picrylhydrazyl), ii) inhibition of linoleic acid peroxidation induced by the dihydrochlroic acid of 2,2-Azabis-2-aminepropane (AAPH). Finally, their inhibitory activity toward soybean lipoxygenase was evaluated, using linoleic acid as substrate. All the samples presented interesting antioxidant activity. They found to strongly inhibit lipid peroxidation as well as soybean lipoxygenase. **Acknowledgements:** Regional Development Fund of the Region of Western Macedonia for financial support

P271 Study on antitumoral activity of some chemical compounds isolated from *Origanum vulgare ssp. vulgare*

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In vivo and in vitro studies have shown that some natural compounds, obtained from plants have regulatory roles on xenobiotic effects. Characterization of these compounds and determination of antimutagenic and anticarcinogenic effects of them has provided an important strategy for decreasing the propagation of cancer disease in human beings. The genus Origanum (Lamiaceae) is represented by 23 species and 6 hybrides in Turkish flora and 14 of them are endemic [1 – 2]. Origanum species are used in infusion form (2%) as diaphoretic, diüretic, carminative and sedative in folk medicine [3]. In this study the chemical composition of Origanum vulgare (Lamiaceae) and its anticarcinogenic effect, and effect mechanism were studied. Plant material was collected from Erzurum-Oltu in flowering period. Powdered dried aerial parts of this plant were extracted with methanol. After evaporation of the solvent, the crude residue was suspended in water and successively extracted with petroleum ether, chloroform and n-butanol, respectively. The organic layers were evaporated to dryness. As a result of chromatographical studies Luteolin-7-O-glucuronide, Luteolin-7-O-xyloside, Rosmarinic acid, Lithospermic acid and Lithospermic acid B were isolated. Antitumor properties of these compounds were investigated by using MCF-7 breast cancer cell sequence. Trypan blue extraction and MTT assay methods were used for the antitumor studies. Luteolin-7-O-glucuronide, Luteolin-7-O-xyloside and Rosmarinic acid have shown moderate antitumoral activity. **Acknowledgements:** This study was supported by grants from the Scientific and Technological Research Council of Turkey (TUBITAK). (Project No: 107T203). **References:** 1. Ietswaart, J.H. (1982) In: "Flora of Turkey and the East Aegean Islands", Ed. P.H. Davis. Univ. Press. Edinburgh. 2. Duman, H. (2000) In: "Flora of Turkey and the East Aegean Islands" (supplement 2), Eds. Güner, A. et al. Univ. Press. Edinburgh. 3. Baytop, T. (1984) Therapy with medicinal plants in Turkey (past and present). Istanbul Üniversitesi Yayinlari. Istanbul.

P272 Antioxidant activity, total phenol and flavonoid contents of *Sideritis montana* L. from Serbia

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In this study were determined the concentration of phenolic compounds, flavonoids and *in vitro* antioxidant activity of water, methanol and acetone extracts, from the whole herb of *Sideritis montana* using spectrophotometric methods. *Sideritis montana* L. (Lamiaceae) is the annual species with low branched trunk, up to 40 cm high. Inhabits sand arid meadows and rocky in the Europe and the Mediterranean [1]. In traditional medicine is known as ironwort and used for the antispasmodic, carminative [2], antimicrobial [3] and antistress [4] effects. Plant material was collected from the Stara Planina Mt. in eastern Serbia. The total phenolic content of the extracts ranged from 47.35 mg/g to 97.85 mg/g dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 24.13 mg/g to 206.4 mg/g, expressed as rutin equivalents. Acetone extract had the highest flavonoid concentration of 206.4 mg/g. Antioxidant activity of extracts were expressed as IC^{50} values (mg/ml) and ranged from 69.37 mg/mL to 229.3 mg/mL. The high contents of phenolic compounds and flavonoids indicated that these compounds contribute to the antioxidant activity. Based on these results, *Sideritis montana* is a potential source of flavonoids as a natural antioxidant substances of high value. **References:** 1. Josifovic, M. (1974) Flora SR Srbije. SANU. Beograd. 2. Tabanca, N. et al. (1998) Turk. J. Chem. 25:201 – 208. 3. Kursat, M. et al. (2009) Turk. J. of Sci. & Tehn. 4(1):81 – 85. 4. Öztürk, Y. et al. (1998) Phytother. Res. 10(1):70 – 73.

P273 Secondary metabolites of *Staehelina uniflosculosa* Sibth. & Sm

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The genus Staehelina (Asteraceae, tribe Cardueae) is represented only by 7 species worldwide. S. uniflosculosa Sibth. & Sm is a Balkan endemic and had not been studied previously. This study is a continuation of the ongoing phytochemical analysis of plants from Asteraceae family. The crude extract of the aerial parts of S. uniflosculosa was fractionated by using several chromotographic methods. So far, four flavonoids (eriodictyol, nepetin, hispidulin and eriodictyol-3′-O-glucopyranoside), one phenolic acid (protocatechuc acid), one phenolic glycoside (4-hydroxyphenyl-1-O-glucopyranoside), one lignan (pinoresinol) and three sesquiterpene lactones (artemorin, tamirin and reynosin) were isolated and identified. Structure elucidation of the pure compounds was achieved by using spectroscopic methods (1D and 2D-NMR). **Acknowledgements:** The authors are grateful to Dr. N. Krigas (Scientific collaborator of Department of Crop Production, Technological Education Institute of Thessaloniki) for the identification of the plant material.

P274 Flavonoids from *Pimpinella kotschyana*

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The genus *Pimpinella* is represented by 23 species in the flora of Turkey, 5 of them are endemic. *Pimpinella* species have carminative, expectorant, sedative, antidepressant, antiseptic, insecticidal, antiviral, antispasmodic, nematocidal, mutagenic, analgesic, antifungal, antibacterial, diuretic, estrogenic, antimalarial, pectoral, stimulant. The essential oils from the fruits of some *Pimpinella* species are also valuable in perfumery and in medicine. Earlier investigations of *Pimpinella* resulted in the isolation of terpenoids, lipids, alkaloids, coumarins, flavonoids and phenylpropanoids. *Pimpinella* are reported to contain iridoids, diterpenoids, essential oils, ketosteroids and flavonoids. In this study, dried and powdered flowering stems of Pimpinella kotschyana were extracted with MeOH. Methanolic extract was dissolved in H_2O and partitioned with CH_2Cl_2 followed by *n*-BuOH. Firstly, the *n*-BuOH phase (57 g) was fractionated on a silica gel column. The subfractions were further chromatographed over silica gel, reversed phase silica gel and Sephadex LH-20 to give two new acylated flavonol glycosides together with four known flavonol glycosides: Kaempherol 3-*O*-α -L-2″- *E*-feruloyl, 3″- *E*-p-coumaroyl) rhamnopyranoside, kaempherol 3-*O*-α-L-(3″-*E*-feruloyl) rhamnopyranoside, kaempherol 3-*O*-rutinoside, quercetin-3-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-rutinoside, isorhamnetin 3-O-α-L- rhamnopyranoside. The structure elucidation of the isolated compounds were done by spectroscopic methods [1D and 2D NMR spectra and MS data interpretation] and by comparison of their physical and spectroscopical data with literature values. This is the first report describing the isolation of flavonoids from the aerial parts of *Pimpinella kotschyana*.

P275 A comparative study of the effects of oleuropein and its dialdehydic form (oleacein) on human neutrophil oxidative burst

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Polyphenols extracted from extra virgin olive oil are known to play a role in preventing atherosclerotic damage by inhibiting LDL oxidation. Olive phenolics include phenolic alcohols (hydroxytyrosol), secoiridoids (oleuropein) and its dialdehydic forms (oleacein) [1]. Oleuropein is well known to possess antioxidant activity but little is known about oleacein activity. Taking into account, that neutrophils are suggested to be implicated in vascular and heart diseases [2], we analysed the effect of oleuropein and oleacein on neutrophil oxidative burst after receptor-mediated (N- formyl-methionyl-leucyl-phenylalanine; f-MLP) and non-receptor-mediated (phorbol-12-myristate-13-acetate; PMA) stimulation. We also performed cell free assays in order to determined the scavenger potential of tested compounds on selected reactive oxygen species (ROS). The comparison of inhibition of ROS production showed that oleacein strongly inhibits the oxidative burst in human neutrophil, in comparison with oleuropein. The cell free experiments demonstrated that oleacein is a strong HClO scavenger, which may be connected with the presence of dialdehydic groups. These results indicate that oleacein down-regulated the PMN responsiveness and may contribute to the protective effect of extra virgin olive oil against vascular and heart diseases.

Table 1

ROS	Oleuropein [$IC_{50;}$ μM]	Oleacein [$IC_{50;}$ μM]
f-MLP induced	11.9	1.6
PMA induced	> 20	3.8
O_2^-	21.1	17.1
H_2O_2	> 50	> 50
HClO	NA	1.5

References: 1. Ervili, M, et al. (2002) Eur J Lipid Sci Technol 104: 602 – 613. 2. Ernst, E, et al. (1987) JAMA 257: 2318 – 2324.

P276 Antitumor activities of some *Bellis perennis* L. fractions

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Bellis perennis L. has been used traditionally in the treatment of wounds, catarrh, rheumatism, arthritis, liver, kidney and respiratory tract disorders [1 – 3]. In this study, antitumor activities of nine different fractions (F.1, 3 – 4, 5, 6 – 7, 8, 9, 10 – 11, 12, 13 – 15) of B. perennis at different concentrations (10000, 1000 and 100 mg/l) were evaluated using the Agrobacterium tumefaciens Potato Disc Tumor Bioassay modified by McLaughlin's group [4]. The inhibition of A. tumefaciens-induced tumors (or crown gall) in potato disc tissue is an assay based on antimitotic activity and can detect a broad range of known and novel antitumor efffects [5,6]. The validity of this bioassay is predicted on the observation that certain tumorigenic mechanisms are similar in plants and animals. It has been shown that the inhibition of crown gall tumor initiation on potato discs and subsequent growth showed good correlation with compounds and extracts active in the 3PS (P388) (in vivo murine leukemia) leukemic mouse assay [6,7]. All tested fractions at all concentrations showed tumor inhibitions. However, 100% tumor inhibition was observed with F.10 – 11 at 10.000 mg/l similar to Camptothecin (positive control). **References:** 1. Grieve, M. (1982). A Modern Herbal, New York. 2. Chevallier, A. (1996). The Encyclopedia of Medicinal Plants, London. 3. Dobelis, IN. (1990). Magic and Medicine of Plants, New York. 4. Ferrigini, N.R. et. al. (1982). J. Nat. Prod. 45:679 – 686. 5. McLaughlin, J.L., Rogers, L.L. (1998). Drug Inf. J. 32:513 – 524. 6. Coker, P.S. et. al. (2003). Phytomedicine, 10:133 – 138. 7. Galsky, A.G. et. al. (1980). Plant Physiol., 65:184 – 185.

P277 Total phenol and flavonoid contents of extracts from different plant parts of *Teucrium montanum* L.

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The contents of total phenolic compounds and flavonoids were determined in acetone extracts from different plant parts of *Teucrium montanum* (mountain germander) using spectrophotometric metods. *Teucrium montanum* L. (Lamiaceae) is a perennial, shrub-like plant with half-ligneous branches, up to 25 cm high and inhabits thermophilic rocks, dry mountain meadows and edges of forests in Europe and Anatoly [1]. It is used as a diuretic and in the treatment of digestive and respiratory diseases and possesses antiinflammatory [2], antioxidative [3] and antimicrobial effect [4]. The phenolic content of the extracts from plant part ranged from 50.36 mg/g to 114.84 mg/g dry weight of extract, expressed as gallic acid equivalents. The total flavonoid content varied from 37.32 mg/g to 88.31 mg/g expressed as rutin equivalents.

The amount of phenolic compounds and flavonoids is very uneven in different parts of the plant. The highest content of phenolic compounds (114.84 mg/g) was recorded in acetone extract of flowers and the highest content of flavonoids (88.31 mg/g) was measured in acetone extract of leaves. Herbal extract from stem contains very low concentrations of phenolic compounds and flavonoids. Obtained values indicate that the flowers and leaves of *Teucrium montanum* species are very rich sources of phenolic compounds. **References:** 1. Josifovic, M. (1974) Flora SR Srbije. SANU. Beograd. 2. Saric, M. (1989) Lekovite biljke SR Srbije. SANU. Beograd. 3. Canadanovic – Brunet, J. et al. (2009) Int. J. of food. Sci. and Techn. 41(6):667 – 673. 4. Vukovic, N. et al. (2008) J Serb Chem 73(3):299 – 305.

P278 Phytochemical analysis of alkaloids from the Icelandic club moss *Diphasiastrum alpinum*

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More than 500 species of club mosses grow all around the world, but only five of them are represented in Iceland: Lycopodium annotinum, Selaginella selaginoides, Diphasiastrum alpinum, Huperzia selago, and Lycopodium clavatum. Club mosses produce a range of secondary metabolites called lycopodium alkaloids and some of them in particular huperzine A have shown interesting anticholinesterease activity (1). The present study is a phytochemical analysis of Diphasiastrum alpinum (Lycopodium alpinum) collected in Iceland. Previous studies on D. alpinum described the isolation of four alkaloids: lycopodine, clavolonine, lycoclavine and des-N-methyl-α-obscurine (2), however our investigation has shown that it contains at least eight alkaloids. The aim of this study was to isolate and elucidate the structures of these alkaloids. The plant extract was subjected to usual fractionation, and structures of the purified alkaloids were determined by 2D NMR spectroscopy including COSY, NOESY, HSQC and HMBC. This resulted in isolation of eight alkaloids including the previously reported lycopodine and clavolonine. Three of the remaining alkaloids are lycopodane type structures with two acetyl groups not previously reported. Lycoclavine and des-N-methyl-α-obscurine found in earlier studies could not be detected in the Icelandic collection of D. alpinum. **Acknowledgements:** The Icelandic Research Fund, The University of Iceland Research Fund, The Icelandic Research Fund for Graduate Students, and Th. Scheving Thorsteinsson Fund. **References:** 1. Wang, et al. (2009) J. Neural Transm. 116: 457 – 465. 2. Miller, et al. (1971) Phytochemistry 10:1931 – 1934.

P279 New monographs proposed for the German homeopathic pharmacopoeia (HAB) for the manufacture of starting materials from bacteria, yeast and fungi

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Fermentation cultures of yeasts (e.g. Candida albicans, C. parapsilosis), fungi (e.g. Aspergillus niger, Mucor mucedo M. racemosus, Penicillium brevicompactum, P. chrysogenum, P. glabrum, P. roquefortii, Fomitopsis pinicola) and bacteria (e.g. Bacillus subtilis, Mycobacterium phlei, Staphylococcus aureus, Streptococcus pyogenes) after preparation were used as active substances (D3, D4, D5 or D6) in different isopathic drug products (drops, injections, tablets, capsules, suppositories and ointments). Isopathy is a special kind of homeopathy [1]. According to isopathic experience during the last 30 years, these drug products were used in the adjuvant treatment of various disorders according to the symptom picture of homeopathic medicine without any severe adverse effects. Possible immunotoxic effects after repeated oral, rectal or subcutaneous application were tested in various guideline studies with GLP compliance in BALB/c mice and Dunkin-Hartley guinea pigs. In general, it can be concluded that the above applications of D3 to D6 dilutions can be regarded as safe. Three valid manufacturing processes are proposed for the German Homeopathic Pharmacopoeia [2]. Preparations according to method A [3] are prepared from cultures of bacteria, yeasts or fungi as well as the fruit bodies of higher fungi. Preparations according to method B [4] are produced from bacteria, yeasts or fungi cultures after acidic or alkaline extraction. Secreted metabolic products of bacteria, yeasts or fungi are prepared by method C [5]. Lyophilisates are identified by capillary electrophoresis (CE), SDS-polyacrylamide gel electrophoresis (SDS-PAGE) or high performance anion exchange chromatography (HPAEC). **References:** 1. Kracke, A., Wiethoff, K. Isopathie – Eine besondere Therapieform, Report Naturheilkunde (in press). 2. German Homeopathic Pharmacopoeia (HAB) draft monograph "H 5.2.6 Isopathics". 3. German Homeopathic Pharmacopoeia (HAB) draft monograph "Prescription A: Mother tinctures made of bacteria fungi or yeasts (isopathics)". 4. German Homeopathic Pharmacopoeia (HAB) draft monograph "Prescription B: Mother tinctures made of bacteria fungi or yeasts after acidic or alkaline extraction (isopathics)". 5. German Homeopathic Pharmacopoeia (HAB) draft monograph "Prescription C: Mother tinctures made of secreted metabolic products of bacteria fungi or yeasts (isopathics)".

P280 NMR spectroscopic investigations of substituted dineolignans from *Manglietia garrettii*

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As part of the search for novel lignan compounds from Magnoliaceae with potential pharmacological activity and with chemotaxonomic interest, the leaves of *Manglietia garrettii* CRAIB (Thai name montha doi) grown in the temperate house of the botanical garden in Graz were phytochemically examined. In addition to the four known neolignans magnolol, honokiol, 5′-methylhonokiol, and obovatol two new isomeric neolignans, garrettilignan A and garrettilignan B, were isolated from the dichloromethane extract and their structures were determined by NMR spectroscopy and MS. Each of the two new compounds comprised 54 carbon atoms in six phenyl propane units, three terminal 4-allylphenol subunits, two 4-allyl-1,2,6-trihydroxyphenyl subunits together with a 4-trihydroxypropyl-1,2,6-trihydroxyphenol. As five subunits were connected via arylether bridges, a constitutional analysis with HMBC experiments was not possible. Instead, a combination of chemical modification (acetylation), which revealed the positions of three phenolic hydroxyl groups, HMBC and selective NOE experiments was used to determine the connections of the subunits in garrettilignans A and B. Common feature of both compounds is a core built up of two obovatol units, one of them is substituted by two additional 4-allylphenol moieties. The difference between these isomeric compounds is found in the linkage of the two obovatol units. In conclusion, garrettilignans A and B belong to the rare class of substituted dineolignans. **References:** 1. Pinto, M. M. M.; Kijjoa, A.; Mondranondra, I.-O.; Herz, W. Planta Med. 1990, 56, 417 – 418.

P281 Influence of fungal napthalenone derivatives on immune cells in an in vitro model of inflammation

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Marine fungi are a promising source for bioactive compounds [1]. The fungal strain 222 has been isolated from wood collected at the coast of the Greifswalder Bodden, Baltic Sea, Germany and produces structurally new naphthalenone derivatives, balticols A to F. They possess antiviral activities [2]. Since other naphthalene compounds are known for their anti-inflammatory activities we investigated whether the balticols have an influence on inflammatory immune cells. Balticols (1 and 10 µg/ml) were added to rat mononuclear cells (F344-MNC) which were cultured alone or together with H9c2-cardiomyocytes. The latter represents a model of inflammation similar as observed after myocardial infarction. MNC's were collected after 48 h and analyzed for T-, B-, NK-, TH-cells and CTL's by flow cytometry. Dexamethasone (Dexa, 10 – 9 mol/l) served as positive control. None of the balticols except balticol E changed the number of control MNC's. The proportion of T-cells was decreased by balticol B and D, but ICAM-1+T-cells increased. Balticol D decreased TH- and increased B-cells as Dexa which additionally decreased CTL's. None of the substances influenced NK cells. After co-culture with cardiomyocytes TH-cells were decreased while CTL's and ICAM-1+T-cells increased. Balticol D partly anticipated the decrease of TH. Balticol E decreased T-cells, especially TH-cells, but stimulated ICAM-1+T-cells. Dexa anticipated the increase of CTL's, had no influence on the proportion of TH-cells and diminished ICAM-1+T-cells. In summary, balticols B, D and E influence unstimulated MNC's. Unambiguous anti-inflammatory effects

were detected using Dexa and balticol E which exerts its effect due reduction of T-cells. **References:** 1. Saleem M et al. (2007) Natural Products Report 24: 1142 – 1152. 2. Shushni MAM et al. (2009) Chemistry & Biodiversity 6: 127 – 137.

P282 Determination of in vitro "anti-aging"-effects of *Ganoderma pfeifferi*

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Although the term "anti-aging" is often used, it is not well defined. We used a perfusion cell culture model [1] for the sensitive detection of real "anti-aging" effects and demonstrate here the results obtained with the new medicinal mushroom *Ganoderma pfeifferi* BRES. *G. pfeifferi* is a European relative of the medicinal mushroom *Ganoderma lucidum* (CURTIS: FR.) P. KARST (Reishi, LingZhi) used in Asian medicine for the prophylaxis and treatment of several disorders including aging processes [2]. A perfusion cell culture was characterised by the continuous addition of fresh nutrient medium and the withdrawal of an equal volume of used medium, allowing the realization of cell cultivation conditions that are approximated as closely as possible to the in vivo situation. We used a human amniotic epithelial cell line (FL cells, ATCC, CCL 62). Glucose consumption and lactate production were continuously measured as parameters of cell viability. Because the proliferation of the cells was inhibited by mitomycin the cell number was nearly the same over the whole time of the experiment. Application of extracts of *Ganoderma pfeiffer*i or of *Ganoderma Maresome*® [3] caused a significant increase in the glucose consumption of the cells and prolonged their life span in comparison to the untreated controls. Whereas the control cells died about 210 h after starting of the perfusion cell culture the treated cells live more than 270 h. The effects occur also after prophylactic application of the mushroom preparation and those of Ganoderma pfeifferi are stronger than those of G. lucidum. **References:** 1. von Woedtke T et al. (2002) Pharmazie 57: 270 – 274. 2. Lindequist U et al. (2010) Med Monatsschr Pharm 33: 40 – 48. 3. Lukowski G et al. (2003) PCT WO 03/072118 A1; 4.09.2003.

P283 Phenolic compounds in the ethyl acetate fraction of a standardized willow bark extract

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Recent pharmacological studies on willow bark extracts have shown that there is more than the effect of salicin and its derivatives alone [1]. Presumably, polyphenols like flavonoids contribute considerably to the analgetic and antirheumatic overall effect [2]. In order to investigate these flavonoids concerning absorption behaviour and pharmacology, a standardized willow bark extract (Steigerwald STW 33-I, extracting agent: water, drug:extract ratio 16 – 23:1 comprising total salicylalcohol derivatives 23 – 26% m/m) was fractionated and the polyphenol enriched ethyl acetate fraction used for isolation. All detectable flavanones and flavanonols were purified by open column chromatography, flash chromatography and RP-HPLC. Structure determination was carried out by mass spectrometry and NMR. Naringenin-7-glucoside, naringenin-5-glucoside, eriodictyol-7-glucoside, naringenin-5-O-(6″-*trans*-p-coumaroyl)-glucoside, dihydrokaempferol, taxifolin, naringenin, eriodictyol and the chalcones isosalipurposide and 6″-*trans*-p-coumaroyl-isosalipurposide were isolated, as well as catechol. Additionally, the phenolglucoside populoside B was identified for the first time in *Salix sp*. All compounds are under investigation concerning their anti-inflammatory activity in an ICAM-1 assay. The absorption behaviour of the flavonoids by using an in-vitro Caco-2 monolayer model and application of the whole extract in comparison to the flavonoid enriched fraction and the single compounds are under current investigation. **References:** 1. Khayyal MT. et al. (2005) Arzneimittelforschung 55: 677 – 87. 2. Nahrstedt A. et al. (2007) Wien Med. Wochenschr. 157: 348 – 51.

P284 Development of self-microemulsifying liquid for oral delivery of tetrahydrocurcumin (THC)

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Curcumin, extracted from the rhizomes of turmeric, has been reported to possess anti-oxidative, anti-inflammatory and anti-carcinogenic activities. Tetrahydrocurcumin (THC) is one of curcumin metabolites. THC exhibits similar pharmacological activities as curcumin; nevertheless, it shows the more potent antioxidant than curcumin [1, 2]. However, the pharmacological effect of THC is limited due to its low aqueous solubility. Self-microemulsifying drug delivery systems (SMEDDS) have been used for the improvement of oral bioavailability of insoluble lipophilic compounds. SMEDDS are isotropic mixtures of oils and surfactants that form fine oil-in-water microemulsions upon mild agitation in aqueous media such as GI fluids [3]. The optimized SMEDDS used for THC formulations in liquid form contained 70% mixtures of two surfactants: Cremophor EL and Labrasol (1:1), and 30% mixtures of oil: Labrafac PG and Capryol 90 (1:1). The liquid THC-SMEDDS rapidly formed fine oil-in water-microemulsions, with particle size of less than 50 nm. The in vitro rate and extend of release of THC from liquid-SMEDDS was about 16-fold higher than that of unformulated THC. Our studies demonstrated the potential use of self-microemulsifying drug delivery system for the delivery of hydrophobic compounds, such as THC by the oral route. **Acknowledgements:** Financial support from Thailand Research Fund under the Royal Golden Jubilee Ph.D. programme (PHD/0150/2549) and the Royal Thai Government through Prince of Songkla University. **References:** 1. Anand, P. et al. (2008) Molec. Pharm. 4:807 – 818. 2. Yoysungnoen, P. et al. (2008) World J. Gastroenterol. 14:2003 – 2009. 3. Constantinides P.P. (1997) Int. J. Pharm. 158:57 – 68.

P285 Enhanced cellular uptake of paclitaxel in the presence of macelignan, a phytoestrogen and its implication in cancer chemotherapy

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This study investigated the effect of macelignan, a phytoestrogen isolated from *Myristica fragrans*, on the P-glycoprotein-mediated drug efflux as well as CYP3A4-medated drug metabolism and subsequently its in vivo implication on the bioavailability of paclitaxel. The inhibition effect of macelignan was negligible over the concentration range of 0.01 – 100 μM in rat liver microsome while its estimated IC50 value was 93.63 μM in human liver microsome, implying that the interaction of macelignan with CYP3A4 might be insignificant at the physiologically achievable concentrations. In contrast, macelignan (20 μM) increased the cellular accumulation of paclitaxel by approximately 1.7 fold in NCI/ADR-RES cells overexpressing P-gp, while it did not alter the cellular accumulation of paclitaxel in OVCAR-8 cells lacking P-gp. The effect of macelignan on the systemic exposure of paclitaxel was also examined in rats after the intravenous and oral administration of paclitaxel in the presence and the absence of macelignan. The concurrent use of macelignan significantly ($p < 0.05$) enhanced the oral exposure of paclitaxel in rats while it did not affect the intravenous pharmacokinetics of paclitaxel, implying that macelignan might be more effective to improve the intestinal absorption rather than reducing hepatic elimination. In conclusion, macelignan appeared to be effective to improve the cellular accumulation as well as oral exposure of paclitaxel mainly via the inhibition of P-gp-mediated cellular efflux, suggesting that the concomitant use of macelignan may provide a therapeutic benefit in improving the anticancer efficacy of paclitaxel. **Acknowledgements:** This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2009 – 0083757).

P286 Chemical and biological studies on the methanol extract of *Gynura pseudochina var. hispida*

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In continuation of our search for bioactive natural products from Thai medicinal plants as potential anti-inflammatory, anticancer and antiox-

idant agents [1], *Gynura pseudochina* (L.) *var. hispida* Thv. (GPH) (Asteraceae) was studied for potential NF-kB inhibitory compounds. Traditionally the fresh leaves and rhizomes have been used externally to treat inflammations of the skin as well as for viral infections and has been investigated as a potential AIDS remedy [2 – 4]. Extraction of the leaves was carried out by cold extraction using petroleum ether, ethyl acetate and methanol. Separation and isolation were achieved using Sephadex LH-20 column chromatography, TLC and HPLC. For the structure elucidation, 1D and 2D NMR and ESI-MS experiments were used. Quercetin-rutinoside, 3, 5- dicaffeoyl quinic acid, 4, 5 dicaffeoyl quinic acid and 3-, or 5- caffeoyl quinic acid were isolated and found to possess NF-kB inhibitory properties with the IC50 values ranging from 25 to 83 µg/ml. All are reported from this species for the first time. *Gynura spp.* is a member of the Senecioneae, and *G. segetum* and *G. divaricata* were reported to contain hepatotoxic pyrrolizidine alkaloids (PAs) [5]. Consequently, the presence of PAs in the methanol extract of GPH was assessed. TLC with spray reagents were used and the results were compared with the PAs fraction separated from Comfrey (*Symphytum officinale* L.). No hepatotoxic PAs were found to present in the methanol extract of GPH. **References:** 1. Siriwatanametanon, N. et al. (2010). J. Ethnopharmacol. *in press*. 2. Saralamp, P. et al. (2000). Medicinal plants in Thailand. Amarin printing & publishing. Bangkok, Thailand. 3. Lemmens, R.H., Bunyapraphatsara, N. (2003). Plant Resources of South-East Asia 12(3). Backhuys Publishers. Leiden, the Netherlands. 4. Woradulayapinij, W. et al. (2005). J. Ethnopharmacol. 101(1 – 3): 84 – 89. 5. Jiang, Y. et al. (2006). Asian J. Pharmacodynamics & Pharmacokinetics. 6(3): 187 – 192.

P287 Polyketide synthases in St. John's wort (*Hypericum perforatum* L.) leaves: immunochemical studies and immunofluorescence localization

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Hypericum perforatum (St. John's wort; Clusiaceae) is an important medicinal plant, which is widely used as an antidepressant. The plant is characterized by the presence of different types of secretory tissue including translucent glands, black nodules and secretory canals. *Hypericum* species are ideal experimental systems for studying the biosynthesis of a diversity of aromatic polyketides. Two type III polyketide synthases (PKSs) involved are benzophenone synthase (BPS) and chalcone synthase (CHS), for which cDNAs were cloned and characterized. The present work describes immunochemical studies and immunofluorescence localization of these PKSs in *H. perforatum* leaves. Both enzymes were heterologously expressed in *E. coli* as 6xHis-tagged proteins and GST-fusion proteins. Polyclonal antibodies were raised against the 6xHis-tagged PKSs in rabbits and the IgG fractions were isolated. The specificity of the antibodies was examined using immunoblotting and immunotitration techniques. Protein extracts from different stages of *H. perforatum* leaves were subjected to SDS-PAGE and immunoblotting. While BPS expression was low, a high CHS level was found in young leaves. The tissue-specific localization of BPS and CHS was studied in different developmental stages of *H. perforatum* leaves using the immunofluorescence technique and confocal laser scanning microscopy. BPS was observed to a low extent in mesophyll cells of young leaves and strongly expressed in the glandular cells of large translucent glands present inside the leaves. CHS was strongly expressed in the mesophyll cells of young leaves and was absent from glands.

P288 Identification of the xanthone of the capitulae from *Syngonatnhus nitens* (Eriocaulaceae) by liquid chromatography tandem mass spectrometry

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Syngonanthus nitens (Bong. Ruhland) or Golden grass is a grass-like species of Eriocaulaceae which exists in the region of Jalapão, state of Tocantins, Brazil. The handcrafts made from coils of S. nitens scapes represent important source of income in Jalapão [1]. The present study aimed to investigate the chemical profile of the methanol extract from the capitulae of S. nitens using HPLC-ESI-IT-MS, negative mode. Capitulae and scapes of S. nitens were collected in Jalapào city, Tocantins State, and authenticated by Dr. Paulo T. Sano from IB-USP (voucher SPF189975). Using this analytical approach three xanthone was detected 1,3,6-trihydroxy-2-methoxyxanthone (1), 1,5,6- trihydroxy-3 – 7-dimethoxyxanthone (2) and 1,3,6 – 8-tetrahidroxy-2,5-dimethoxyxanthone(3). Previous studies of Eriocaulaceae those [2] did not mention the presence of xanthone in Syngonanthus genus. Current studies provide subsidies to try to better explain the relation between Leiothrix and Syngonanthus genus in Eriocaulaceae. **Acknowledgements:** FAPESP, CNPq, CAPES and PROPG-UNESP. **References:** 1. Schmidt, IB. (2005) Etnobotânica e Ecologia Populacional de Syngonanthus nitens: Sempreviva utilizada para artesanato no Jalapão, Tocantins. Dissertação (Mestrado em Ecologia), Universidade de Brasília. 2. Santos, LC. et al. (2001) Phytochemystry. 56:853 – 856.

P289 Quantification of rutin in some plants of family Lamiaceae using high performance liquid chromatography with electrochemical detection

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Rutin is the glycoside of the flavonol quercetin and the disaccharide rutinose widely distributed in various medicinal plants. **Objectives:** In this study, using high performance liquid chromatography with electrochemical detection (HPLC-ED) system, quantitative analysis of rutin was carried out in water extracts of sage, mint, rosemary, thyme (*Thymus vulgaris* L., *Thymus serpyllum* L., and *Thymus sibthorii* Benth.), marjoram, oregano, and lemon balm. **Method:** The drug (1 g) was powdered and extracted with pure water (9 ml). Afterward 1 ml of that extract was decanted and centrifuged. Supernatant was used for analysis. The standard solution was rutin dissolved in pure water. HPLC conditions were following: Mobile phase methanol-acetonitrile-water-acetic acid (20+10+70+1); ED detector with range 50nA, potential +0.840 V, filter 0.02 Hz; flow rate 1 ml/min; temperature 25 °C. **Results:** The amounts of rutin in mg/g were: sage 11.8, mint 1.92, rosemary 1.61, thyme (*Thymus vulgaris* L. 24.9, *Thymus serpyllum* L. 9.0, *Thymus sibthorii* Benth. 22.64), marjoram 4.44, oregano 11.16, and lemon balm 0.25. **Conclusion:** The highest amount of rutin was in *Thymus vulgaris* L. following *Thymus sibthorii* Benth., and sage.

P290 Quantification of rosmarinic acid and caffeic acid in some plants of family Lamiaceae using high performance liquid chromatography with electrochemical detection

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Rosmarinic acid (RA) and caffeic acid (CA) are simple phenolic coumpounds widely distributed in the plants. Various phenolic acids and phenolic glycosides of many different types are widerspread in nature

and are to be found in most classes of natural compounds having aromatic units. In this study, using HPLC-ED system, quantitative analysis of RA and CA was carried out in water extracts of some herbs and leaves of sage, mint, coleus, rosemary, thyme (T*hymus vulgaris* L., *Thymus serpyllum* L., and *Thymus sibthorii* Benth.), marjoram, oregano, and lemon balm. All examined plants belonging *Lamiaceae* family. The drug (1 g) was powdered and extracted with pure water (9 ml). Afterward 1 ml of that extract was decanted and centrifuged. Supernatant was used for analysis. The standard solutions were RA, and CA dissolved in mobile phase. HPLC conditions were following: Mobile phase methanol-acetonitrile-water-acetic acid (20+10+70+1); ED detector with range 50nA, potential +0.840 V, filter 0.02 Hz; flow rate 1 ml/min; temperature 25 °C. The content in (mg/g) of RA was in the sage 5.45 and CA 0.73, mint 5.1 and CA 0.01, coleus, 0.46 and CA 0.10, rosemary 14,64 and CA 0.96, thyme: (*Thymus vulgaris* L. 4.27 and CA 0.66, *Thymus serpyllum* L., 17.7 and CA 1.36, *Thymus sibthorii* Benth. 5.5 and CA 0.95), marjoram 11.36 and CA 0.31, oregano 5.82 and CA 0.7, and lemon balm 5.1 and CA 0.14. The highest content of RA and CA was found in the leaves of *Thymus serpyllum* L., and rosemary.

P291 Screening of 50 medicinal plant extracts for quercetin-3-rutinoside

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The flavonoid rutin (quercetin-3-rutinoside), is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose. **Objectives:** In this study, using HPLC-ED system, quantification of rutin was carried out in different extracts of medicinal plants. **Methods:** Analyses of rutin were performed on the leaves and flowers of 50 medicinal plants from Bosnia of rue, buckwheat, rose, sage, calendula mariogold, chamomile, elder, dandelion, feverfew, lemon balm, linden, thyme, valerian, stinging nettle, cloves, dog rose, pansy, parsley, cowslip, rose e.t.c. Rutin was extracted with hot water. Supernatant was used for analyses. The standard solution was rutin. HPLC conditions: Mobile phase methanol-acetonitrile-HPLC water-acetic acid (20+10+70+1); Potential: +0.840 V; Flow rate 0.8 ml/min; Column: ODS hypersil. **Results:** Content of rutin (mg/g) was highest in the leaves of rue (86.0) and follow flowers of buckwheat (53.5), the leaves from buckwheat (20.0), flowers of pansy (33.5) and flowers of rose (10.0). In all other plants the content of rutin was lower than 0.5 mg/g. The lowest content rutin found in the leaves lemon balm 0.25 mg/g. **Conclusion:** The high concentration of rutin in flowers and leaves of rue, buckwheat, pansy and rose give more importance to rue, buckwheat, pansy and rose as medicinal and diet plants for theirs use to decreasing of capillary fragility.

P292 Total sulphur and organosulphur compounds in garlic and ramsons plant organs at the end of vegetative period

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In this study was analysed total sulphur and content of organosulphur compounds such alliin, diallyl disulfide (DD), reduced glutathione (GSH) and L-cysteine in the bulb and leaves of garlic and ramsons at the end of vegetative period. Total sulphur content was determined by ion chromatography in the form of sulphate ion. Analysis of alliin, DD, GSH and L-cysteine was performed by HPLC using UV-VIS, electrochemical and fluorescence detectors. Sulphur content (mg/g) in leaves and bulb of garlic was: bulb 0,63 and leaves 0,66. Content of alliin: bulb $4{,}8 \times 10^{-2}$ µg/g, leaves $3{,}8 \times 10^{-2}$ µg/g, and DD: 12,97 mg/g, but in the leaves of garlic DD was below of the limit of detection. Content of L-cysteine in the bulb of garlic was 15,82 mg/g, leaves 2,31 mg/g, while the content of GSH in the bulb of garlic was 21,9 mg/g and leaves 12,69 mg/g. Total sulphur content (mg/g) in leaves and bulbs of ramsons at the end of vegetative period was: bulb 0,93 and leaves 0,74. Content of alliin: bulb $23{,}2 \times 10^{-2}$ µg/g, leaves $7{,}3 \times 10^{-3}$ µg/g. The content of DD in the bulb of ramsons was 1,78 mg/g, while in the leaves content of DD was below of limit of detection. L-cysteine in the bulb of ramsons was 14,51 mg/g and leaves 0,94 mg/g, GSH in the bulb of ramsons was 14,51 mg/g and leaves 8,94 mg/g. In general the contents of total sulphur and organosulphur compounds in the bulb of garlic and ramsons are higher than in leaves.

P293 Content of some phenolic acids and rutin in the leaves and roots of *Symphytum officinale* L.

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Symphytum officinale L. is a perennial flowering plant of the genus Symphytum in the family *Boraginaceae* wich contains allantoin. **Objectives:** In this study, using HPLC-ED system, analysis of chlorogenic acid (CGA), gallic acid (GA), rosmarinic acid (RA) and caffeic acid (CA), and rutin was carried out in water extracts in the leaves and roots of S. officinale. **Methods:** Analyses of CGA, GA, RA, CA and rutin were performed in the leaves and root of S. officinale The drug (1 g) was powdered and extracted with pure water (9 ml). Afterward 1 ml of that extract was decanted and centrifuged. Supernatant was used for analysis. The standard solutions were of CGA, GA, RA, and CA dissolved in mobile phase, and rutin was dissolved pure water. HPLC conditions were following: Mobile phase methanol-acetonitrile-water-acetic acid (20+10+70+1); ED detector with range 50nA, potential +0.840 V, filter 0.02 Hz; flow rate 1 ml/min; temperature 25 °C, Column: ODS hypersil. **Results:** The content in (mg/g) of CGA was in the root of *S. officinale* 1.27, GA 0.05, RA 0.85, CA 0.15, and leaves GA 0.20, RA 1.15, CA 0.29. The content of CGA in leaves, and rutin in root was below limit of detection by this method. **Conclusion:** The highest content of RA, CA, GA and rutin was determined in leaves of *S. officinale*, while only was in roots determined CGA.

P294 Screening of sage and thyme plant extracts for some phenolic acids and rutin

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Objectives: In this study, using HPLC-ED system, analysis of chlorogenic acid (CGA), gallic acid (GA), rosmarinic acid (RA) and caffeic acid (CA), and rutin was carried out in water extracts of sage and different thyme. **Methods:** Analyses of CGA, GA, RA, CA and rutin were performed of herbs sage and thyme. The drug (1 g) was powdered and extracted with pure water (9 ml). Afterward 1 ml of that extract was decanted and centrifuged. Supernatant was used for analysis. The standard solutions were of CGA, GA, RA, and CA dissolved in mobile phase, and rutin was dissolved pure water. HPLC conditions were following: Mobile phase methanol-acetonitrile-water-acetic acid (20+10+70+1); ED detector with range 50nA, potential +0.840 V, filter 0.02 Hz; flow rate 1 ml/min; temperature 25 °C, Column: ODS hypersil. **Results:** The content in (mg/g) of CGA was in the sage 0.36, GA 0.09, RA 5.45, CA 0.73, and rutin 11.8, thyme: (*Thymus vulgaris* L. CGA 1.21, GA 0.3, RA 4.27, CA 0.66, and rutin 24.9, *Thymus serpyllum* L. CGA 4.6, GA 0.95, RA 17.27, CA 1.36, and rutin 17.27, *Thymus sibthorii* Benth. CGA 1.44, GA 0.3, RA 5.5, CA 0.95, and rutin 22.64). **Conclusion:** The highest content of CGA, RA, CA and GA was determined in herbs of *Thymus serpyllum* L., and highest content of rutin was determined in *Thymus vulgaris* L.

P295 Processing, analytical caracteristics and skin anti-ageing properties of a purified sea buckthorn extract

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Sea buckthorn (*Hippophae rhamnoides* L. – Elaeagnaceae) is a spiny shrub native to Europe and Asia. The entire plant has been used for centuries in traditional medicine, but lately the interest has been focused on the berries, particularly for their nutritional value. They are rich in organic acids (mainly malic acid), carotenoids, phytosterols and flavonoûds. The berries are processed into 3 types of extracts: the pulp juice for the food market, pulp and seed CO2 extracts mainly for the cosmetic market. We focused on the potential use of sea buckthorn juice in cosmetic application, particularly for its organic acids. The juice commercialized for food products is difficult to use in cosmetics because of its odour and colour impacts on cosmetics products. In order to lessen this problem, we used ion exchange resins to purify the organic acids from a commercial sea buckthorn juice. The organic acids were analysed by HPLC – UV. After ion exchange resin treatment, the organic acids represent the majority of the soluble solids present in the juice. Malic acid concentration is about 3 times higher than in the previous commercial juice. Quinic acid is also present in important concentration, along with ascorbic acid. In addition to the organic acids, the treated juice still contains some flavonoûds. The anti-ageing activities of this organic acid rich sea buckthorn extract were demonstrated on dermal cells.

P296 Oil content determination in wild chamomile populations from Banat area

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Chamomile (Matricaria recutita L.) has wide ecological amplitude and this species geological occurrence in partial all over the world. Plant habits and the creation of secondary metabolites in the chamomile are affected by the endogenous and exogenus factors, which can be divided in: morfo- ontogenetic variability and genetic variability. With regard to the essential oil content the populations were very heterogeneous even those, which came from the same habitat. The trial was carried out in the Romanian Timis County. We studied spontaneous chamomile populations around the following localities: Carani, Gavojdia, Ghiroda and Remetea. These populations were compared with the native chamomile cultivar Margaritar, cultivated on the experimental plot of the Banat University of Agricultural Science Timisoara. Comparing the values of volatile oil content of the Margaritar chamomile cultivar with the values yielded by some spontaneous populations from the Banat area we can see that compared to the Margaritar chamomile cultivar the closest value of volatile oil content was in the Remetea and Ghiroda populations (1.06%), with a difference of only 0.02% volatile oil accumulated. The values obtained in the Carani area and in the Gavojdia area are lower, i.e. 0.99 and 1.03% respectively volatile oil. One of the causes might be the slightly low temperatures in these areas. These results are particularly important not only for the valorisation of these populations but also for their improvement in order to get new Romanian chamomile cultivars, taking into account that at present the only cultivar available on the market is the Margaritar chamomile cultivar.

P297 A comparative analysis between historical *iatrosophia* texts and modern plant use in the monasteries of Cyprus

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The *iatrosophia* is a genre of historical medical Greek texts rooted in the Byzantine Empire [1]. Here we use a diachronic approach based on the medicinal knowledge contained in six *iatrosophia* related to Cyprus from the 17th to 20th century mostly written by monks [2]. We juxtapose this ancient knowledge with modern knowledge collected in an ethnobotanical field study involving 21 monasteries. In both the ancient and the modern dataset gastrointestinal and respiratory tract disorders are among the most important conditions treated (at least 35% of the medicinal use reports). The most remarkable change is the decline of uses in dermatology (from 22% to less than 7%) and the appearance of cardiovascular and blood system uses (from practically zero to 14%) in the modern dataset. In both datasets the majority of the twenty most frequently reported plants are cultivated vegetables, fruits, aromatic herbs and other crop plants with about half belonging to the Lamiaceae, Rosaceae and Liliaceae. *Mentha spicata* L., *Crataegus azarolus* L. and *Salvia fruticosa* Mill. are used for medicinal purposes in at least 16 of the 21 monasteries. While *M. spicata* scores among the top ten with the highest numbers of use reports in the historical texts, *S. fruticosa* is less important and *C. azarolus* not mentioned at all. The present analysis highlights changes and similarities in plant usage patterns of two closely related traditional systems separated in time and adds to our understanding of the dynamics involved the development of herbal medicine use in the (Eastern) Mediterranean. **Acknowledgements:** This study was supported by a grant from the A. G. Leventis Foundation. **References:** 1. Touwaide, A. (2007) In: Bowers, B.S. (Ed.) The Medieval Hospital and Medical Practice. Ashgate. Hampshire. 2. Lardos, A. (2006) J Ethnopharmacol 104: 387 – 406.

P298 Quantitative HPLC analysis of rosmarinic acid and luteolin 5-*O*-β-glucoside in the aerial parts of *Thymus praecox* Opiz subs. *großheimii* (Ronniger) Jalas var. *großheimii*

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There are 39 (64 taxa) *Thymus* species in Turkey, 27 taxa of which are endemic [1 – 3]. *Thymus praecox* subs. *großheimii* var. *großheimii* is used as a herbal tea for cold, stomachache, cough, infections in Erzurum Province (Turkey). In our previous study we have isolated two triterpenic acid compounds (ursolic acid and oleanolic acid), three phenolic compounds (rosmarinic acid, ethyl rosmarinate and methyl rosmarinate), one flavon glycoside (luteolin 5-*O*-β-glucoside), and one monoterpene diglycoside (thymoquinol 2,5-*O*-β-diglucopyranoside) from the aerial parts of the plant. In this study, the HPLC methods were applied for quantitative analysis of rosmarinic acid and luteolin 5-*O*-β-glucoside. Analysis were performed on an Agilent 1200 HPLC system, equipped with a DAD detector. For all analysis, RP-C 18 column (250 x 4.6 mm, 5 µm particle size, ACE®) was used and maintained at 30 °C. Solvents used for separation were 0.05% aqueous phosphoric acid (v/v) (A) and acetonitrile (B) for rosmarinic acid; 1% acetic acid (v/v) (A) and methanol (B) for luteolin 5-*O*-β-glucoside. Flow rate and sample volume were set to 1.0 ml/min and 100 µl, respectively. Detection wavelength was 330 nm for rosmarinic acid, 350 nm for luteolin 5-*O*-β-glucoside. The contents of the rosmarinic acid and luteolin 5-*O*-β-glucoside were 15.2 mg/g and 57.8 mg/g of dry weight, respectively. **Acknowledgements:** The authors would like to thank Erzurum Regional Hygiene Institute for the technical support. **References:** 1. Jalas J. (1982) *Thymus* L., in Flora of Turkey and the East Aegean Islands, Vol. 7, pp. 349 – 382, edited by P.H. Davis, University Press, Edinburgh, UK. 2. Davis P.H., Mill R.R., Kit T. (1988) Flora of Turkey and the East Aegean Islands, Vol. 10, pp. 209 – 504, University Press, Edinburgh, UK. 3. Güner A., Özhatay N., Ekim T., Baser K.H.C. (2000) Flora of Turkey and the East Aegean Islands, Vol. 11, p. 209, University Press, Edinburgh, UK.

P299 The content of total phenolics and arbutin in some fruits from Bosnia

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The aim of this study was to determine total phenolics with spectrophotometric method and arbutin using HPLC-ED method in the fruit extracts of blueberry, red currant, cowberry, sour cherry and wild cherry. The fruits were homogenized and extracted with water. Afterward of

that extracts were centrifuged. Supernatants were used for futher analysis. The content of total phenols was estimated by a spectrophotometric method using gallic acid as a standard. HPLC-ED conditions: Mobile phase: EDTA, sodium acetate, acetic acid, methanol 50%, and water up to 1 L; ED detector with range 50 nA, potential +0.750 V, filter 0.02 Hz; flow rate 0.9 ml/min; temperature 25 °C. Pure arbutin was dissolved in mobile phase and served as standard solution. It was estimated that total content of phenols in wild cherry was 7,5 mg/g, in blueberry 5,5 mg/g, in cowberry 5,1 mg/g, red currant 2,7 mg/g and sour cherry fruits had 2,2 mg/g. The content of arbutin in blueberry was 21 μg/g, red currant 26 μg/g, cowberry 63 μg/g and sour cherry 20 μg/g. The content of arbutin in wild cherry was below limit of detection. All examined fruits contain uroantiseptic arbutin. The highest content of total phenolics was found in the fruits of wild cherry and blueberry.

P300 Casticin and rotundifuran content of subcritical *Vitex agnus-castus* CO_2 fruit extracts compared with classical Soxhlet extracts

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The ripe fruits of *Vitex agnus-castus* L. are traditionally used as herbal medicine for the treatment of various conditions in women, like premenstrual syndrome, menopause and disrupted lactation. The dopaminergic activity of some extracts is assumed to be a synergistic effect of diterpenes and other constitutents [1]. The aim of the present study was to compare subcritical liquid CO_2 extracts of *Vitex agnus-castus* dried fruits with soxhlet extracts using three different solvents, namely n-hexane, dichloromethane and methanol. Subcritical liquid extraction (25 – 26 °C, 62 – 64 bar) was used because of its suitability for use in laboratory scale and minor costs compared to supercritical fluid extraction (SFE), which has been used in prior studies [2]. The so-called periodic or soxhlet-type subcritical liquid extraction [3] allowed to stop extraction at certain time points (15, 45, 90 min and 12 hours), yielding 0.01 g, 0.14 g, 0.25 g and 0.28 g extract, respectively compared to 1.8 g n-hexane, 1.3 g dichloromethane and 0.96 g methanol classical soxhlet extracts. HPLC-DAD and external standards were used for the quantification of the flavonoid casticin and the diterpene rotundifuran [4]. The highest casticin content was found in the n-hexane soxhlet extract. Overall, concentrations of casticin varied between 0,15 and 106,7 mg/100 g in the dried fruits and between 0,13 and 1,18% in the extracts. Rotundifuran content was below limit of quantification for the three soxhlet extracts, but showed relatively high amounts (11,75 – 301,01 mg/100 g drug) in the subcritical CO_2 extracts in comparison with literature [2], [4]. **Acknowledgements:** Reference standards (casticin, rotundifuran) were a kind gift from Bionorica AG. **References:** 1. Meier B. et al. (2000) Phytomedicine, 7(5):373 – 381. 2. Cossuta D. et al. (2008) J. of Supercritical Fluids, 47: 188 – 194. 3. Naik S.N. et al. (1989) Fluid Phase Equilibria, 49: 115 – 126. 4. Hoberg E. et al. (2000) Planta Med., 66(4): 352 – 355.

P301 Phytochemical studies on the aerial parts of *Origanum minutiflorum* (Lamiaceae)

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The genus *Origanum* (Lamiaceae) is represented by about 38 species worldwide [1]. There are 22 *Origanum* species (27 taxa) in Turkey, 13 taxa of which are endemic [2 – 3]. *Origanum minutiflorum* is an endemic and commercial species for Turkey [2]. It is used as a herbal tea in Turkey. In this study, two triterpenic acids (ursolic acid (**1**) and oleanolic acid (**2**)) and three phenolic compounds (rosmarinic acid (**3**), 4-(3,4-dihydroxybenzoyloxymethyl)phenyl-β-D-glucopyranoside (**4**), and 3-(3,4-dihydroxyphenyl)-2-hydroxy-propionic acid (danshensu) (**5**)) were isolated from the methanol extract of the aerial parts of *Origanum minutiflorum* using several chromatographic methods. The structures of the pure compounds were elucidated by using ^{1}H-NMR and ^{13}C-NMR Spectroscopy, and ESI-MS. **Acknowledgements:** The authors would like to thank Prof. Dr. Mehmet Koyuncu (Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara) for identification of the plant material and Prof. Dr. Ihsan Çalis (Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara) for structure elucidation of the compounds. **References:** 1. Evans W.C. (1989) Trease and Evans' Pharmacognosy, 13th ed. Bailliere Tindall: London, Great Britain. 2. Ietswaart J.H. (1982) *Origanum* L., In: Flora of Turkey and the East Aegean Islands, (edited by P.H. Davis), Vol. 7, pp. 297 – 313, University Press, Edinburg, UK. 3. Duman H. (2000) *Origanum* L., In: Flora of Turkey and the East Aegean Islands (edited by Güner A, Özhatay N, Ekim T, Baser KHC), Vol. 11, p. 207, Supplement 2, University Press, Edinburgh, UK.

P302 New sesquiterpene lactones from *Inula montbretiana* DC

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The genus *Inula* (Asteraceae) comprises more than 100 species distributed in Asia, Europe and Africa. Many of these species have been used as traditional medicines and were reported to possess anti-inflammatory, anti-infective and other activities [1]. Sesquiterpene lactones (STL) have frequently been reported as active constituents. In the course of our studies on STL in Asteraceae, we have now investigated for the first time *Inula montbretiana* DC, a species occurring in Turkey. From the dichloromethane extract of aerial parts we isolated several esters of 9β-hydroxy-parthenolide, whose structures were determined by HR-ESI-MSMS and extensive NMR measurements. A mixture of **1a-2b** was obtained in crystalline form which was separated by prep. HPLC to yield diastereomeric mixtures of **1a+1b** and **2a+2b**. Compounds **3** and **4** were obtained by CC from further fractions. While **3** and **4** have previously been described as constituents of *Inula verbascifolia* [2], compounds **1a** and **1b** as well as **2a** and **2b** are new natural products, to the best of our knowledge. Further compounds of this type were detected in the extract by UHPLC-ESI-MSMS; their isolation is still ongoing. Studies on the anticancer and anti-protozoal activity of the isolated compounds are in progress.

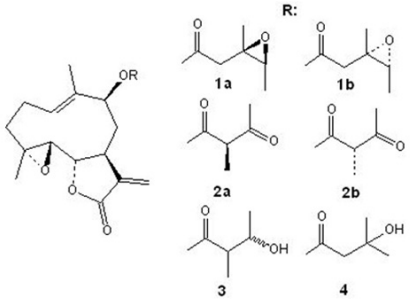

Fig. 1

Acknowledgements: The presented work was performed at IPBP, Münster, during an ERASMUS fellowship. **References:** 1. Zhao, Y-M. et al. (2006) Chem. Biodivers. 3: 371 – 384. 2. Harvala, E. et al. (2002) J. Nat. Prod. 65: 1045 – 1048.

P303 The investigation on the terpenoids and flavonoids of an endemic *Taraxacum* species from Turkey

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Taraxacum genus (Asteraceae) has about 2000 species in the world. The 48 species are naturally found in Turkey [1]. Some species of these plants are wellknown in Turkish folk medicine and are used medicinaly [2]. Many biological activities of Taraxacum genus were determined in the earlier reports. Actually, the terpenoids and flavonoids play a role of showing the most of these biological activities. Taraxacum farinosum Hausskn. & Bornm., an endemic Taraxacum species, is mainly distributed in Central Turkey. And this plant has not been investigated so far. The plant materials were collected in a flowering stage during the spring. The ethanolic extracts of root and herb samples were examined

by RP-HPLC. They were found to be rich in terpenoid and flavonoid contents. From root extract of the plant taraxasterol was isolated.The antibacterial and antifungal activities of the root and herb extracts were also screened against six bacterial, and six fungal strains. Agar well diffusion and agar tube dilution techniques were performed for evaluation of antimicrobial activity of the extracts. Taraxacum farinosum root extract was active to Staphylococcus aureus and Salmonella typhi. The root and herb extracts of the plant have shown significant activity against Microsporum canis and Trichophyton longifusus. **References:** 1. Davis, P.H. (1982) Flora of Turkey and the East Aegean Islands, University Press, Edinburgh. 2. Baytop, T. (1984) Therapy with Medicinal Plants in Turkey (Past and Present), Publication f Istanbul University, Istanbul.

P304 A new acylated neohesperidose derivative from *Geranium purpureum*

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In the flora of Turkey, the genus *Geranium* L. (Geraniaceae) is represented by 35 species [1, 2], some of which are traditionally used as antidiarrheal, antihemorrhoidal, antidiabetic, hemostatic, stomachic, diuretic as well as for the treatment of stomach ulcers and internal bleeding [3]. Moreover, leaves and tubers of some *Geranium* species are consumed as food in Anatolia [1]. As a part of our ongoing phytochemical studies on the Turkish *Geranium* species [4, 5], we have investigated aerial parts of *Geranium purpureum* Vill.

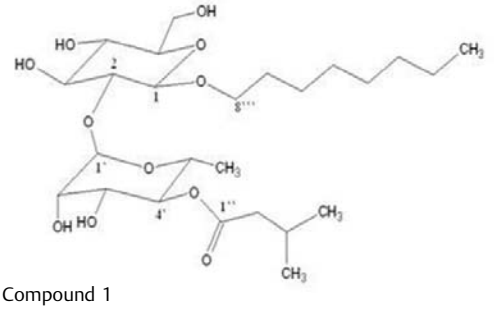

Fig. 1: Compound 1

A new acylated neohesperoside derivative, 1-octyl-4′-isovaleroyl-neohesperoside **(1)** along with known compounds, quercetin-3-rutinoside **(2)** and gallic acid **(3)** isolated from *Geranium purpureum*. The structure elucidation of the isolated compounds was performed by spectroscopic analysis including 1- and 2-dimensional NMR experiments (^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY) as well as ESI-TOF-MS spectrometry. This is the second report of occurance of acylated neohesperidose derivatives in the nature and this type of compounds have been isolated only from *Geranium caespitosum* in the plant kingdom up to now. **References:** 1. Davis, PH. (1966) Flora of Turkey and East Aegean Islands; Davis P. H. (Ed.), Vol 2. Edinburgh University Press. Edinburgh. 2. Güner, A. (2000) Flora of Turkey and East Aegean Islands Supll. II. Güner A., Özhatay N., Ekim T., Baser KHC. (Eds.), Edinburgh University Press. Edinburgh. 3. Baytop, T. (1999) Theraphy with Medicinal Plants in Turkey, Nobel Tip Kitabevi. Istanbul. 4. Söhretoglu, D. et al. (2009) Turk. J. Chem., 33:685 – 692. 5. Söhretoglu D. et al. (2009) Helv. Chim. Acta, 92: 520 – 524.

P305 In vitro toxicity and antioxidant activities of *Hedychium gardnerianum* from S. Miguel (Azores)

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There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources. Numerous studies on essential oils have been performed in order to investigate the effects of these fragrances on many biological systems. Hedychium gardnerianum, an infestant plant in the Azores (Portugal) is used in many popular applications but not as natural medicinal drug. Therefore, essential oils from the leaves of three different places in S. Miguel Island were extracted by hydrodistillation and assayed for their antioxidant and toxicity properties, using the stable free radical diphenylpicrylhydrazyl test. [1] and Artemia salina cytotoxicity assay [2], respectively. All the fractions assayed show high antioxidant activity with EC50 values between 8.46 and 28.76 μg/mL with the essential oil from Furnas the most active. A. salina LC50 toxicity values are within the range 300.1 and 379.2 μg/mL with the essential oil from Fogo with the higher toxic effect. Our results indicate that the higher antioxidant activity the lower the toxicity in A. salina. These results are a very good preliminary approach for the antitumor and pesticidal activity of these samples. However, further studies must be performed in order to elucidate the mechanism of action of these essential oils on cytotoxicity tests. **References:** 1. Molyneux P (2004). Songklanakarin J. Sci. Technol. 26(2). 2. Solis P, Wright C, Anderson M, Gupta M, Phillipson D (1993). Planta Med. 59:250 – 252.

P306 The use of bark from *Stryphonodendron barbatiman* martius tree in cutaneous wounds

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Several herbal compounds have been used in different cutaneous wounds aiming to decrease the time of cicatrization. The use of bark from *S. barbatiman* tree represents a very common kind of treatment of wounds in animals and human in Brazil. In this work, the application of S. barbatiman bark extract was carried to analyze the cicatrization process of cutaneous wounds of 2 cm, which were produced surgically on dorse of 20 mice. In the first group, 15 animals were treated using ointment formulation containing 10% of ethanol extracts of *S. barbatiman*. In the control group, 5 animals do not received treatment. The wounds were measured to each 7 days until the 21th day. The treatment showed to be effective, and on 21th day the wounds were completely cicatrized, whereas the animals that not received any treatment presented discrete decrease of wound size at the same time. Histological analysis from tissues obtained from animals that received treatment showed the epithelial tissue formation from 10th day. On the other hand, only from 18th day, little epithelial tissue formation was observed in the control group, without total cicatrization on the 21th day. This data suggest that the use of bark from *S. barbatiman* tree could represent an efficient and rapid method of wound treatment.

P307 *In-vitro* lipid peroxidation as progression of liver injury at the cellular level

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Jaundice is a condition, which affects the liver of the individual. It is not a disease on its own as the tissue damage and endotoxemia enhances the formation of free oxygen radicals and reactive oxygen metabolites which increase lipid peroxidation through the accumulation of hydrophobic bile acids. In this study, nineteen plants were recorded during an ethnobotanical survey for jaundice therapy: *Alstonia boonei* De Wild. (Apocynaceae), *Cajanus cajan* (Linn.) Millsp (Papilionoideae), *Calliandra portoricensis* (Jacq) Benth (Mimosaidaea), *Celastrus paniculatus* Linn. (Celastraceae), *Cochlospermum tinctorium* Perr. (Cochlospermaceae), *Curcuma longa* Linn. (Zingiberaceae), *Curculigo pilosa* (Schum. & Thonn.) (Hypoxidaceae), *Cymbopogon citratus* (DC). Stapf. (Poaceae), *Enantia chlorantha* Oliv. (Annonaceae), *Gossypium barbadense* Linn. (Malvaceae), *Kigelia africana* (Lam.) (Bignoniaceae), *Lawsonia inermis* Linn. (Lythraceae), *Lophira alata* Banks (Ochnaceae), *Mangifera indica* Linn. (Anacardiaceae), *Morinda lucida* Benth. (Rubiaceae), *Phyllantus amarus* Schum. & Thonn. (Euphorbiaceae), R*auwolfia vomitoria* Afzel. (Apocynaceae), *Sarcocephalus latifolius* Sm.(Rubiaceae). Free radical scavenging activity was evaluated using DPPH radicals and inhibition of lipid peroxidation

accessed with thiobarbituric acid (TBA) method in two poly unsaturated fatty acid (PUFA) models of *Clarias gariepinus* and *Scomber japonicum* fish homogenates calculated as MDA equivalent/gm of tissue. *L. alata* had highest antioxidant activity r (80.95%) and *P. amarus* lowest (1.53%). As the concentration of extract increases, the absorbance increases, while TBARS value decreases as 6.7305×10^{-5} to1.0384×10^{-5} and 8.2304×10^{-5} to 5.4100×10^{-5} (mg/tissue) in *C. gariepinus* and *S. japonicum* fish models respectively. Thus, TBARS determination provides a measure of membrane lipid peroxidation and may provide a direct assessment of the progression of liver injury at the cellular level.

P308 Isolation and characterisation of arabinogalactan-proteins from cell cultures of *Pelargonium sidoides* DC

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Pelargonium sidoides is a traditional medicinal plant from South Africa. An aqueous-ethanolic formulation of the roots is approved for the treatment of acute bronchitis. The main effects could be related to antibacterial activities and the stimulation of the non-specific immune system by the main components of Pelargonium sidoides: coumarins, phenols and tannins [1]. Due to wild harvesting, Pelargonium sidoides is an endangered species. Therefore the propagation of the plant material by cell cultures and the extraction of ingredients are interesting tasks. Calli were established from roots and shoots of germinating seeds and the maintenance of callus and suspension cultures in different media was investigated. In suspension cultures of Echinacea purpurea a secretion of arabinogalactan-proteins (AGPs) with weak immunomodulatory activities has been shown [2]. From suspension cultures of Pelargonium sidoides high amounts of pure AGPs could be isolated by precipitation with β-glucosyl Yariv reagent. To study potential therapeutic benefits the characterisation of these AGPs is necessary. Quantification of neutral sugars by acetylation pointed out arabinose (Ara) and galactose (Gal) as dominating monosaccharides in a ratio of 1: 1.7 – 1.8. Colourimetric determination of uronic acids revealed an amount of 5 – 8%. Linkage type analysis showed that the main components are 1,3,6-Gal(p), 1,3-Gal(p) and 1-Ara(f) as well as minor amounts of 1,6-Gal(p), 1,4-Gal(p) and 1,5-Ara(f). **References:** 1. Kolodziej H (2008) Planta Med 74:661 – 666. 2. Classen B et al. (2006) Phytomed 13:688 – 694.

P309 Cytotoxicity of aristolochic acid I in HepG2 cells: comparison of different cell viability assays

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Application of herbal preparations in the context of Traditional Chinese Medicine for example becomes more and more popular. The focus of research is on the one hand the pharmacological activity of such herbal preparations and on the other hand whose potential toxicological risk for the human organism. A number of methods have been developed to determine cytotoxicity, each of them using specific approach to detect different aspects of cell viability, such as proliferation and metabolic functions. In this study the natural product aristolochic acid I (AAI), a component of Aristolochia species, was analyzed for its toxic effects on the human hepatoma cell line HepG2. Four methods (cell counter, MTT assay, BrdU assay, colony forming ability) were compared. HepG2 were exposed to AAI for 24 h. BrdU assay and colony forming ability were more sensitive than MTT assay and the determination of cell viability using a cell counter. Especially for risk assessment of natural products as well as herbal preparations the results point out the importance of the deliberate selection of cytotoxicity assay.

P310 The effect of some flavonoids and pyruvate on 6-hydroxydopamine and 3-hydroxykynurenine induced neurotoxicity

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Oxidative stress has been strongly implicated as one of the cause in cell death in many neurodegenerative disorders. Due to antioxidative properties in vitro, the use of flavonoids and other polyphenolic compounds synthetised by plants are considered to be a promising strategy to treat Alzheimer's disease and Parkinsons's disease. In the present study, we tested the protective effects of some flavonoids and sodium pyruvate on 6-hydroxydopamine (6-OHDA) and 3-hydroxykynurenine (3-HK) neurotoxcicity in retinoic acid differentiated and non differentiated human neuroblastoma cells SK-N-SH and SH-SY5Y. Sodium pyruvate prevented 6-OHDA and 3-HK induced cell viability reduction. However, neither pre-treatment with flavonoids nor simultaneous addition had any protective effects in the 6-OHDA and 3HK model of neurotoxicity. The ability of sodium pyruvate to exhibit neuroprotection in the 6-OHDA and 3-HK toxicity may be related to the antioxidant properties and the capability to penetrate into the cell.

P311 Triterpenoid saponins of the Caryophyllaceae and Illecebraceae family

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The closely related plant families of Caryophyllaceae and Illecebraceae are reviewed for their saponins. An overview with special attention on the contained sapogenins and their linkage of sugar moieties is provided. Gypsogenin, Gypsogenic acid and Quillaic acid turned out to be widely spread in the family of Caryophyllaceae. Gypsogenin is found in 46% of the examined species. The occurrence of Gypsogenin is 1.5-fold higher than that of Gypsogenic acid or Quillaic acid, which occur with the same frequency. The genus Gypsophila L. of the family of Caryophyllaceae has the highest accumulation of Gypsogenin. 75% of the examined species comprehend Gypsogenin. It occurs 3-fold more often than Gypsogenic acid or Quillaic acid in this genus. In contrast all examined species of the family of Illecebraceae lack of Gypsogenin. Since certain bisdesmosidic Gypsogenin-based saponins of Gypsophila paniculata L. recently showed the ability to drastically amplify the toxicity of cellular membrane-impermeable type I ribosome-inactivating proteins (type I RIPs), the analysis reveals other possible natural sources for further testing [1, 2, 3]. **References:** 1. Weng, A. et al. (2008) Chem.-Biol. Interact. 176:204 – 211. 2. Weng, A. et al. (2009) Chem.-Biol. Interact. 181:424 – 429. 3. Hebestreit, P. et al. (2006) Toxicon 47:330 – 335.

P312 Inhibitory profile of latex-proteases in the genus *Euphorbia*

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The latex of some plant families such as Apocynaceae, Asclepiadaceae, Asteraceae, Caricaceae, Convolvulaceae, Euphorbiaceae, and Moraceae are known to contain endopeptidases. Proteolytic enzymes from plant latex have received special attention in the pharmaceutical industry and biotechnology due to their property of being active over wide range of temperature and pH. Nearly half of the commercially available enzymes are proteases, frequently used in food processing, tenderization of meat, brewing, cheese elaboration, bread manufacturing, leather and textile industries [1]. In the genus Euphorbia (Euphorbiaceae) 64 different species are mentioned to have proteolytic activity in their latices [2]. In this investigation the latex of 23 species of the genus Euphorbia, which are not characterised before, were collected in the Botanical Garden Berlin. To determine proteolytic activity we used the fluorogenic substrate BODIPY FL- casein (Moleculare Probes, Inc., USA) [3]. All tested samples show proteolytic activity. To investigate the type of endopeptidases, the latex samples were pre-incubated with specific inhibitors for serine (AEBSF (4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride)-, cysteine(E64 (4-(2-Aminoethyl)benzenesulfonylfluoride hydrochloride)) -, aspartatic (Pepstatin A)- and metalloprotease (EDTA) and the remaining activity was determined. 18 plants were strongly inhibited only by serine specific inhibitor, one plant was not influenced by any inhibitor and 4 plants were influenced by at least two inhibitors and had still a remaining proteolytic activity. **References:** 1. Domsalla, A., Melzig, M.F. (2008) Planta Med 74:1 – 13. 2. Domsalla, A., Melzig, M.F. (2010) Pharmazie 65:227 – 230. 3. Menges, D.A. et al. (1997) Anal Biochem 251:144 – 147.

P313 Quantification of isoflavones in nutritional supplements of red clover by HPLC and HPTLC analysis

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Two methods for the quantification of the five main isoflavones in red clover containing nutritional supplements using high performance liquid chromatography and high performance thin layer chromatography were developed. Validation of both methods was performed according to the ICH guidelines. The HPLC method was found to be rapid (25 min for a single run) and offered a good linearity, sensitivity and repeatability but caused high operating costs due to the sophisticated instrumentation and extensive amounts of solvent for the whole validation process. For the quantitative determination of the five isoflavones by HPTLC, a mobile phase of dichloromethane-acetic acid-ethyl acetate (12:2:1, v/v/v) was used which afforded sufficient separation. HPTLC allowed simultaneous fast quantification of the isoflavones comparable to the HPLC method and efficient distinction between different nutritional supplements. Therefore, this inexpensive method could be used in the quality control of nutritional supplements of red clover.

P314 Resveratrol, a naturally occurring stilbene with antileishmanial activity

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Research over the past several decades has revealed that resveratrol exerts pleiotropic health beneficial effects including potent antioxidant, anti-inflammatory, anti-aging, cardioprotective and neuroprotective activities. As a phytoalexin, resveratrol possesses considerable capacity to control fungal, bacterial and viral infections in plants, and possibly in infectious conditions in humans. Our interest in natural products with anti-infective potentials and a recent report on the antileishmanial activity of resveratrol prompted the present study. Using transgenic Leishmania major expressing green fluorescent protein (GFP) and FACS analysis, resveratrol revealed pronounced direct toxicity for extracellular promastigotes, with a maximal killing rate of ca. 97% at a sample concentration of 45 µg/mL. When L. major GFP-infected BMM? were exposed to the test compound (5 – 75 µg/mL), the resulting GFP signal was similarly reduced in a concentration-dependent manner (from 95% to 5%). However, cell cytotoxicity invariably increased with concentrations? 25 µg/mL as evident from the number of propidium iodide positive events. The MTT-assay provided an IC50 of 83 µM (20 µg/mL) for non-infected BMM? This finding contrasts with the reported absence of toxic effects up to 45 µg/mL in a microscopic study. The Diff-Quick stain verified changes in the morphology of infected cells. Although resveratrol is generally considered to be well tolerated [1], the method of assessment appears to be a critical issue as well as the kind of cells used in experiments [2, 3]. In conclusion, resveratrol showed potent antileishmanial activity in the range of 15 – 20 µg/mL, while higher concentrations produced adverse effects on the host cells. **References:** 1. Cottart, CH. et al. (2010) Mol. Nutr. Food Res. 54, 7 – 16. 2. Kedzierski, L. et al. (2007) Parasitol Res. 102: 91 – 97. 3. Kedzierski, L. et al. (2004) The Journal of Immunology 172: 4902 – 4906.

P315 Characterization of the anti-adhesive principle of a *Pelargonium sidoides* root extract (EPs® 7630) in the interaction of group A-streptococci and human laryngeal epithelia cells

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Recently, intriguing experimental research has shown an anti-adhesive capability of the aqueous-ethanolic root extract EPs® 7630 that prevents docking of group A-streptococci on human laryngeal epithelial HEp-2 cells [1]. The present work aimed to gain further insight into the underlying biologically active principle and to identify the components that account for the anti-adhesive activity of this herbal medicine. In a validated flow cytometry-based assay [2], fluorescent-labelled group A-streptococci were incubated with human epithelial (HEp-2) cells. Only after pre-treatment of S. pyogenes, EPs®7630, a methanol-soluble (MSF) and a methanol-insoluble fraction (MIF) inhibited the adhesion of the pathogen to the host cells by ca. 45%, ca. 30% and ca. 34%, respectively. This finding indicated that the anti-adhesive effects were due to interactions with binding factors on the bacterial surface. Treatment of the extracts with skin powder produced polyphenol-free samples. That these samples were devoid of any anti-adhesive activities clearly indicated that the present proanthocyanidins played a decisive role as anti-adhesive components. Comparative studies with chemically defined proanthocyanidins including A- and B-type oligomers identified the presence of pyrogallol B-ring elements of constituent flavanyl units (prodelphinidin nature) as important structural feature of the anti-adhesive principle of this herbal medicine. However, the targets on the bacterial cell surface remain unknown. **References:** 1. Conrad A. et al., (2007) Phytomedicine 14 (Suppl. VI), 52 – 59. 2. Sethman CR et al. (2002) J Microbiol Methods 51, 35 – 42.

P316 Micronutrient and hydro priming of seed to overcome salt stress during germination in cumin (*Cuminum cyminum* L.)

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In production of medicinal plants, seed germination is a very important problem. The treated seeds (control, hydropriming and Zn_2SO_4) of Cumin (*Cuminum cyminum* L.) were evaluated at germination and seedling growth for tolerance to salt (NaCl and Na_2SO_4) conditions at the same water potentials of 0.0, −0.3, −0.6, −0.9 and −1.2 MPa. Electrical conductivity (EC) values of the NaCl solutions were 0.0, 6.5, 12.7, 18.4 and 23.5 dS.m-1, respectively. The objective of the study was to determine factors responsible for germination and early seedling growth due to salt toxicity or osmotic effect and to optimize the best priming treatment for these stress conditions. Results revealed that germination delayed in both solutions, having variable germination with different priming treatments. Germination, shoot and weight, root and shoot length were higher but mean germination time and abnormal germination percentage were lower in NaCl than Na_2SO_4 at the same water potential. The root/shoot weight and R/S length increased with increase in osmotic potential in both NaCl and Na_2SO_4 solutions. NaCl had less inhibitor effect on seedling growth than the germination. It was concluded that inhibition of germination at the same water potential of NaCl and Na_2SO_4 resulted from salt toxicity rather than osmotic effect. Hydropriming increased germination and seedling growth under salt stress. This protocol has practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions. **Keywords:** Cumin, salt stress; zinc, hydropriming; germination

P317 The interaction effect of water stress and manure on yield components, essential oil and chemical compositions of cumin (*Cuminum cyminum*)

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Water stress enhances essential oils content in medicinal plants. Manure in soil prepares essential elements and increases quality and quantity of plant products. To study the effects of water stress and manure application on yield components, oil percentage and its main constituents on *Cuminum cyminum*, this experiment was conducted at the Agricultural Research Station of Zahak, Zabol, south east of Iran during 2003 and 2004 in a complete randomized block in factorial design with four replications. Treatments including irrigation intervals (I1: two times irrigation, I2: three times irrigation and I3: four times irrigation that are irrigation in germination, seedling, flowering and seed filing stages) and manure application (F1: without manure application, F2: 20 t/ha

manure application). The chemical composition of the essential oil was examined by GC and GC-MS and they were significantly affected by water stress and manure ($P < 0.05$). Three irrigation times with manure treatment produced the highest number of umbrella per plant, seed and biological yield and the lowest 1000 – seed weight and number of seed per umbrella. The effect of water stress and manure were significant on essential oil and its constituents. Three irrigation times with manure treatment caused the highest amount of cumin aldehyde and ρ-cymene and the lowest of β-pinene, γ-terpinene and α-pinene. Results showed that a relationship exists between the main constituents of cumin essential oil under water and manure application. Key word: Cuminum cyminum, manure, irrigation, oil and oil constituents

P318 Production of secondary metabolites in intact plants and cultured cells of *Thymus vulgaris* L.

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Thymus vulgaris L. is a traditional herbal medicine used widely as an expectorant, antitussive, and antibacterial agent. In this study the analysis of the chemical composition of essential oil from the upper parts of plant and calli of *Thymus vulgaris* L. was done for detection of the presence of major components. Growing of the calli of *Thymus vulgaris* L. and its secondary metabolites production were studied and compared with those in the intact plants. *Thymus* seeds were first sterilized by shaking in 3% (W/V) aqueous hydrogen peroxide, 5% Na hypochlorite and 80% aqueous ethanol solution respectively, and then kept in sterile petri dishes containing autoclaved agar (0.8%) at 25 – 27 °C under dark aseptic condition. seedlings were developed in two weeks. Then they were transferred to sterile Murashige and Skoog (MS) culture media containing 2, 4-D (3 mg/L), IAA (1 mg/L) and Kinetin (0.2 mg/L) as plant growth regulators. The amorphous masses (calli) were produced and subcultured every 30 – 35 days. The essential oils of plant specimens were obtained using clevenger apparatus within 4 hours and then analyzed by GC/MS. for calli, the GC/FID used for Thymol detection in dichloromethane extract. The results obtained from the GC/MS & GC/FID of essential oil of the upper parts of the plant and the calli indicated that the major components produced by the plant include thymol, para-cymene, and gamma-terpinene and the calli is able to produce thymol among mentioned components. Phytochemical tests indicated that some tannins were produced by calli, too.

P319 Chemical composition of essential oil and hydrolat of *Geum iranicum*

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Geum iranicum is an endemic plant of the genus *Geum* (Rosaceae) that has 5 species in Iran [1]. Some species of *Geum* such as *G. urbanum*, *G. rivale* and *G. japonicum* are used as medicinal plants in folk medicines [2]. In Iranian folk remedy, infusion of the aerial part of *G. iranicum* is employed for diarrhea and other gastrointestinal disorders. The decoction of the whole plant is mixed with wheat flour and used as poultice for frostbite [3]. The essential oils and hydrolats of the aerial part and root of *G. iranicum* grown at Shirvan, in the northeast of Iran, were obtained by hydrodistillation and analyzed by GC and GC/MS. The essential oil and hydrolat of the root were characterized by a high amount of eugenol (83.9%, 65.4%) and myrtenol (2.3%, 9.9%) respectively, whereas the essential oil of the aerial part of the plant had tridecanal (5.9%) and tricosane (3.9%) as characteristic constituents. Eugenol (45.7%) and linalool (7.3%) were identified as major components in the hydrolat of the aerial part of *Geum iranicum*. **References:** 1. Mozaffarian, V. (1996) A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran. 2. Vollmann, C. (1995) Flavour Fragr J. 10:173 – 178. 3. Abutorabi, H. (2001) D. Pharm. dissertation No.4247, Faculty of Pharmacy, Tehran University of Medical Sciences, Iran.

P320 Effect of phenological stages on chemical compositions of essential oils of *Dracocephalum kotschyi* Boiss. growing wild in Dizin of Iran

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The volatile compounds of D. *kotschyi*, a valuable endemic medicinal and aromatic plant in Iran, were analyzed by GC and GCMS at three different phenological stages. About 98.2%, 97.5% and 99.1% of compounds were identified at vegetative, flowering and fruiting stages, respectively. Average yields of essential oil were 0.67, 0.8 and 0.3% (v/w), for vegetative, flowering and fruiting stages, respectively. The major compounds at the vegetative stage were – (E)βocimene 53.28% and Nerol 36.38%, at the flowering stage were – (E)βocimene 47.24%, Nerol 15.53%, Methyl geranate 8.3%, αpinene 7.98% and Geraniol 5.92. At the fruiting stage – (E)βocimene 33%, αpinene 16.34%, Geraniol 14.77% Geranial 11.4% and Methyl geranate 11% were identified as major compounds. Therefore the vegetative stage is suggested as the best time collection, to obtain highest amounts of – (E)βocimene and Nerol that are two major compounds of essential oil from fragrance materials. Also in order to Geraniol and Geraniol compounds flowering and fruiting stage is as suggestible stages for gathering. **Keywords:** *Dracocephalum kotschyi,* essential oil, phenological stage, chemical compositions, (E)βocimene, Nerol and αpinene

P321 Effect of aquatic and hydro-alcoholic extracts of some medicinal plants on after germination stages of *Rumex cirspus* L.

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Use of natural and safe components in organic agriculture is increasing currently. In this context, the use of plant extracts as natural materials is a new idea for management of weeds. For this purpose the allelopathic effects of aqueous and hydroalcoholic extracts of Teucrium polium, thyme (Thymus kostchyanus) and watercress (Nasturtium officinalis) was evaluated on Rumex cirspus L. at the vegetative stage (post emergence). The effects of extracts were evaluated on plant roots and leave of plants. Plants root treated by hydro-alcoholic extracts cause to increase plant dejection after 24 h maintaining in distilled water. At this stage plant treated by watercress hydro-alcoholic and Teucrium polium water extract had high dejection effect and low dejection was observed in plants treated by control. Only aquatic watercress extracts treated on leave have shown burning effects. It seems that inhibitory effects on root of plants to be due to active ingredients in medicinal plants, Teucrium polium and Nasturtium officinalis. Our experiment have shown that some of medicinal plant could be as a useful natural herbicides, However future studies are necessary to fully understand the reasons by which medicinal plants extract may affect as herbicide in order to commercial application. **Keywords:** allelopathy, aquatic extract, hydro-alcoholic extract, Thymus kostchyanus, Nasturtium officinalis, Rumex cirspus L

P322 Bioactivity and sterols from *Gracilariopsis Persica* and *Sargassum oligocystum*

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Gracilariopsis Persica (Rhodophyta) and *Sargassum oligocystum* (Heterokontophyta) are two of the most abundant algae from Persian Gulf (1). In this study the cytotoxic effects of the mentioned algae on the Brine Shrimp Larvae (BSA) were evaluated (2) and the main sterols were identified. Separation and purification of the compounds was carried on silica gel column chromatography and HPLC to obtain 4 pure compounds 1 – 4. Structural elucidation of the constituents was based on the data obtained from H-NMR, 13C-NMR and Mass spectroscopy. The separated compounds from *G. Persica* were identified as cholesterol (1), 22-dehydrocholesterol (2) and the isolated constituents from *S. oligocystum* were identified as cholesterol (1), 22-dehydrocholesterol (2), fucosterol (3) and ostreasterol (4) based on the spectral data compared to those reported in literatures (3). The results of BSA indicated that the ethyl acetate extract of G. Persica showed a cytotoxic effect against *A. salina nauplii* (LC 50 = 4 µg/ml). The MeOH extract of *G. Persica* showed no activity but the aqueous methanol extract was less effective (LC 50 = 40 µg/ml) compared to berberine hydrochloride as a positive control (LC 50 = 26 µg/ml). The chloroform extract of S. oligocystum showed a cytotoxicty effect against *A. salina* nauplii (LC 50 = 159 µg/ml).

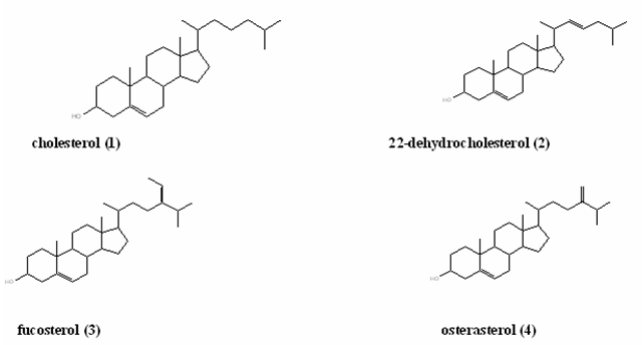

Fig. 1: Sterols from Gracilariopsis Persica and Sargassum oligocystum.

Acknowledgements: This research has been supported by Tehran University of Medical Sciences and Health Services grant **References:** 1. Bellorin, AM. et al. (2008) J. Phycol. 44: 1022 – 1032. 2. Saeidnia, S. et al. (2009) Phcog. Res. 1: 428 – 430. 3. Gohari, AR. et al. (2008) J. Med. Plants 7: 47 – 55.

P323 *Curculigo orchioides* Gaertn. extract improves sexual performance in diabetic male rats

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Sustained hyperglycaemia is considered as a major cause of sexual and erectile dysfunction in human population [1]. *Curculigo orchioides* Gaertn. is considered as a sexual tonic in Ayurvedic system of medicine with potent antioxidant and adaptogenic properties [2]. The aqueous extract of the herb was evaluated for its effectiveness against streptozotocin induced hyperglycaemic stress and subsequent sexual dysfunction due to hyperglycaemia in male rats. The body and organ weights of the animals were recorded. Behavioural analysis of rats was undertaken to observe the effect on mount, ejaculation, intromission (latencies and frequencies), and hesitation time ($p < 0.05$). This deleterious effect of sustained hyperglycaemia and associated stress was prominently ameliorated in animals treated with aqueous extract of *C. orchioides*. Hyperglycaemia also resulted in a reduction of the serum testosterone levels, *in vivo* sperm count, seminal fructose content, and serum testosterone level which was ameliorated by *C. orchioides* ($p < 0.05$). Antioxidant and anabolic activities of the extract under investigation could be a major attribute in preserving the sexual functions in hyperglycaemic male rats. The study validates the use of *C. orchioides* in traditional medicine for curing diabetes induced sexual dysfunction and compromised sexual potency [3]. **Acknowledgements:** University Grants Commission, New Delhi **References:** 1. Chauhan, N.S. et al. (2007) Fitoterapia: 530 – 534. 2. Thakur M. et al. (2009) Archives of Sexual Behavior: 1009 – 1014. 3. Lakshmi V et al. (2003) J Ethnopharmacol: 181 – 184.

P324 A new flavonoidal compound from *Diospyros lotus* L. leaves

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In our complementary study of the chemical constituents from aqueous-methanolic extract of *Diospyros lotus* L. leaves, in addition to seven flavonoid compounds (myricetin 3-O-β-glucoside, quercetin 3-O-α-rhamnoside, quercetin 3-O-β-glucoside, quercetin 3-O-β-galactoside, rutin, quercetin 3-O-β-glucuronoide, kaempferol 3-O-α-rhamnoside), a new natural product quercetin 3-O-β-glucuronide-3′-O-[6′ ′ ′-O-galloyl)-5″-O-galloyl]-O-β-D-glucoside was isolated for the first time. The aqueous-methanolic extract of *D. lotus* leaves and some isolated compounds proved a significant antioxidative effect and antitumor activities[1] as well as a significant hepatoprotective effect [2]. **References:** 1. Monica Rosa Loizzo, Ataa Said, Rosa Tundis, Usama W. Hawas, Khaled Rashed, Francseco Mechichini. Antioxidant and Antiproliferative Activity of Diospyros lotus L. Extract and Isolated Compounds. Plant Foods Human Nutrition Journal 2009, 64, 264 – 270. 2. A. Said, Usama W. Hawas, Salwa M. Nofal, K. Rashed and Antje Huefner. Pharmaco-Chemical Studies on the Aqueous Methanolic Extract of Diospyros lotus Leaves. Research Journal of Phytochemistry 2009, 3(1), 1 – 12.

P325 Sunless tanning product from green coffee beans

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The purpose of this study was to develop sunless tanning products from natural source, avoiding skin risk from sunbath and synthetic chemical. The Maillard or browning reaction, to form brown-black compounds called melanoidins was the principal of this product. Chlorogenic acid, which was extracted from green coffee beans, was brought to the change the colour of keratin in stratum corneum. It was studied that concentration of reacting color directly varied to chlorogenic acid concentration (the more concentration of chlorogenic acid, the more keratin color changed, from light brown to dark brown, with 95% confidence). The green coffee beans used in the study were Robusta (*Coffea canephora* Pierre ex A. Froehner, syn. *C. robusta* L. Linden), and were extracted with a mixture of isopropanol and water (60:40). The extract yield was 29%. It was analysed by UV-spectrophotometry and characterised by NMR-spectrophotometry. Cream with good consistency was formulated with 5% w/w chlorogenic acid, 25% w/w palm oil. Tween 60 and Span 60 of 6.1:3.9 was emulsifying agent, and other necessity. The physical evaluation, accelerated stability study, and efficacy of tanning effect to bovine skin were performed. In conclusion, sunless tanning product from green coffee beans was potentiated to be continuously studied as a commercialised product. **Keywords:** skin tanning, sunless tanning products, Maillard reaction, keratin, chlorogenic acid, green coffee beans

P326 Role of nitric oxide and hydrogen peroxide in artemisinin increase of *Artemisia annua* L. hairy roots induced by an oligosaccharide elicitor from endophytic *Fusarium* sp. SZ1

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Artemisia annua L. is presently the sole natural source of antimalarial drug artemisinin. Endophytic fungi, which spend their entire life spans inside the healthy *A. annua* plant, exhibit intimate impact on the growth and physiology of their hosts. An oligosaccharide elicitor (OE) of one endophytic fungi (*Fusarium* sp. SZ1) induced multiple responses in *A. annua* hairy roots, including rapid generation of nitric oxide (NO) and reactive oxygen species (ROS), sequentially followed by enhancement of artemisinin production. The purpose of this work was to characterise NO and ROS and their relationships with the induced artemisinin. The OE at 0.3 mg total sugar/mL induced a rapid nitric oxide synthase (NOS)-dependent NO and H_2O_2 production in the cultures, which exhibited a biphasic time course, reaching the first plateau within 1.5 h and the second within 8 h after the elicitation. The NO donor sodium nitroprusside (SNP) potentiated OE-induced H_2O_2 production and OE-induced NO was suppressed by NADPH oxidase inhibitor diphenylene iodonium (DPI) and a scavenger of H_2O_2, catalase (CAT). The inhibition of NOS activity by DPI and CAT, NADPH oxidase activity by NOS inhibitor and NO scavenger show that the OE-induced NO production and H_2O_2 production are interdependent. Moreover, the OE-induced NO and ROS were involved in stimulating the bioconversion from artemisinic acid to artemisinin. These results suggest that the mutual effects between NO and ROS play a signal role in the elicitor-induced responses and secondary metabolism activities in *Artemisia annua* L. **Acknowledgements:** Supported by the National Natural Science Foundation of China (30772731)

P327 Composition and antibacterial activity of the essential oil from aerial parts, stems, flowers, and leaves of *Ferulago contracta* Boiss. et Hausskn. from Iran

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The genus *Ferulago* comprises some 35 species, seven are found in Iran [1]. Since ancient times, *Ferulago* species have been used in folk medicine as sedative, tonic, digestive, aphrodisiac, and in the treatment of intestinal worms and haemorrhoids in different regions [2]. More over the plants of the genus *Ferulago* have been employed against ulcers, snake bite, as well as headache and diseases of the spleen [3]. The chemical investigation of some *Ferulago* species have shown coumarins, flavonoids, sesquiterpenes, and aromatic compounds [4]. The essential oil obtained by hydrodistillation from the aerial parts, stems, flowers, and leaves of *Ferulago contracta* Boiss. et Hausskn., which is endemic to Iran, was analysed by GC and GC/MS. β-Phellandrene (15.0%, 15.3%, and 25.0%) and α- phellandrene (14.4%, 11.5%, and 25.0%) were the main constituents of the aerial part, stem, and flower oil of *F. contracta* respectively. The other main components of the aerial parts' oil of the plant were β-eudesmol (10.9%) and (E)-β-ocimene (10.0%), the latter being also the main component (11.3%) of the stem oil. The leaf oil of the plant was characterised by higher amounts of β-eudesmol (24.5%), spathulenol (16.2%) and citronellol (11.9%). **References:** 1. Rechinger, K.H. (1987). *Ferulago* in: Flora Iranian Umbellifarea, No. 162. Edits., Rechinger K.H. and Hedge I.C, Akademische Druck- und Verlagsanstalt, Graz, Austria, 427 – 433 pp. 2. Aklam, E. (1999). Pharmaceutical Botanical Investigation of *Ferulago* Species Growing in Western Turkey. Ph.D Thesis, Istanbul Univ., Istanbul. 3. Demetzos, C., Perdetzoylou, D., Gazouli, M., Tan, K and Economakis, C. (2000). Planta Med., 66, 560. 4. Jimenez, B. Grande, M.C. Anaya, J., Torres, P and Grande, M. (2000). Phytochemistry, 53, 1025.

P328 Isolation of 3-butyliden-4,5-dihydrophthalide and steroids from *Kelussia odoratissima*, a Persian food seasoning

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Kelussia odoratissima Mozaff. is wild growing in central Iran and being called "karafs koohi" [mountain celery], is vastly used as yogurt seasoning [1]. It is believed in folk medicine to be effective as anti-inflammatory, antiulcer, anti-tussive, sedative and to treat gastrointestinal and cardiovascular diseases. Some of the claimed effects have been tested and approved; e.g. it had beneficial effects to prevent development of fatty streak [2]. Despite its wide use, there is no previous phytochemical analysis on the plant. Here, we report the isolation of two sterols and one phthalide from Kelussia odoratissima. Grounded fruits were extracted with hexane using a Soxhlet apparatus. After winterization, the extract was purified using mplc, eluting with heptane-ethylacetate (10:0 to 0:10) to get fractions A1-N1. Fraction G1 rendered mass of crystals which were recrystallized repeatedly in hexane to get crystals of stigmasterol and sitosterol. Fractions H1-L1 were put together and purified using second mplc eluted with heptane:ethyl acetate (8:2 – 6:4), resulted in fractions A2-R2. Fraction E2 rendered mass of crystals which were recrystallized to get 3 as 3-butyliden-4,5-dihydrophthalide (Z-ligustilide). Using HNMR, CNMR and mass spectra, structures were elucidated. Since, plant sterols are effective on blood cholesterol [3] and inflammatory processes [4] in human body, and phthalides could decreased platelet aggregation [5,6], and were effective on Helicobacter pylori [7] and inflammation [8], a rational relation between the major plant constituents and its proposed ethnopharmacological effects may be postulated. **References:** 1. Mozaffarian, V. (2003) Bot. Zhurn. (Moscow & Leningrad) 88(2): 88 – 94. 2. Asgary, S. et al (2004) Phytother. Res. 18: 370 – 372. 3. Katan, M.B. et al. (2003) Mayo. Clin. Proc. 78:965 – 78. 4. Boukes, G.J. et al. (2008) African J. Biotech. 7:1624 – 1629. 5. Zhang, L. et al. (2009) Yakugaku Zasshi. 129:855 – 859. 6. Cao, Y.X et al. (2006) Vascular pharmacology. 45:171 – 176. 7. Dekker, K.A. et al. (1997) J. Antibiotic 50: 833 – 839. 8. Zheng, G.Q. et al. (1993) Nutr Cancer. 19:77 – 86.

P329 Pharmacokinetic study of lancemaside A in mice and anti-inflammatory effect of its metabolite, echinocystic acid

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To understand the anti-inflammatory effect of lancemaside A isolated from *Codonopsis lanceolata* Trautv. (family Campanulaceae), which ameliorates TNBS-induced colitis [1], we orally administered lancemaside A to mice and performed its pharmacokinetic study in mice by LC-MS/MS. Orally administered lancemaside A was metabolized to echinocystic acid via lancemaside X by intestinal microflora in mice by intestinal microflora and/or intestinal tissues and then absorbed it into the blood. Echinocystic acid also down-regulated inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) as well as inflammatory mediators, NO and PGE2 in LPS-stimulated peritoneal macrophages. Orally and intraperitoneally administered echinocystic acid suppressed the production of pro-inflammatory cytokines, TNF-α and IL-1β, in LPS-injected mice. These results suggest that orally administered lancemaside A may be metabolized to echinocystic acid by intestinal microflora, and echinocystic acid be absorbed into the blood and shall express anti-inflammatory effects. **Acknowledgements:** Financially supported by a grant (09172 KFDA 996) from Korean Food and Drug Administration (2009). **References:** 1. Joh, E.H. et al. (2010) Int J Colorectal Dis. 25:545 – 551.

P330 The hexane fraction of an *Ardisia crispa* ethanolic extract shows anti-arthritis effects in a rat arthritis model

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Ardisia crispa has been claimed by local villagers to have medicinal properties, and it is widely used in treating dysmenorrhea, rheumatism, orchitis, skin problem, coughs, fractured bones, sprains and treatment for women afterbirth [1]. To date, there is no report documented on *Ardisia crispa*'s effect on chronic inflammation especially on anti-arthritic properties. The hexane fraction of an ethanolic extract of *Ardisia crispa* root (ACHE) was used in this study. Complete Freund's adjuvant (CFA) was injected onto plantar aponeurosis of right paw of rat to induce chronic arthritis. The following day onwards, ACHE at 3, 10, 30, and 100 mg/kg and also indomethacin as positive control were administered to the rats. Paw volume of rat was measured with a plethysmometer for 14 days. Ankle tissue was collected for ELISA test of tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β). The result show that ACHE at all doses (3.10.30 and 100 mg/kg) have significant ($p < 0.05$) inhibition of oedema by 45.3%, 64.31%, 73.12% and 49.55% respectively. At 3, 10 and 30 mg/kg, ACHE significantly reduced TNF-α by 45.2%, 45.7% and 25.1% respectively when compared with control. For IL-1β, only 10 mg/kg and 30 mg/kg of ACHE elicited a significant ($p < 0.05$) inhibition of this mediator in local tissue by 45.9% and 36.5% respectively. The efficacies of those doses were comparable to the effect of indomethacin (34.6%). Thus, it can be concluded that *Ardisia crispa* posseses anti- arthritic effect on specific FCA-induced arthritis model in rats.

P331 Anti-infammatory and free radical scavenging activity of *Juniperus communis* L. 1753 var. *communis* leaves and cones extract

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All over the world plants from the *Juniperus* genus have always been regarded as a well-known traditional remedy and spice. These plants are extensively used in the folk medicine for healing various disorders: common cold, urinary and kidney infections, dermatological disorders, bronchitis, pneumonia, dysentery, hemorrhage, rheumatic arthritis, stomachache, diarrhea and for regulation of the menstruation and in relieving menstrual pains [1,2,3]. However, there are only few literature data about their pharmacological activity and chemical composition. In this study antioxidant properties of methanol extracts of leaves and cones of the *Juniperus communis* L. 1753 var. *communis*, were determined using assays which measure free radical scavenging ability toward DPPH and superoxide anion radical. Additionally, inhibition of lipid peroxidation, reducing power (FRAP) and total flavonoid and flavonoid content were determined. The extract has been characterized regarding composition by LC-MS/MS where several flavonoids have been investigated, but only luteolin-7-*O*-glc was determined in leaves (96.19 μg/g of dw) of examined species. The anti-inflammatory activity, considering inhibitory potency toward COX-1 and 12-LOX enzymes, was determined by novel optimized method which is using LC-MS/MS technique for the quantification of products of COX-1 and 12-LOX metabolism [4]. Both extracts showed markedly anti-inflammatory activity, with leaves having somewhat higher potency concerning both assays, reaching IC_{50} at 1.50 mg/mL towards COX-1 and IC_{50} at 1.81 mg/mL towards 12-LOX. According to obtained results examined *Juniperus communis* L. 1753 var. *communis*. species could be regarded as a promising source of bioactive natural compounds, which can be used both as food supplement and as remedy. **Acknowledgements:** The Ministry of Sciences and Environmental Protection, Republic of Serbia (Grant No. 142036) supported this research work. We thank Goran Anackov, PhD for the plant specimen determination. **References:** 1. Thomas, P.A et al. (2007) J. Ecol. 95: 1404. 2. Akkol, E.K. et al. (2009) J. Ethnopharmacol. 125: 330. 3. Miceli, N. et al. (2009) J. Agr. Food Chem. 57: 6570. 4. Beara, I.N. et al. (2010) J. Pharm. Biomed. Anal. doi:10.1016/j.jpba.2010.02.014.

P332 New clerodane diterpenoids from *Salvia adenophora* Fernald (Lamiaceae)

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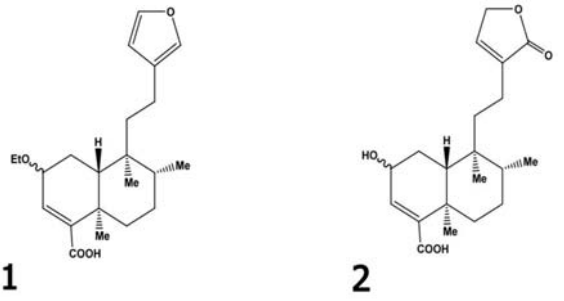
The surface exudate of the aerial parts of *S. adenophora* [1], that showed germination total inhibition [2] against *Papaver rhoeas* L. and *Avena sativa* L. at 5 mg/L in Petri dish experiments, subjected to column chromatography on Sephadex LH-20 and silica gel and to HPLC-MS and MS2 experiments followed by semi-preparative RP-HPLC, yielded two new clerodane diterpenoids (1 and 2), identified by IR and NMR analysis, including TOCSY, COSY, HSQC, HMBC and ROESY experiments, ESI-TRAP-MS and HR-MS analysis.



1 **2**

Fig. 1

References: 1. Epling, C. (1940) A Revision of Salvia, subgenus Calosphace. In: Repertorium Specierum Novarum Regni Vegetabilis. Vol. 110. Fedde F., University of California Press: Berkley, California. 2. Chiapusio. G., Sanchez A.M., Reigosa M.J., Gonzalez L., Pellissier F. (1997), Journal of Chemical Ecology, 23, 2445 – 2453.

P333 Antioxidant activity of *Passiflora edulis* and *Passiflora alata* fruits

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Flavonoids are the major constituents of *Passiflora edulis* (PE) and *P. alata* (PA) fruits [1, 2], widely cultivated in Brazil, for the production of processed or fresh juice [3]. We evaluated the radical-scavenging ability of methanol extracts (DPPH· method) [4] and the antioxidant activities on PMA-stimulated equine neutrophils and on purified equine MPO of PA and PE fruit pulp extracts and PE rinds (healthy and infected with PWV virus) [5]. The radical-scavenging ability followed the order: rutin > resveratrol > healthy PE peels > PE peels infected by PWV > PE pulp > PA pulp. The total juice extract of PE, which was measured by lucigenin-dependent chemiluminescence (CL), had a stronger inhibitory effect on ROS production than did PA, but only at a concentration of 1 mg mL-1, while PE rind extracts showed dose-dependent inhibitory effects on CL response which were slightly stronger with healthy rind. MPO activity was assessed by SIEFED (specific immunologic extraction followed by enzymatic detection) [6], and all the extracts showed dose-dependent inhibitory effects, although the rind extracts showed the highest efficacy. These results indicated that the PWV disease may alter the antioxidant content, and that fruit rind showed higher free radical scavenging ability and antioxidant activities than total juice on the oxidant response of equine PMN, including ROS production and MPO activity. These findings suggest the potential of passion fruit rind, a by-product of the passion fruit processing industry, as a possible functional food or as a raw material for the development of phytomedicines. **Acknowledge-**

ments: CAPES, CNPq, FAPESP. References: 1. Pereira, C.A.M., Vilegas, J.H.Y. (2000) Rev. Bras. Med. 3: 1 – 12. 2. Dhawan, K. et al. (2004) J. Ethnopharm. 94: 1 – 23. 3. Mercadante, A.Z. et al. (1998) J. Agric. Food Chem. 46: 4102 – 4106. 4. Trevisan, F. et al. (2006) Plant Dis. 90: 1026 – 1030. 5. Brand-Williams, W. et al. (1995) Lebensm-Wiss. Technol. 28: 25 – 28. 6. Franck, T. et al. (2006) J. Vet. Diagn. Invest. 18: 326 – 334.

P336 ?? Anti-diabetic activity of *Pseuderanthemum palatiferum* Radlk. leaf aqueous extract

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Fresh leaves of *Pseuderanthemum palatiferum* Radlk. were claimed to cure various disease including diabetes mellitus in Thai people. However, hypoglycemic and anti-diabetic activities of *P. palatiferum* have not been reported. This study examined the hypoglycemic and anti-diabetic effects of *P. palatiferum* leaf aqueous extracts by an oral glucose tolerance test (OGTT) in normal and streptozotocin-induced diabetic rats [1]. A single oral administration of the aqueous extract at doses of 0.25 and 0.50 g/kg significantly decreased the blood glucose level in STZ-induced diabetic rats but not in normal rats. A reference drug, glibenclamide, at a dose of 5 mg/kg showed a significant blood glucose lowering effect both, in normal rats and STZ-induced diabetic rats. Phytochemical screenings to examine compounds responsible for the biological activity were carried out by color and precipitation tests [2]. It was found that the extract contained flavonoids, phenolic compounds, non-volatile lactones and saponins. References: 1. Peungvicha, P. et al. (1998) J. Ethnopharmacol. 60:27 – 32. 2. Farnsworth, NR. (1996) J. Pharm. Sci. 55(3):225 – 69.

P337 ?? Triterpenoids as antiproliferative agents in classical and atypical multidrug resistant cancer cells

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Resistance of cancer cells to multiple classes of structurally and mechanistically unrelated antitumor drugs can be defined as multidrug resistance (MDR), and it is one of the major causes of chemotherapy failure. The most significant mechanism of MDR, referred as typical or classical MDR, is that resulting from altered cell membrane transport due to overexpression of the transporter protein P-glycoprotein (Pgp/MDR1) that act as an drug efflux pump. Conversely, MDR cells without overexpression of this transporter protein are referred as atypical MDR cells and their resistance has been associated with enhanced expression of alternative transporter protreins, altered DNA topoisomerase II activity or other mechanisms. According to some authors, atypical MDR may also result from altered expression of some metabolizing enzymes [1]. Therefore, a promising approach to overcome MDR is the development of compounds that are selectively cytotoxic to resistant cancer cells. Continuing our search for anticancer agents from plant sources [2], the aim of this work was to evaluate the antiproliferative activity of several cucurbitane-type triterpenoids, isolated from *Momordica balsamina* a medicinal African plant also used as food. The study was carried out with human cancer cell lines derived from three tumor entities: gastric (EPG85 – 257), pancreatic (EPP85 – 181) and colon (HT-29) carcinomas. Furthermore, different multidrug-resistant variants of these cancer cell lines with over-expression of MDR1/P-gp or no MDR1/P-gp expression were also investigated. When comparing with the parental cell lines, some of the multidrug resistant variants showed increased sensitivities to the studied compounds. Acknowledgements: The authors wish to thank the Science and Technology Foundation, (FCT, grant SFRH/BD/22321/2005). References: 1. Teodori, E. et al (2006) Curr Drug Targets 7: 893 – 909. Ramalhete, C. et al (2009) Bioorg. Med. Chem. 17: 6942 – 6951.

P336 Further insights into *Ficus carica* latex metabolome

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Ficus carica products are widely used both as food and as medicine in the Middle East. All *Ficus* species possess latex-like material within their vasculatures, affording protection and self-healing from physical attacks. *F. carica* latex has been traditionally used in the treatment of gout, ulcers and warts, among other situations, given its proteolytic and keratolytic effects, associated to its viscosity [1]. In this work fatty acids and phytosterols profiles were established. Combined fatty acids were hydrolyzed and all free compounds were derived to their methyl ester forms, prior to GC-MS analysis. Fourteen compounds were determined, being saturated fatty acids, namely arachidic, palmitic and behenic acids, the ones present in highest contents. Phytosterols profile of saponified sample was characterized by HPLC-DAD, which allowed the determination of seven compounds. β-Sitosterol, lupeol and lanosterol were the major phytosterols. This study provides further knowledge to the richness of latex metabolome, namely in bioactive compounds. Acknowledgements: Fundação para a Ciência e a Tecnologia, Andreia P. Oliveira (SFRH/BD/47620/2008). Fundação Calouste Gulbenkian, Branca M. Silva. References: 1. Oliveira, A.P. et al (2010) J. Agric. Food Chem. 58: 3393 – 3398.

P337 Anxiolytic effects of fractions obtained from *Passiflora incarnata* L. in the elevated plus maze in mice

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The anxiolytic effects of passion flower (Passiflora incarnata L.) have been confirmed in several pharmacological studies [1 – 3], however, the compounds responsible for this effect are still a matter of debate. The purpose of this study was to characterize the putative anxiolytic-like activity of fractions prepared from a hydroethanolic extract using the elevated plus-maze (EPM) in mice. The fractions were prepared as published recently [4], yielding into a butanol, petroleum ether and chloroform fraction. Male BL6/C57J mice were either treated orally with each fraction in three different concentrations according to their percent amount in the extract or the positive control diazepam (1.5 mg/kg). From the tested fractions, the butanol fraction showed significant increases in the number of open arm entries in the EPM in concentrations of 2.1 mg/kg and 4.2 mg/kg corresponding to 150 and 300 mg/kg of the original extract. The highest activity was found for the chloroform fraction in doses of 0.17 mg/kg (10.0 ± 1.9, $p < 0.001$) and 0.34 mg/kg (6.6 ± 0.86; $p < 0.05$) which corresponds to a total extract dose of 150 and 300 mg/kg respectively. Interestingly, the petroleum ether fraction did not show any effects in the elevated plus maze. A sedative effect of each of the fractions could be excluded, since none of the compounds had an influence on the total distance that the animals covered during the observation period. Our results suggest that the active principle of passion flower seems to be in the chloroform fraction and to a lower extend in the butanol fraction. References: 1. Soulimani, R. et al. (1997) J Ethnopharmacol 57(1): 11 – 20. 2. Grundmann, O. et al. (2009) Pharmazie 64: 63 – 64. 3. Grundmann et al. (2008) Planta Medica 74(15): 1769 – 1773. 4. Holbik, M. (2010) Diploma Thesis, University of Vienna.

P338 Determination of alpha-mangostin in rat plasma by HPLC-MS and its application to pharmacokinetic studies

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Garcinia mangostana L. (Clusiaceae) is a plant that originates from Southeast Asia and has been used traditionally to treat diarrhea, dysentery, inflammation, skin disorders, and wounds. Products manufactured from *G. mangostana* are increasingly popular as a botanical dietary supplement in the United States, because of their potent antioxidant properties. The xanthone α-mangostin is one of the major bioactive secondary metabolites in mangosteen. Until now, studies on the disposition, absorption, bioavailability, and metabolism of α-mangostin are limited. In the present study, a HPLC-MS assay has been established for the determination of α-mangostin in rat plasma, with bergamottin as internal standard. The recovery percentage of α-mangostin from the plasma samples was 93.19%. The calibration curve was linear over the range of 20 – 2000 ng/ml. The total run time was 8 min. The intra- and inter-assay variability was less than 9.32% and 9.87%, respectively. The accuracies determined at the concentrations of 70, 850 and 1800 ng/ml for α-mangostin were within +15%. The validated method, which is rapid, simple, and precise, was used successfully to support pharmacokinetic studies in rats after oral and intravenous (i.v.) injection. Non-compartmental analysis suggested a short half-life of 4 minutes and a low oral bioavailability of only 4.24% for α-mangostin. Further investigations are in preparation to link the pharmacokinetics of α-mangostin with the threshold of its reported antitumorigenic or antiproliferative effects.

P339 Assays for the cultivation of two native plant species largely utilized as aphrodisiac in the central region of South America

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The two vegetable species more frequently used as aphrodisiac in the central region of South America are: A-Catuaba [*Anemopaegma arvense* (Vell.) Stellfeld & J. F. Souza, (Bignoniaceae)] and B-Nó-de-Cachorro [*Heteropterys aphrodisiaca* Machado, (Malpighiaceae)]. These are native plants withdrawn from the natural environment which is getting degraded. In order to prevent extinction of species A and B, a viable alternative is to cultivate them. To contribute for the definition of a rational production system of these two species, an effective single (AA or BB) or consorted (AB or BA) cultivation system was investigated in Cáceres, MT, Brasil, from Dec 2004 to Aug 2006), through a series of experiments, executed in two phases: "I" – "nursery" (during 142 days, plants spacing of 12 cm); "II" "definitive cultivation" (during 218 days, plants spacing of 50 cm). In the nursery, the growth (cm) of the A species was greater in the consorted (12.93) than in the single (9.92) system while the B species grew less in the consorted (11.33) than in the single system. In the definitive cultivation, the height of the B species (23.85) was not affected by the system while the A species grew less in the consorted (8.61) than in the single (11.70) system. The A species behaved in opposite ways in the single and consorted systems when grown in nursery or definitive cultivation while the B species was affected by the cultivation system only when grown in the nursery. Different responses of plants are strategies developed to adapt to adverse conditions in distinct development stages. This is expressed through alelopathic effects, adaptative capacities, morphological and physiological characteristics as well as phytochemical constitution, important for the explotation of these species. **Acknowledgements:** Institutional support- UNEMAT, FAPEMAT and EMPAER-MT; Suport provided for all PLAMED team-project: Bonila MGO, Carniello MA, Carvalho, AM, Ramos PR et al.

P340 Effects of pear alcoholic fermentation beverage on airway hyperresponsiveness and immunoglobulin production in asthmatic mice

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Objective: To investigate the inhibitory activity of alcoholic fermentation beverage using pear, Bae Ro Mi In (BRMI), in asthma, we evaluated the airway hyperresponsiveness and immunoglobulin production in asthmatic mouse model. **Methods:** The airway hyperresponsiveness was measured by enhanced pause (Penh). Antigen specific total antibody and subclasses such as IgG1, IgG2a and IgE were measured by ELISA. Prednisolone (PD, 5 mg/kg) was used as positive control. **Results:** Oral administration of BRMI did not attenuate airway hyperresponsiveness. But, production level of OVA-specific total antibody significantly decreased by BRMI. Especially, BRMI suppressed the OVA-induced serum IgE level compared to non-treated control group. PD decreased airway hyperresponsiveness and production levels of total antibody and IgE in serum respectively.

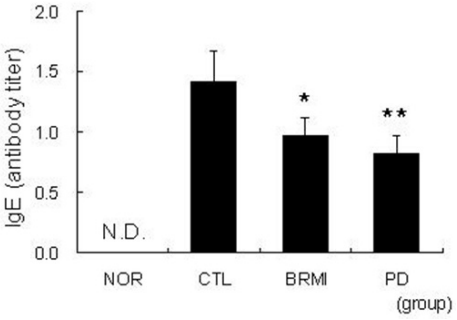

Fig. 1: Effects of BRMI on OVA-specific IgE production in serum. Amount of OVA-specific IgE was measured using ELISA method. NOR: naive group, CTL: asthma induced control group, BRMI: BRMI administered group PD: prednisolone administered group. N.D.: not detectable. Results are presented as mean ± SD. *P < 0.05, **P < 0.01 as compared with control group. (n = 8)

Conclusion: These findings demonstrate that BRMI could be decreased levels of OVA-specific total antibody and IgE production in asthmatic mice. BRMI likely exerts its preventive or cure effect through regulation of antigen-specific antibody production in asthma. **Key words:** Pear beverage, asthma, immunoglobulin

P341 Changes of phenolics and antioxidant activity during hawthorn (*Crataegus pinnatifida* Bunge) fruit ripening

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The antioxidant activity, total phenolic compounds and four major phenolics in hawthorn fruits (during three ripening stages) were investigated. Hawthorn has been used as a folk medicine in Korea for the treatment of various cardiovascular disease, arteriosclerosis and hypertension [1]. Many recent clinical studies have demonstrated that hawthorn extract show a protective effect on cardiovascular diseases [2]. In our study, we used *Crataegus pinnatifida* Bunge fruit to measure the phenolic compounds and antioxidant activity. Using HPLC, four phenolic compounds including (−)-epicatechin (EC), chlorogenic acid (ChA), hyperoside (HP) and procyanidin B_2 (PC-B_2), in hawthorn fruit were evaluated. Hawthorn fruits of five clones (selected from different area of Korea, C 1, C 8, C 15, J and P), grown in the Korea Forest Research Institute (Suwon) were utilized. The finding results were that the clone of J had highest EC (1122.6 mg/100 g) and HP (100.6 mg/100 g) content at 2nd ripening stage and PC- B_2 (863.9 mg/100 g) content at 2nd ripening stage in the fruit ripening. C 8 had highest ChA (377.0 mg/100 g) content at 2nd ripening stage. In our research, the free-radical scavenging activities of five clones (C 1, C 8, C 15, J and P) were 51.9, 37.6, 42.2, 70.0, and 25.5% at 2nd stage, respectively. Total phenolic content in fruit at 2nd

ripening stage of five clones were 137.6, 102.9, 133.5, 179.0, and 52.5 mg/g, respectively. **References:** 1. Chang, Q. et al. (2006) Food Chem. 98:426 – 430. 2. Liu, T. et al. (2010) Food Chem. 119: 1656 – 1662.

P342 Centelloside production in *Centella asiatica* cell suspension cultures elicited with methyl jasmonate

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Centella asiatica is a herbaceous plant used in medicine for its wound-healing and anti-inflammatory properties [1]. Its bioactive compounds are ursane-type triterpene saponins known as centellosides. With the aim of biotechnologically increasing the production of these compounds, *C. asiatica* cell suspensions were established and treated with two concentrations of methyl jasmonate (100 μM and 200 μM), an elicitor that induces the biosynthesis of many secondary metabolites [2]. The cell suspensions were established from 20 g friable calli cultured on a shaking 200 ml MS medium [3]. The maximum centelloside production was observed in the stationary growth phase, reaching 160 μg/gPS in the control and 1.110 μg/gPS in the methyl jasmonate-elicited cultures. The effect of the elicitor was greatest during the first 4 days of treatment and it did not change the centelloside pattern, madecassoside being the main compound, followed by asiaticoside. RT-PCR analysis of the *beta*-amyrin synthase gene (the specific oxidosqualene cyclase that leads to centelloside formation) showed higher levels of expression in the elicited cultures than in the control, peaking at 20 h. The maximum content of centellosides was obtained at day 15 of culture, showing a time lag between the gene activation and centelloside biosynthesis. In the 200 μM methyl jasmonate-elicted cultures the expression level of the gene remained much lower than in the 100 μM-elicited cultures and the centelloside production did not increase compared to the control. Thus, methyl jasmonate elicitation in this type of culture was dosis-dependent and its inducing role was apparent at low concentrations. **Acknowledgements:** This work was partially supported by grants from Spanish Ministerio de Ciencia e Innovación (BIO2008 – 01210) and the Generalitat de Catalunya (2009SGR1217) **References:** 1. Skopinska-Rózewska *et al.* (2002) Central-Europ J Immunol. 27: 142. 2. Gundlach *et al.* (1992) Proc Nat Acad Sci USA 89: 2389. 3. Murashige and Skoog (1962) Physiol Plant. 15: 473.

P343 The disconnection approach: integrationism and reductionism in the study of medicinal plants

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Nowadays there have been numerous discussions about the reductionist and integrationist (or holistic) approaches in the search of new biologically active compounds [1]. In this study, we propose the combination of both approaches to investigate the mode of action of the medicinal plant *Tithonia diversifolia* (Hemsl). A. Gray (Asteraceae) through the "disconnection approach", i.e. the receipt of different plant extracts from a biological matrix and further dereplication. Leaves of *T. diversifolia*, which are traditionally used as anti-inflammatory remedy, were used to obtain the following extracts: aqueous crude extract by infusion of entire leaves; leaf rinse extract [2] by rinsing entire dried leaves with acetone; 70% methanol extract of powdered leaves without glands. The essential oil was obtained by steam distillation of leaves. The extracts and their main isolated compounds were evaluated using *in vivo* (subchronic toxicity in rats, paw oedema and croton oil assays in mice) and *in vitro* (MPO activity, NO, IL, TNF-α, etc.) models while the essential oil was evaluated by the croton oil assay. The dereplication using HPLC-DAD-MS/MS indicated flavonoids, chlorogenic acids and sesquiterpene lactones (STLs) in the extracts while mono and sesquiterpenes were identified in the essential oil by GC-MS. The extracts and the oil showed anti-inflammatory activity while the products containing STLs were found to be toxic; not only STLs are involved in the anti-inflammatory activity as expected [3]. As conclusion, using the disconnection approach it was possible to find out which classes of compounds are related to a certain biological property as well as to propose modes of action. **Acknowledgements:** FAPESP, CAPES and CNPq. **References:** 1. Peterson, RT (2008) Nat. Chem. Biol. 11: 635 – 638. 2. Ambrósio, S.R. et al. (2008) Phytochemistry 69: 2052 – 2060. 3. Valério, D.A.R. et al. (2007) Eur. J. Pharmacol. 562: 155 – 163.

P344 New benzenoids and anti-inflammatory constituents from the stem wood of *Zanthoxylum nitidum*

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Zanthoxylum nitidum (Rutaceae) is a scandent shrub, distributed from India to North Australia and Southeast China [1]. Alkaloids, lignans, flavonoids, and their derivatives were isolated from this plant in previous studies. Many of these compounds were found to exhibit significant bioactivities. Investigation of EtOAc-soluble fraction of the stem wood of *Z. nitidum* has led to the isolation of three new benzenoids, (*E*)-4-(4-hydroxy-3-methylbut-2-enyloxy)benzaldehyde (**1**), (*E*)-methyl 3-(4-((*E*)-4-hydroxy-3-methylbut-2-enyloxy)phenyl)acrylate (**2**), and (*Z*)-methyl 3-(4-((*E*)-4-hydroxy-3-methylbut-2-enyloxy)phenyl)acrylate (**3**), together with seventeen known compounds. The structures of new compounds **1-3** were determined through spectral analyses including extensive 2D NMR data. The anti-inflammatory effects of compounds isolated from the stem wood of *Z. nitidum* were evaluated by suppressing fMet-Leu-Phe/cytochalasin B (fMLP/CB)-induced superoxide anion generation and elastase release by human neutrophils. Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls for superoxide anion generation and elastase release, respectively. From the results of our anti-inflammatory tests, the following conclusions can be drawn: (a) (*S*)-6-Acetonyl-8-*O*-demethyldihydrochelerythrine, decarine, (+)-episesamin, (−)-sesamin, and *N*-methylflindersine exhibited potent inhibitory activities ($IC_{50} \leq 4.69$ μg/mL) on human neutrophil superoxide anion generation. (b) Decarine, (+)-episesamin, (−)-sesamin, and *N*-methylflindersine exhibited potent inhibition ($IC_{50} \leq 2.56$ μg/mL) against fMLP-induced elastase release. (c) *N*-Methylflindersine was the most effective among the isolated compounds, with IC_{50} values of 0.35 and 0.41 μg/mL, respectively, against fMLP-induced superoxide anion generation and elastase release. **References:** 1. Chang, C.E. et al. (1993) Rutaceae in Flora of Taiwan, 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, Vol.3: 537 – 544.

P345 New aporphine alkaloids and cytotoxic constituents from the root of *Illigera luzonensis*

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Illigera luzonensis Merr. (Hernandiaceae) is a scandent shrub distributed in Philippines, Ryukyus, and southern Taiwan [1]. Aporphine alkaloids, benzylisoquinoline alkaloids, lignans, and their derivatives are widely distributed in plants of the family Hernandiaceae [2], [3]. Many of these compounds exhibit various biological activities, such as cytotoxic, anti-platelet aggregation, vasorelaxing, antioxidant, and antiplasmodial activities [2], [3]. In our studies on the anti-cancer constituents of Formosan plants, many species have been screened for *in vitro* cytotoxic activity, and *I. luzonensis* has been found to be an active species. Five new aporphine alkaloids, (*S*)-*N*-butyrylcaaverine (**1**), (*S*)-*N*-propionylcaaverine (**2**), (*S*)-*N*-acetylcaaverine (**3**), (6a*R*,7*R*)-*N*-butyrylnorushinsunine (**4**), and (6a*R*,7*R*,*E*)-*N*-(but-2-enoyl)norushinsunine (**5**), and fifteen known compounds have been isolated and identified from the root of *I. luzo-*

nensis. Among the isolates, (−)-yatein exhibited cytotoxicities, with IC_{50} values of 0.81, 0.20, and 0.59 μg/mL, respectively, against DLD-1, CCRF-CEM, and HL-60 cell lines. (6a*R*,7*R*)-*N*-Butyrylnorushinsunine (4) exhibited cytotoxicities, with IC_{50} values of 5.45 and 3.66 μg/mL, respectively, against DLD-1 and IMR-32 cell lines. In addition, *N*-phenethylcinnamamide exhibited cytotoxicities, with IC_{50} values of 6.84 and 5.79 μg/mL, respectively, against DLD-1 and CCRF-CEM cell lines. **References:** 1. Li, H.I. et al. (1996) Hernandiaceae. In: Flara of Taiwan, 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan: Vol. 2: 500 – 503. 2. Conserva, L.M. et al. (2005) The Alkaloids, Academic Press, San Diego, Vol. 54: 175 – 243. 3. Lakshmi, V. et al. (2009) Rec. Nat. Prod. 3: 1 – 22.

P346 In vitro hepatic biotransformation of cepharanthine, a bisbenzylisoquinoline alkaloid

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Cepharanthine is a major biscoclaurine (bisbenzylisoquinoline) alkaloid isolated from the tuber of Stephania rotunda Lour., a Cambodian creeper belonging to the family of Menispermaceae [1]. Cepharanthine exerts various biological effects including antiplasmodial [1], cytotoxic [2] and antioxidant [3] activies, but the metabolism of this compound has not been elucidated to date. The aim of the present investigation was to study in vitro phase I metabolism of cepharanthine using liver microsomes from different species. Incubation of cepharanthine with human liver microsomes led to the formation of one major metabolite, detected by HPLC-DAD. Enzyme kinetics were investigated with pooled human liver microsomes. The apparent Michaelis-Menten constants for the human major metabolite were Km = 37.7 μM and Vm = 22.7 nmol/mg/h. We conducted inter-individual variability study using 20 different human liver microsomes. Results showed marked inter-individual differences in human cepharanthine metabolism. In addition, the biotransformation of this alkaloid was investigated using liver microsomes from different animal species (rats, mice, rabbits, guinea-pigs). Experiments showed important inter-species variability of cepharanthine metabolism

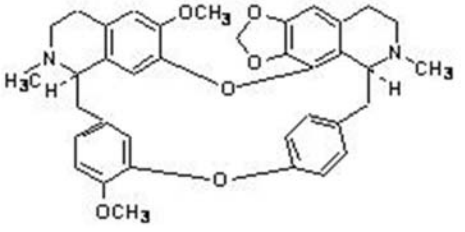

Fig. 1

References: 1. Chea, A. et al. (2007) J. Ethnopharmacol. 112:132 – 137. 2. Bun, S.S. et al. (2009) Phytother. Res. 23:587 – 590. 3. Gülçin, I. et al. (2010) J. Enzyme Inhib. Med. Chem. 25:44 – 53.

P347 Naphthalenone derivatives from *Berrya ammonilla* with inhibitory activity on superoxide generation and elastase release by neutrophils

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Berrya ammonilla Roxb. (Tiliaceae) is a large tree, distributed in southern India, Ceylon, Philippines, and Taiwan [1]. The plants of the family Tiliaceae are rich in flavonoids with flavones, flavanones, flavans, and biflavans as the major constituents, some of which have demonstrated cytotoxic and antiplatelet aggregation activities. In our studies on constituents of Formosan plants for *in vitro* inhibitory activity on neutrophil proinflammatory responses, *B. ammonilla* has been found to be an active species. Five new naphthalenone derivatives, berryammone A (1), berryammone B (2), berryammone C (3), 6-O-methylberryammone C (4), and 4-O-methylberryammone C (5) and eleven known compounds (6 – 16) have been isolated and identified from the stem of *B. ammonilla*. The structures of these new compounds were determined through spectroscopic and MS analyses. Compounds 1, 2, 3, 5, (+)-pinoresinol (6), and 3-*epi*-betulinic acid (12) exhibited inhibition ($IC50 \leq 1.58$ μg/mL) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds 1, 2, and 5 also inhibited fMLP/CB induced elastase release with IC_{50} values ≤ 1.21 μg/mL.

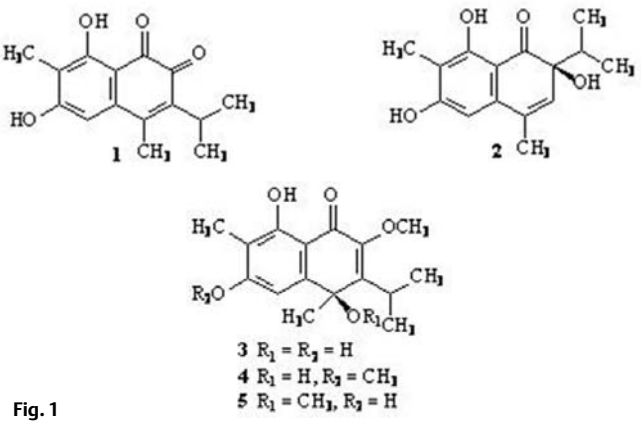

Fig. 1

Acknowledgements: This work was supported by grant from the National Science Council of the Republic of China. **References:** 1. Robson, N.K.B. Tiliaceae in Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan: 1996; Vol. 3: 694 – 714.

P348 *Ex vivo* protective effect of rosmarinic acid and *Mentha* x *villosa* water extract on tissue injury induced by ischaemia/reperfusion

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Ischaemia/reperfusion (I/R) is known to induce tissue injury associated with increased production of reactive oxygen species (ROS). Thus compounds with antiradical activity may prove beneficial as they help to reduce or even to prevent this damage. The aim of the study was to test the effect of the water extract of leaves of *Mentha* x *villosa* Huds (EMV, 1.3 mg/10 ml)) and its main phenolic active compound – rosmarinic acid (RA, 5×10^{-4} mol/l) on changes in ROS production induced by I/R in intestinal and vascular tissues. In anaesthetised rats, ischaemia was induced *in vivo* by clamping the onset of the superior mesenteric artery (SMA) for 60 min followed by 30 min reperfusion1. After sacrifying animals, the ileum and SMA were removed. Tissue segments were placed into the testing solutions with or without EMV and RA and incubated for 30 min (SMA) or 60 min (ileum). Tissues of sham-operated animals served as a control. Production of ROS by segments of the ileum and SMA was determined using luminol enhanced chemiluminiscence (CL). After I/R, CL in SMA increased from control values of 7.9 ± 1.66 to 15.21 ± 2.49 mV/mg w.w., in intestinal segments from 33.74 ± 6.47 to 58.53 ± 11.73 mV/100 mg w.w. Both, EMV and RA reduced intestinal and vascular CL to control values. Moreover in *in vitro* experiments, the antioxidant activity of EMV and RA was tested using DPPH test. $SC_{50\%}$ values were 7.26 ± 0.09 μg/ml and 1.72 ± 0.13 μg/ml, respectively. In ABTS test $SC_{50\%}$ were 15.07 ± 0.47 μg/ml and 1.93 ± 0.03 μg/ml, respectively. The results confirmed the attenuation of increased ROS production induced by I/R in SMA and ileum by rosmarinic acid and *Mentha* x *villosa* extract, and thus indicate their probable protective effect on tis-

sues. **Acknowledgements:** VEGA grant MS SR 2/0050/09 **References:** 1. Sotníková, R. et al. (2005) Biologia (17) 60: p 145 – 147.

P349 Albactam, a novel β- lactam derivative from the flowers of *Albizia lebbeck* with platelets anti-aggregatory activity *in vitro*

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Genus *Albizia* is used in folk medicine to treat several inflammatory pathologies such as asthma, arthritis and burns. It is also reported that *A. lebbeck* flowers are commonly used to treat anxiety, depression and insomnia in traditional Chinese medicine. *Genus Albizia* is characterized by different classes of secondary metabolites such as triterpenoidal saponins, flavonoids and alkaloids. Phytochemical investigation of the alcoholic extract of the inflorescences afforded a novel β-lactam derivative, albactam. It showed an anti-aggregatory activity against adenosine diphosphate and arachidonic acid-induced Guniea Pigs' platelets aggregation *in vitro* at a dose 208 μg/ml and 172 μg/ml respectively. Moreover, monoterpene derivative (epoxy linalol), two tritepenes (β- amyrin and 11α, 12α-oxidotaraxerol), two ceramides and rutin have been isolated. Structural elucidation was achieved utilizing different spectroscopic techniques, including 1D and 2D NMR.

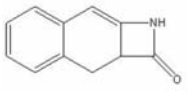

Fig. 1: Albactam

Acknowledgements: The authors are indebted to Prince Sultan Bin Abdulaziz International Program for Distinguished Research Scholarships for financial support.

P350 Ellagic acid derivatives and a ferulic acid ester derivative from the leaves and twigs of *Pachycentria formosana*

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Pachycentria formosana Hayata (Melastomataceae) is an endemic creeping shrub distributed in forests at altitude 300 – 2100 m throughout Taiwan. The plants of the family Melastomataceae are rich in tannins, flavonoids, and triterpenoids as the major constituents, some of which have demonstrated cytotoxic and anti-oxidant activities. Investigation on CH_2Cl_2- soluble fraction of the leaves and twigs of P. formosana has led to the isolation of two new ellagic acid derivatives, 5-hydroxy-5′-methoxy-3,4,3′,4′-tetra-O-methylellagic acid (1), 3,4,3′,4′-di-O-methyleneellagic acid (2) and a ferulic acid ester derivative, (2Z,2′Z)-tetracosane-1,24-diyl bis(3-phenylacrylate) (3) together with thirteen known compounds. The structure of new compounds were determined through spectral analyses including extensive 2D nuclear magnetic resonance data. Among the isolates, oleanolic acid and octacosanoyl ferulate exhibited inhibition (IC_{50} values = 3.12 and 6.30 μg/mL, respectively) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). **Acknowledgements:** This work was supported by grant from the National Science Council of the Republic of China. **References:** 1. Chang CE, Hartley TG. Rutaceae in Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan: 1993; Vol. 3: 905 – 28.

P351 Impact of protein binding on thymoquinone's analytical detection

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Background and Aims: Much data is reported for the promising anticancer activity of Thymoquinone (TQ), an active component of *Nigella sativa* L. (Ranunculaceae). However, no analytical methods have been reported for its quantification from blood or serum. The aims of this study are: 1) to develop a method for the isolation and detection of TQ from spiked serum, and 2) to determine the impact of protein binding on TQ's analytical detection. **Methods:** Solid phase extraction/liquid-liquid extraction/protein precipitation, ultracentrifugation-HPLC, and Mass Spectrometry were used. **Results:** *In vitro*, TQ prepared in ACN or PBS was detected by a C_{18} reversed-phase HPLC-UV assay using an isocratic mobile phase of water: ACN (45:55% v/v) at a flow rate of 1 ml/min. The limit of detection was 0.05 μg/ml. Interestingly, in spiked serum the average recovery of TQ was 3.25% when 10 μg/ml TQ was used and 75% when 100 μg/ml TQ was used. The low recovery was due to high protein binding. The percentage of binding was > 95% within 10 min of incubation. Further investigation showed that bovine serum albumin (BSA) and alpha-acid glycoprotein play a major role in this binding. Binding studies showed that TQ bind covalently to cystein-34 of the amino acid sequence of BSA. **Conclusion:** This data provides evidence that due to the high percentage of binding (covalent/non-covalent) HPLC methods using unlabeled TQ cannot be used for TQ detection in serum. This finding should be taken in consideration when further pharmacokinetic/pharmacodynamic profiling of TQ is to be performed.

P352 Antioxidant activity and major constituents of methanol extract of *Polygonum hyrcanicum*

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The genus *Polygonum* (Polygonaceae) is one of the widely grown medicinal plants in Iran. Different species of *Polygonum* were shown to possess various medicinal activities such as anti inflammation, neuro protection, reduction of age-related degenerative changes, cardiovascular protection and treatment of hepatic disorders. It is considered that the beneficial effects afforded by *Polygonum* treatments, are partly mediated by their antioxidant properties. This study is designed to investigate radical scavenging activity of *Polygonum hyrcanicum* Rech. f. and to identify major constituents of the effective fraction. *P. hyrcanicum*. Aerial parts of the plant were extracted with hexane, ethyl acetate and methanol respectively. DPPH free radical scavenging assay was employed to study antioxidant activities. Major constituents of methanol extract, as the most antioxidant containing fraction, were purified using different chromatographic methods. Finally, 7 flavonols involving myricetin (1), myricetin-3-O- rhamnopyranoside (2), myricetin-3-O- α-L-arabinofuranoside (3), quercetin (4), quercetin-3-O-α-L-arabinofuranoside (5), quercetin-3-O-galactopyranoside (6), quercetin-3-O-(3"-O-acetyl- arabinofuranoside) (7), and 2 structurally related flavanols, catechin (8) and gallocatechin (9), were identified. Among different extracts of *Polygonum hyrcanicum* which were tested for their radical scavenging properties in this study, methanol extract exhibited noticeable antioxidant activity (IC 50 = 68.6 μg/ml) which is comparable with 16 μg/ml vitamin E. Main constituents of the mentioned fraction involved highly hydroxylated flavonols, myricetin and quercetin, their glycosilated derivatives and their structurally related catechins which are known as potent antioxsidants and possess various interesting medicinal properties.

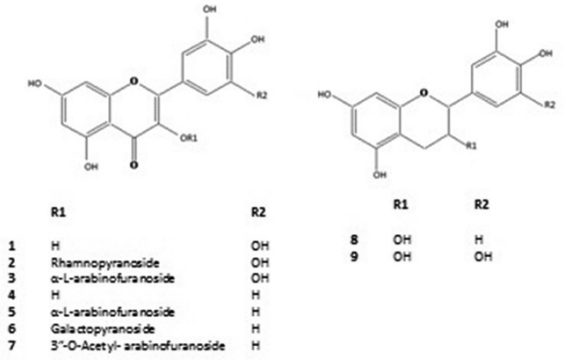

Fig. 1

P353 Stemona alkaloids from the roots of *Stemona sessilifolia* Miq.

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Plants belonging to the genus Stemona (family Stemonaceae) are noted for producing a series of alkaloids with unique structures, most of which are characterized by incorporating a pyrrolo[1,2-a]azepine core. Of the genus Stemona plants, Stemona japonica (Blume) Miq., S. tuberosa Lour., and S. sessilifolia (Miq.) Miq. have been used in China and Japan as an insecticide and also as a remedy for cough, and their biological activities are considered to be related to their alkaloid components. In our studies on the chemical constituents of S. sessilifolia [1 – 4], we isolated eleven new alkaloids, sessilifoliamides A – J and sessilifoliamine A, with novel alkaloid skeletons. In this meeting, we represent their isolation and structure determination.

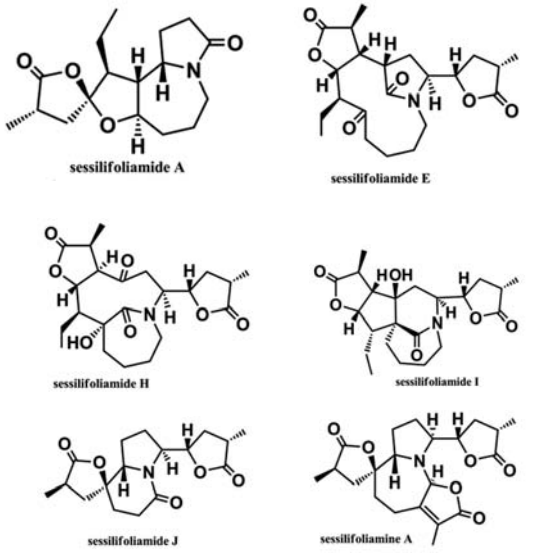

Fig. 1

References: 1. Tetrahedron 59: 7779 – 7786 (2003). 2. Tetrahedron 63: 1008 – 1013 (2007). 3. Heterocycles 71: 2035 – 2040 (2007). 4. Tetrahedron Letters 49: 7376 – 7379.

P354 New thymol derivatives and cytotoxic constituents from *Eupatorium cannabinum subsp. asiaticum*

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Eupatorium cannabinum L. subsp. *asiaticum* Kitam (Compositae) is a perennial herb, distributed in Himalaya, China, and Taiwan [1]. Thymol derivatives [2], sesquiterpene lactones [3], diterpenes [4], flavonoids [4], pyrrolizidine alkaloids [4], and their derivatives are widely distributed in plants of genus *Eupatorium*. Many of these compounds exhibit anti-inflammatory, cytotoxic, and antibacterial activities [5]. Investigation on the *n*-hexane-soluble fraction of the aerial part of *E. cannabinum* subsp. *asiaticum* has led to the isolation of two new thymol derivatives, cannabithymol A (**1**) and cannabithymol B (**2**), together with five known compounds, including three thymol derivatives, (*E*)-2-methylbut-2-enolic acid 2-(2-acetoxymethyl-oxiranyl)-5-methyl-phenyl ester (**3**), 8-methoxy-9-*O*-angeloylthymol (**4**), and (*Z*)-3-acetoxy-2-hydeoxy-2-(2-hydroxy-4-methylphenol)propyl-2-ethylbut-2-enoate (**5**), a benzofuran, euparin (**6**), and a coumarin, 2*H*-chromen-2-one (**7**). The structures of the two new compounds were determined through spectral analyses including extensive 2D NMR data. Among the isolates, (*E*)-2-methylbut-2-enolic acid 2-(2-acetoxymethyl-oxiranyl)-5-methyl-phenyl ester (**3**) exhibited cytotoxic activity, with IC_{50} values of 0.02 and 1.02 μg/mL, respectively, against DLD-1 and CCRF-CEM cell lines. **References:** 1. Chang, C.E. et al. (1993) Compositae in Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan. Vol. 2: 804 – 1101. 2. Tori M. et. al. (2001) J. Nat. Prod. 64: 1048 – 1051. 3. McPhail A.T. et al. (1975) J. Chem. Soc. Perkin II 1798 – 1801. 4. Paolini J. et al. (2007) Phytochem. Anal. 18: 235 – 244. 5. Woerdenbag, H.J. (1986) Pharm. Weekbl. Sci. 8: 245 – 251.

P355 Cladieunicellins A and B, two new eunicellin-based diterpenoids from an Indonesian octocoral *Cladiella* sp.

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Two novel eunicellin-based diterpenoids, designated as cladieunicellins A (1) and B (2), were obtained from an Indonesian octocoral identified as *Cladiella* sp. The structures of eunicellins 1 and 2 were established by spectroscopic methods. These two compounds are the first metabolites of eunicellin-related natural products found to possess 2-hydroxybutanoxy groups. Eunicellin 1 exhibited significant cytotoxicity toward CCRF-CEM tumor cells (ED_{50}= 3.61 μg/ml) and moderate cytotoxicity towards DLD-1 (ED_{50} = 8.50 μg/ml) and P388D1 (ED_{50} = 8.32 μg/ml) tumor cells. Eunicellin 2 was also found to exhibit moderate cytotoxicity towards CCRF-CEM (ED 50 = 4.65 μg/ml) and DLD-1 (ED_{50}= 10.15 μg/ml) tumor cells.

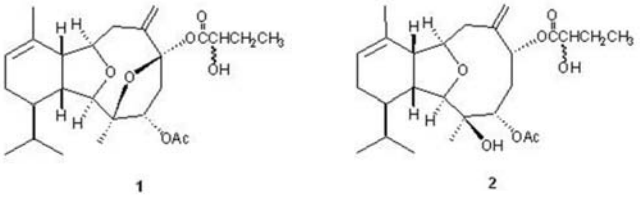

Fig. 1

P356 Studies on the chemical constituents and their biological activities of the fruit of *Garcinia multiflora*

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Garcinia multiflora (Champ.) is a small evergreen tree of the Guttiferae family, distributed in South China, Hong Kong, and Taiwan [1]. Xanthones, flavonoids, benzophenones, and their derivatives are widely distributed in plants of the genus *Garcinia*. Many of these compounds exhibit cytotoxic, anti-inflammatory and antitubercular activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for in vitro anti-inflammatory activity, and *G. multiflora* has been found to be one of the active species. Six new benzophenone derivatives, 13,14-didehydoxyisogarcinol (1), garcimultiflorone A (2), garcimultiflorone B (3), 13-hydroxy-garcimultiflorone B (4), garcimultiflorone C (5), and garcimultiflorone D (6), together with eleven known compounds (7 – 17) have been isolated and identified from the fruit of *G. multiflora*. The structures of these new compounds were determined through spectral analyses including extensive 2D NMR. Among the isolates, garcimultiflorone B (3) exhibited potent inhibition with IC_{50} values of 0.11 ± 0.04 and 0.14 ± 0.02 μM, respectively, against fMLP/CB-induced superoxide anion generation and elastase release. In addition, δ-tocotrienol (10) showed the antitubercular activity with MIC value of 30.0 μM against *Mycobacterium tuberculosis* H_{37}Rv *in vitro*.

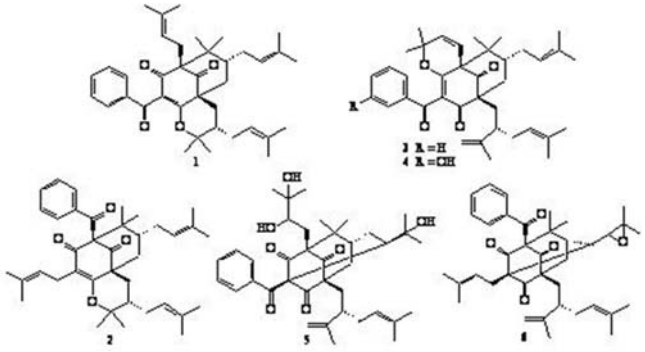

Fig. 1

Acknowledgements: This work was supported by grant from the National Science Council of the Republic of China. **References:** 1. Chang, C.E., Hartley, T.G. (1993) Guttiferae in Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan 2: 694 – 714

P357 Anti tumor effects of standard- and triterpenoid enriched mistletoe extracts on murine melanomas

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The European mistletoe (*Viscum album* L.) contains a variety of water soluble (mistletoe lectins, viscotoxins) and -insoluble (triterpenoids) substances with anti-cancer effects. This makes mistletoe derived extracts and compounds interesting for treatment of cancers with low response rates like melanoma. Mistletoe therapy is the most important complementary therapy in central Europe (1) but up to date the standard preparations contain only water soluble substances of the plant. Triterpenoid extracts from mistletoe (80% oleanolic acid and 4% betulinic acid) are well characterized (2) and solubilization with 2-hydroypropyl-beta-cyclodextrin makes them available for cell culture experiments and cancer treatment in animal models as so called solubilized triterpene extracts (STE). Experimental setup: B16.F10 melanoma cells were inoculated subcutaneous (sc) into the flanks of C57BL/6 mice. Treatment with mistletoe extracts (sc injections) were started three days after tumor inoculation for ten cycles (every second day). The tumor size was determined by calliper measurement every second day. We show here, that high dose mistletoe treatment slows down melanoma growth and prolongs survival of the treated animals. Mistletoe extracts containing STE are more effective in treating melanoma than normal extracts. Histological examinations of the tumors and surrounding tissue show increased tumor necrosis, infiltrating immune cells in the tumor surrounding tissue and decreased neoangiogenesis compared to control animals. We show here, that standard mistletoe extracts show anti-cancer effects on melanoma. Interestingly, novel mistletoe preparations containing STE from mistletoe in addition to the water soluble substances show improved anti-cancer effects. **Acknowledgements:** Software AG Stiftung, Darmstadt, Germany. **References:** 1. Horneber, M.A. et al. (2008) Cochrane Database Syst Rev 2:CD003297. 2. Jäger, S. et al. (2009) Molecules 14:2016 – 31.

P358 Effects of dimethylacrylshikonin and epoxyshikonin on melanoma cell lines

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Cancer is one of the most common causes of death worldwide. Much research has been performed on how to fight this disease. However, current therapeutic concepts have still limited effectiveness because of resistances of tumours and lethal side effects. Therefore, identifying and developing new anti-cancer drugs are still important research objectives. A petrol ether extract of *Onosma paniculatum* showed strong growth inhibition, cell cycle influence and activation of caspase 3 in melanoma cells from primary (SBc-L2, WM35) and metastatic (WM9, WM164) lesions [1]. We now isolated and identified the active compounds and started different pharmacological tests to reveal the mechanism of action. Four active shikonin derivatives were isolated using preparative HPLC. The two main compounds, dimethylacrylshikonin and epoxyshikonin, showed IC_{50} values of: SBc-L2: 1.1 μM and 15.5 μM; WM35: 2.3 μM and 22.9 μM; WM9: 2.7 μM and 18.8 μM; WM164: 8.3 μM and 53.2 μM, respectively. Their effects on cell cycle and appearance of a sub-G1 peak were analyzed by flow cytometry. Both increased the sub-G1 DNA content and the percentage of G2/M-phase cells, accompanied by a decrease of G1-phase cells. Moreover, in SBc-L2 and WM164 cells, the influence of dimethylacrylshikonin on expression levels of different apoptosis involved genes were examined using real-time PCR. In SBc-L2 cells, expression of extrinsic (Fas-Ligand, Trail) and intrinsic (Bim, noxa, bid) apoptotic genes were induced, whereas, in WM164, only intrinsic apoptotic genes (bmf, noxa, bik, bax) where induced. Our data suggest that dimethylacrylshikonin and epoxyshikonin inhibit cell growth of tumor cells and may ultimately lead to cell death through apoptosis induction. **Acknowledgements:** This work was supported by the "Fonds zur Foerderung der Wissenschaftlichen Forschung" P21114. **References:** 1. Rinner, B. et al. (2010) J Ethnopharmacol. in press.

P359 *In silico* guided search for anti-viral properties of a natural methylenecyclopropane glucoside

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(2E)-2-(6-hydroxyhexyliden)cyclopropyl-β-glucopyranoside (1) was isolated from the rhizomes of the tree fern *Metaxya rostrata*, Metaxyaceae, a Costa Rican traditional herbal remedy against intestinal diseases [1]. Literature research for similarity of the structural characteristics resulted in different related compounds with the very uncommon structural part of methylenecyclopropane [2,3,4], which showed anti-viral activitiy against Epstein-Barr-, Human Herpes-, Hepatitis B-, Human Immunodeficiency- and Human Cytomegalo-virus in vitro. Thus, an in silico guided search for possible anti-viral activities of (1) was realized. Pharmacophoric profiling against over 2200 models representing over 280 putative pharmacological targets [5] suggested α-glucosidase as potential target for (1). A structural similarity search in the MDL Drug Data Report (MDDR) database [6] confirmed this result making α-glucosidase a promising target for biological evaluation of compound (1).

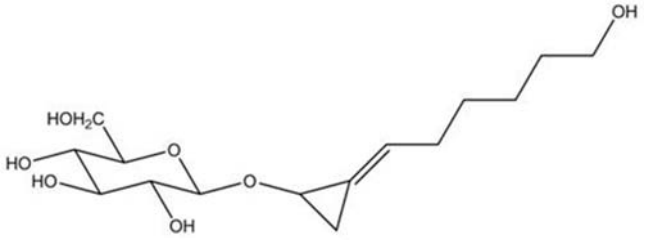

Fig. 1: (2E)-2-(6-hydroxyhexyliden)cyclopropyl-β-glucopyranoside (1)

References: 1. Kainz, K.P. et al. (2010) J. Nat. Prod.: submitted. 2. Rybak, R.J. et al. (2000) Antimicrob. Agents Chemother. 44/6: 1506 – 1511. 3. Qiu, Y.L. et al. (1998) J. Med. Chem. 41: 10 – 23. 4. Uchida, H. et al. (1999) Antimicrob. Agents Chemother. 43/6: 1487 – 1490. 5. Rollinger, J.M. et al. (2009) Planta Med. 75: 195 – 204. 6. Keiser, M.J. et al. (2007) Nat. Biotech. 25: 197 – 206.

P360 Evidence for the absorption of a Cimicifuga/Hypericum fixed combination after oral administration by determination of specific marker substances in rat plasma

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Cimicifuga racemosa as an alternative to hormone therapy is the most extensively studied medicinal herb used for the alleviation of menopausal symptoms. Cimicifuga/Hypericum film-coated tablets (CHF) is a fixed combination containing extracts from the herbal substances *Hypericum perforatum* and *C. racemosa*. The absorption of constituents from *H. perforatum* has already been demonstrated after oral intake of monopreparations. Only few data are available for the absorption of Cimicifuga-components. No data at all exist for the fixed combination [1,2,3]. The purpose of this study was to prove the absorption of CHF after oral administration, using valid analytical methods. Hypericin was selected as biomarker for *H. perforatum* and caffeic acid derivatives (ferulic acid, cimicifugic acid B) as marker substances for *C. racemosa*. Wistar rats received single, 10-fold und 100-fold human equivalent dosages (HED) of CHF-granulate via gavage. At defined time points blood plasma samples were taken and analysed by HPLC with fluorescence detection. The absorption of the Hypericum-specific marker hypericin was demonstrated after application of the single and 10-fold HED of CHF. Ferulic acid, a marker compound of *C. racemosa*, could be quantified in the plasma samples in the case of the 10- and 100-fold HED. An enzymatic pretreatment of the plasma samples enabled the determination of cimicifugic acid B, another Cimicifuga-specific compound. The resulting plasma concentrations of all three marker compounds were dependent from the administered dosage. These results prove the absorption of CHF by the detection of specific marker compounds in the plasma of rats after oral application of CHF. **References:** 1. Schulz, H.U. et al. (2005) Arzneimittelforschung 55:15 – 22. 2. Si, D. et al. (2008) J Pharm Biomed Anal 47: 140 – 145. 3. Einbond, L.S. et al. (2009) Fundam Clin Pharmacol 23:311 – 321.

P361 Antioxidant activity and inhibitory effect of *L. viridis* extract on Fe^{2+}-induced lipid peroxidation in brain homogenates

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The brain is particularly susceptible to oxidative stress damaging effects due such events as the high consumption of oxygen, limited concentration of antioxidants and a relatively high degree of polyunsaturated fatty acids that are particularly good substrates for peroxidation reactions [1 – 3]. Oxidative stress could lead to damage biological target molecules, affecting the cellular function and integrity [4]. The ability of natural antioxidants, mainly phenolic compounds, to protect cells from oxidative stress has been previously demonstrated [5]. In this work, the methanol extract from *Lavandula viridis* L'Hér. (Lamiaceae), a xerophytic aromatic shrub endemic to the south-west Iberian Peninsula [6], was investigated for its effect on deoxyribose degradation, its reducing properties, Fe^{2+}-chelating ability and total phenol content. The capacity of this extract to prevent Fe^{2+}-induced lipid peroxidation in mouse brain (*in vitro*) was also evaluated. *L. viridis* extract showed Fe^{2+} chelating activity, reducing power and the ability to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose in a dose-dependent manner. This extract also revealed a high phenol content (893.01 ± 17.09 μmol gallic acid equivalents/g extract) evaluated by Folin-Ciocalteu method. Moreover, in brain homogenates, the methanol extract of *L. viridis* caused a high decrease in the MDA production in both the basal and the Fe^{2+}-induced lipid peroxidation. The effective protective properties of *L. viridis* could be attributed to its higher phenol content, Fe^{2+} chelating ability, reducing properties and HO· radical scavenging ability. The findings suggest that methanol extract from *L. viridis* could be a potential source of natural antioxidants. **Acknowledgements:** C. Patrícia and S. Gonçalves acknowledge a grant from Portuguese Science and Technology Foundation (FCT, SFRH/BD/63505/2009 and Grant SFRH/BPD/31534/2006, respectively) **References:** 1. Sah, R. et al. (2002) J Neurochem 80: 383 – 391. 2. Shulman, R.G. et al. (2004) Trends Neurosci 27: 489 – 495. 3. Halliwell, B., Gutteridge, J.M.C. (2007) Free radicals in Biology and medicine, Fourth Edition. Clarendon Press, Oxford Science Publications. Oxford/UK. 4. Britton, R.S. et al. (2002) Int J Hematol 76: 219 – 228. 5. Stalikas, C.D. (2007) J Sep Sci 30: 3268 – 3295. 6. Nogueira, J.M.F., Romano, A. (2002) Phytochem Anal 13: 4 – 7.

P362 Phytochemical investigation of *Johreniopsis seseloides* aerial parts from Iran

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Johreniopsis seseloides (C.A. Mey.) Pimenov belongs to the family of the Apiaceae, subfamily Apioidae, tribe Peucedaneae. The *Johreniopsis* genus has 4 species (*J. oligactis. J. scopari, J. seseloides* and *J. sticticaulis*) in Iran. Since there is no report about the secondary metabolites of *J. seseloides*, it was collected from Kordestan Province in August 2007 and aerial parts were finely powdered in a mill. 600 g of sample was percolated with 80% methanol. After evaporation of the solvent, the gummy residue (crude extract) was re-extracted with chloroform (chloroform extract) and the residue was named the methanol extract. A white amorphous substance was precipitated from the total hydroalcholic extract and identified as a free carbohydrate, mannitol. The methanol extract (70 g) was investigated for flavonoids and the chloroform extract (22 g) for coumarins and other compounds. Flavonoids are potent antioxidants and have important activities, such as dietary anti-carcinogens and anti-inflammatory compounds. Flavonoids are also significant to plants, serving as signal molecules in various developmental processes. Coumarins are chemical compounds found in many plants especially in the genera of the Apiaceae. They have clinical value as the precursor for several anticoagulants, notably warfarin. Furocoumarins are used therapeuti-

cally for the treatment of vitiligo and psoriasis. In addition to mannitol, also quercetin, rutin, isorhamnetin 3-O-glucoside, a chromon (3,5,7-trihydroxy-chromone), coumarin (1), 6 – 7-dimethoxy-coumarin or scoparone (2), bergapten (3), oxypeucedanin hydrate (4) and a triterpene (α-amyrin) were isolated from the methanol and chloroform extracts of this plant with different chromatographic methods and identified with spectroscopic methods.

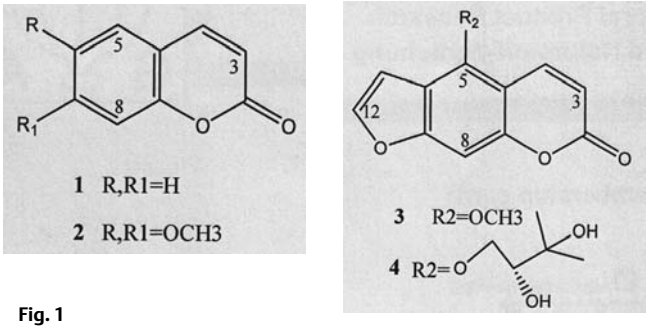

Fig. 1

P363 Effect of *Smallanthus sonchifolius* extracts on croton oil-induced oedema and neutrophil migration to the ear skin tissue of mice

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Smallanthus sonchifolius (yacón) is an Andean species whose leaves have glandular trichomes rich in sesquiterpene lactones (STLs). The chemical profile of the leaf rinse extract (RLE) of yacón showed several STLs [1]. The major STL isolated were quantified [2] and the *in vitro* anti-inflammatory effect of the two major STLs was evaluated [1]. However, STLs are known for their oral toxicity and the study of topical applications can be an alternative to the use of these anti-inflammatory substances. Thus, the aim of this work was to evaluate the *in vivo* topic anti-inflammatory potential of yacón. The RLE was obtained from entire dried leaves rinsed with acetone. After liquid-liquid partition with *n*-hexane the resulting hydromethanolic fraction was evaluated using the croton oil ear oedema assay in mice [3]. Subsequently, fragments of ears were used for myeloperoxidase (MPO) determination in order to evaluate leukocyte migration to the subcutaneous tissue. IR spectrum and HPLC-UV-DAD analysis demonstrated that the RLE is a STL-rich extract, which showed anti-oedematogenic activity (Image 1A) followed by inhibition of neutrophil migration to the inflamed tissue in all tested doses (Image 1B). Plant extracts that decrease oedema and leukocytes migration can be an useful alternative to the treatment of topical inflammatory injuries. Thus, the STL-rich extract of yacón can be a promising phytotherapeutic agent for topic application in the inflammatory process.

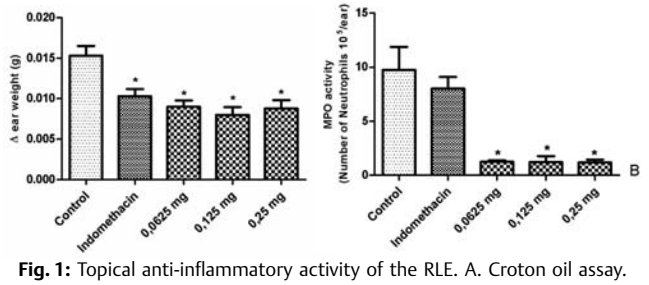

Fig. 1: Topical anti-inflammatory activity of the RLE. A. Croton oil assay. B. MPO quantification. Mean ± S.E.M, ANOVA one-way, Tukey's post-test. * P < 0.05 in relation to the control. N = 10 animals.

Acknowledgements: FAPESP, CAPES. **References:** 1. Schorr, K. et al. (2007) Nat. Prod. Commun. 2:367 – 374. 2. Schorr, K. et al. (2005) Phytochem. Anal. 16:161 – 165. 3. Tubaro, A et al. (1985). Agents and Actions. 17:347 – 349.

P364 Prenylated furanocoumarin and flavonoids of *Cervaria cervariifolia* from Iran

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Cervaria cervariifolia (C.A Mey.) Pimenov from tribe of peucedaneae and subfamily of Apioidae family of Apiaceae was collected from Golestan Province of Iran in May 2007. The plant parts were dried at ambient temperature in the shade. The root of *C. cervariifolia* were powdered and extracted by percolation with 80% methanol. The solvent was evaporated under vacuum to give a crude extract (CE). The CE was re-extracted with chloroform (nonpolar fraction). The powdered aerial parts of plant percolated with 80% methanol, hydroalcoholic solution was dried at vacuum and re extracted with hexan and chloroform (discarded). Remainder was a gummy residue (polar fraction). Non-polar fraction of root was chromatographed on silicagel by column chromatography and substances were purified on PTLC with different solvent systems and crystallization. Four coumarins, a terpenoid and a carbohydrate from *C. cervariifolia* were isolated and identified by spectroscopic methods such as 1 H, 13C-NMR, UV, IR and mass spectroscopy. The compounds of polar fraction of aerial parts were isolated with paper chromatography on Watman No. 3 and purified on Sephadex LH-20 with different solvent systems. Four glycosylated flavonols were identified. The isolated compounds were: bergapten (1), xanthotoxin (2), 5-prenyl,12-methoxyfuranocoumarin (3), 12-methoxyfuranocoumarin (4), beta-sitostrole, mannitol, quercetin 3-*O*-glucoside, quercetin 3,7-*O*-diglucoside, kaempferol 3-*O*-glucoside and kaempferol 3,7-*O*-diglucoside. 5-prenyl,12-methoxy-furanocoumarin is reported for the first time.

P365 Tentative LC-MS-MS identification of flavonoids and phenolic acids from *Scandiceae tribe* (Apiaceae) Species

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Tribe Scandiceae comprises 3 genera (*Anthriscus*, *Chaerophyllum* and *Scandix*) with over 60 species, the most of them considered weeds, although some are used as a food or in traditional medicine. The purpose of this work was to identify (by rapid resolution liquid chromatography coupled with electrospray-ionization triple-quad mass spectrometry) flavonoid and phenolic acids profile of 70% methanolic extracts of six species: *A. sylvestris*, *A. cerefolium*, *Ch. bulbosum*, *Ch. hirsutum*, *Ch. temulentum* and *S. pecten-veneris*. By means of targeted MS^2 (Precursor Ion Scan) screening [1 – 3], it was possible to detect over 40 flavonoid *O*-glycosides, mostly hexosides, acetylhexosides and malonylhexosides of luteolin, apigenin, quercetin and methyl luteolin. While luteolin derivatives were present in all investigated species, the presence of other flavonoids was species-dependent and might serve as a chemotaxonomical marker. A number of chlorogenic acids (quinic acid esters with hydroxycinnamic acids) was also identified by MS*2* and pseudo-MS^3 experiments [4,5], including isomeric caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA) and their acetyl-, malonyl-, and acetyl-malonyl-derivatives. The nature of chlorogenic acids was also species-dependent, with *A. sylvestris* being rich in unsubstituted CQA and diCQA, *A. cerefolium* and *Ch. bulbosum* having a high content of malonylated acids, and *Ch. temulentum* and *S. pecten-veneris* containing acetylated derivatives. **Acknowledgements:** This work was supported by a research grant from the Ministry of Science and Technological Development, Republic of Serbia (Grant No. 142036). **References:** 1. Cuyckens, F. et al. (2004) J. Mass Spectrom., 39: 1 – 15. 2. Cuyckens, F. et al. (2000) Analusis 28: 888 – 895. 3. Clifford, M.N. et al. (2005) J. Agric. Food Chem. 53: 3821 – 3832. 4. Ye, M. et al. (2005) Rapid Commun. Mass Spectrom. 19: 1469 – 1484. 5. Zhang, Y. (2007) Rapid Commun. Mass Spectrom. 21: 2971 – 2984.

P366 Radical scavenging activity of phenolic constituents from *Limonium latifolium*

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Limonium latifolium Kuntze (syn. L. gerberi Soldano) or sea lavender is a member of the highly stress-tolerant family Plumbaginaceae found in Brittany (France) littoral. Salt-tolerance capacity of this halophyte may include mechanisms like compartmentation of toxic ions, osmolites accumulation and redox control making this synthetic pathway an interesting target for radical scavenging activities (RSA) [1, 2]. In this study, we investigate the bioactive constituents of *L. latifolium* with RSA using in vitro superoxide anion (O_2^-) and hydroxyl radical (·OH) inhibition assays. Preliminary anti-oxidant screening has shown the scavenging capacity of a crude hydro-alcoholic extract of the two free radicals? OH and O_2^- (62.0% and 68.8% of inhibition at 2 and 25 µg.mL^{-1}, respectively). Bioguided fractionation permit us to isolate 6 major constituents with potent RSA against OH and O_2^{-}. Their identification by HPLC-DAD, NMR and MS data shown that they consist of gallic acid, ethyl gallate, myricitrin, epicatechin gallate, kaempferol 3-*O*-glucoside and myricetin 3-*O*-galactoside, all of them identified for the first time in this species. Thus, the strong anti-oxidative potential of these polyphenols could explain the adaptability of sea lavender to hostile biotopes and may contribute to a potent pharmacological effect. **References:** 1. Plant Sci. 160 (2001) 415 – 423 2. Plant Cell Physiol. 44 (2003) 388 – 394.

P367 Endiandric acid analogs from the roots of *Beilschmiedia tsangii*

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Beilschmiedia tsangii (Lauraceae) is a medium sized evergreen tree, distributed in Tonkin, Vietnam to Yunnan, Kwangsi and Kwangtung, China and south of Taiwan [1]. There are only two species of Beilschmiedia in Taiwan. The stems and leaves of *B. tsangii* and the roots of *B. erythrophloia* have been reported their chemical constituents and bioactivities in our previous studies[2 – 5]. The methanolic extract from the roots of *B. tsangii* showed antitubercular activity against *Mycobacterium tuberculosis* H37Rv *in vitro* and it has never been studied. The aims of this study are the isolation of chemical constituents and antitubercular activity from the methanolic extract of the roots, which was partitioned to get EtOAc layer, H_2O layer and insoluble part. Bioassay-guided fractionation of active EtOAc layer led to the isolation of three new compounds, erythrophlotsangine A (**3**), erythrophlotsangine B (**4**), and endiandric acid K (**7**), together with four known compounds. The structures of isolates were elucidated by spectral 2D NMR analysis. Antitubercular activity assay of the isolates is still in progress.

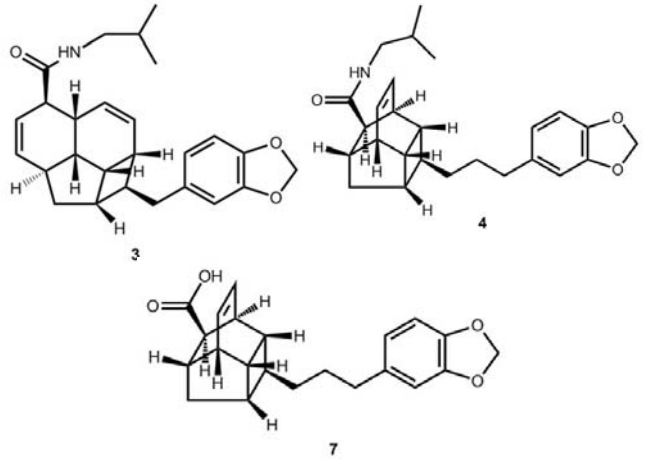

Fig. 1: Compounds **3**, **4** and **7**

References: 1. Liao JC (1996) Flora of Taiwan, Editorial Committee of the Flora of Taiwan, Taipei. 2. Yang, PS. et al. (2008) Helv. Chim. Acta 91: 2130 – 2138. 3. Yang, PS. et al. (2009) J. Nat. Prod. 72: 53 – 58. 4. Chen, JJ. et al. (2006) Planta Med. 72: 351 – 357. 5. Chen, JJ. et al. (2007) Planta Med. 73: 567 – 571.

P368 Flavonoids and benzoic acid derivatives from *Dioclea virgata*

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Plants of the genus Dioclea (Family Leguminosae) are commonly used in Brazilian folk medicine for various diseases. Dioclea virgata is a climbing shrub which is endemic in Bahia (Northeast of Brazil). It leaves are used in popular medicine for treatment of kidney and prostate diseases [1]. Chemical investigation of leaves of Dioclea virgata led to isolation of 7-hydroxy-6-methoxyflavone (1), 7-hydroxy-6-methoxyflavanone (2) and 7-hydroxy-6 methoxyflavanonol (3), as well as 3-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, lupeol and glycoside of stigmasterol. 7-Hydroxy-6-methoxyflavanone (2), isolated from Dioclea violacea by our group, has previously been found to possess immunomodulatory activity by inhibiting the synthesis of NO as well as lymphocyte proliferation [2].

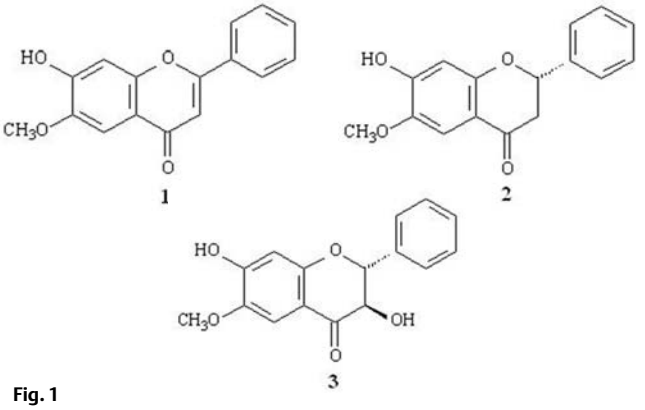

Fig. 1

Acknowledgements: We wish to thank CAPES (Brazil) for financial support and CNPq (Brazil) for a fellowship for C.Q. Alves **References:** 1. David, J.P. et al. (2002) Rev. Brás. Farmacogn. 12: 05 – 06. 2. Barreiros, A. L. B. S. et al. (2000) Phytochemistry, 55, 805.

P369 Stability of tannins and stilbenes against UV-B radiation

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Tannins function as powerful defensive biopolymers against herbivories or microbes by mainly precipitating proteins [1] while stilbenes have a role in plant disease resistance [2]. They have shown to inhibit the growth of bacteria and fungi in vitro. The synthesis of tannins and stilbenes is increased by UV-B radiation in some species [2, 3]. The studies on pine seedlings have revealed that also ozone induces the synthesis of stilbenes in the needles. The aim of this study was to test the stability of tannins and stilbenes in UV radiation in vitro. Standard curves were prepared for tannins (acid-butanol test) and stilbenes (RP-HPLC). The compounds were added in filter paper chips and recoveries were analyzed. The recovery percentage of tannins using an acid butanol test was 99,96 ± 1,23%. Stilbenes were extracted from the paper chips and analyzed by RP-HPLC. Their recovery percentages were 80,95 ± 1,02% and 81,29 ± 0,95% to pinosylvin and pinosylvin monomethyl ether, respectively. The stability tests of tannins and stilbenes will be started during ongoing spring by adding them on the filter paper chips, exposing the chips to UV radiation and analyzing the chips as described above. Results will be presented and discussed. Acknow-

ledgements: Åbo Akademi, Eckerman C & Holmbom B. References: 1. Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.I., Schwartz, C.C. & Mautz, W.W. 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68: 98 – 107. 2. Chong, J., Poutaraud, A. & Hugueney, P. 2009. Metabolism and roles of stilbenes in plants. Plant Science 177: 143 – 155. 3. Rozema, J., van de Staaij, J., Björn, L.O. & Caldwell, M. 1997. UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol. Evol. 12: 22 – 28.

P370 Phytochemical studies on *Galanthus fosteri* Baker

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The genus *Galanthus* L. (Amaryllidaceae) is represented by 14 species (15 taxa) in Turkey [1, 2]. Among these species, *G. fosteri* Baker occurs mainly in south- and north-central Turkey [3]. *Galanthus* species are known to possess Amaryllidaceae alkaloids with interesting chemical structures and biological activities [4,5]. The most well-known alkaloid of this group, galanthamine, has acetylcholinesterase inhibitory activity and therefore it is used in the treatment of mild to moderate Alzheimer's disease [6]. In the course of our ongoing phytochemical studies on Turkish *Galanthus* species, *G. fosteri* has been investigated for its chemical profile. As a result, six known Amaryllidaceae alkaloids, ismine, tazettine, galanthamine, 9-*O*-demethylhomolycorine, sanguinine and lycorine were isolated by using column and preparative thin layer chromatography. The structures of the compounds were elucidated by means of spectroscopic methods (1D, 2D NMR and MS). The present study is the first phytochemical report on *G. fosteri*. Acknowledgements: This study was financially supported by TUBITAK (TBAG-104T272) and EBILTEM (2007-BIL-007). B. Sarikaya was a recipient of TUBITAK research fellowship. References: 1. Davis, A.P., (2000) Galanthus L. In Flora of Turkey and the East Aegean Islands, Vol 11 Edinburgh University Press, Edinburgh. 2. Davis, A.P., Ozhatay, N. (2001) Bot J Linn Soc 137:409 – 412. 3. Davis, A.P. (1999) The genus Galanthus Timber Press Inc., Oregon. 4. Hoshino, O. (1998) The Amaryllidaceae Alkaloids. In The Alkaloids Chemistry and Pharmacology, Vol 51 Academic Press Inc., New York. 5. Unver, N., (2007) Phytochem Rev 6: 125 – 135. 6. Heinrich, M., Teoh, H.L. (2004) J Ethnopharm 92:147 – 162.

P371 Monitoring of essential oils during storage by selected quality parameters

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Essential oils are complex mixtures derived from a considerable number of aromatic plants [1]. While it is well accepted that essential oils are susceptible to chemical alterations during storage [2], comprehensive data on the physico-chemical alterations are still scattered. Although HPLC has been pronounced as a suitable method to detect less volatile and thermolabile substances in essential oils [3,4] extension to stability studies is lacking. Therefore, the aim of the present study was to establish a set of appropriate quality parameters including HPLC to monitor essential oil alterations upon storage. Aliquots of essential oils from lavender, thyme, rosemary and pine were exposed to stress conditions for up to 14 weeks at 40 °C and cool white light in the presence of air. To stop further alterations treated samples were immediately stored frozen at −80 °C until analysis. HPLC profiles showed distinct alterations for all essential oils during storage. Changes were also detectable by TLC. All essential oils exhibited a considerable conductivity increase accompanied by a pH-value reduction except for thyme oil. A remarkable elevation of the peroxide value was also monitored. Hence, in addition to peroxide value, conductivity and pH-value can be suggested as appropriate quality parameters to reveal chemical changes in essential oils during storage. Complementary to GC, HPLC appears to be a suitable tool to shed light on the underlying chemical alterations. Further investigations are in progress to confirm the presented concept by extension to other essential oils. References: 1. Bakkali, F. et al. (2008) Food Chem. Toxicol. 46:446 – 475. 2. Grassmann, J. et al. (2003) Encyclopedia of Food Sciences and Nutrition. Elsevier. Amsterdam, London, New York. 3. Lockwood, G.B. (2001) J. Chromatogr. A 936:23 – 31. 4. Kovar, K.-A. et al. (1980) Arch. Pharm. 313:416 – 428.

P372 HPLC determination of 18β-glycyrrhetinic acid urine excretion profile after ingestion of glycyrrhizin

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Glycyrrhizin is the main pharmacologically active component in licorice. It has a pleasant aromatic sweet taste and is commonly used as flavouring and sweetening agent in confectionery, beverages, chewing gums, and tobacco products. Long term consumption of higher amounts of glycyrrhizin results in serious side effects known as the syndrome of pseudoaldosteronism, which is caused by the glycyrrhizin metabolite 3β-monoglucuronyl-18β-glycyrrhetinic acid (3-MGA). The aim of our study was to determine urine excretion profile of 3-MGA. We analyzed 18β-glycyrrhetinic acid obtained after enzymatic hydrolysis of. Six healthy volunteers ingested 0.6 g of glycyrrhizin. In the first experimental period, glycyrrhizin was ingested in the morning and in the second experimental period, which followed after two weeks, glycyrrhizin was ingested in the evening. Each urine excretion was collected separately and the urine volume measured. Results showed that the maximum amount of metabolite is excreted between 19 and 20 h after glycyrrhizin ingestion. Maximum elimination rates in six individuals were 27 – 110 μg/h. After 4 days, 0.14 – 0.33 % of ingested glycyrrhizin was detected in urine, which is in accordance with literature. There were no significant differences in elimination rates when glycyrrhizin was ingested in the morning or evening. It is often extremely difficult to establish licorice abuse as the cause of mineralocorticoid symptoms. We were able to detect glycyrrhizin metabolites even 4 days after ingestion of a single dose. This suggests a clinically applicable diagnostic HPLC method and provides some important clinical data on glycyrrhizin metabolism.

P373 *In vitro* cytotoxic activity of indole alkaloids from *Croton echioides*

Mello J[1], Novello C[1], Pires M[1], Nocchi S[1], Nakamura T[1], Nakamura C[1], Dias Filho B[1], Marques L[2], Vidotti G[1], Sarragiotto M[1]
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The genus *Croton* (Euphorbiaceae) is a rich source of secondary metabolites such as alkaloids, flavonoids, and terpenoids, which have prominent pharmacological properties [1,2]. *Croton echioides*, a Brazilian medicinal plant, is used in folk medicine because of its aphrodisiac reputation and tonic property. In this study, size exclusion chromatography and preparative MPLC were used to isolate four indole alkaloids from the ethanol stem-bark extract. Their structures were characterized as: *N-trans*-coumaroyl-tryptamine (**1**), *N-trans*-coumaroyl-5-hydroxytryptamine (**2**), *N-trans*-4-methoxycinnamoyl-5-hydroxytryptamine (**3**), and *N-trans*-feruloyl-5-hydroxytryptamine (**4**) by 1D and 2D NMR analysis, EI-MS, and chemical evidence. Each compound was evaluated against HCT-116 colon carcinoma cells [2], and their CC_{50} (μg/mL) were 47.5 (**1**), 72.7 (**2**), 29.4 (**3**), and 53.3 (**4**). These results indicate that the substitution of the hydroxyl group at R_3 by a methoxyl group in 3 increased the cytotoxic activity. The substitution of the group at R_1 had no effect.

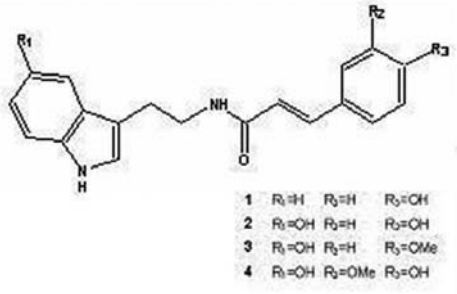

Fig. 1

Acknowledgements: *CAPES, CNPq, INCT_if.* **References:** 1. Rizsk, A. (1987) Bot. J. Linn. Soc. 94: 293 – 326. 2. Payo, H. et al. (2001) Rev. Cubana Farm. 35: 203 – 206. 3. Skehan, P et al. (1990) J. Natl. Cancer Inst. 82: 1107 – 1112.

P374 Conversion of hydrophilic constituents from *Mercurialis perennis* L. by lactic acid fermentation

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Fermented and hydroalcoholic extracts from dog's mercury (*Mercurialis perennis* L.) are used in anti-inflammatory phytomedical remedies. In continuation of our research on lipophilic constituents [1], the hydrophilic compounds from *M. perennis* were studied by HPLC/DAD and GC/MS techniques. Following extraction with aqueous acidified extracts, solvent partitioning with ethylacetate and *n*-butanol yielded two fractions mainly containing glucaric-cinnamic acid derivatives 1 (EtOAc) as well as mono- and oligo-glycosides of kaempferol and quercetin (BuOH) all of which being assigned by LC/MS-MS. In the course of lactic acid fermentation phenolics were converted into smaller molecular units. Since the latter were hardly accessible by LC/MS, identification was performed by GC/MS after extraction with EtOAc and silylation: Dihydrocaffeic- (2, R = OH), dihydroferulic- (2, R = OCH3), dihydrocoumaric- (2 R = H) and 3-phenyllactic acids (3) as well as small volatile phenolics like 4-ethylcatechol (4, R = OH), 4-ethylphenol (4, R = H)) and several glycols were found. The present study marks the first comprehensive report on the hydrophilic compounds from *M. perennis* and their bioconversion upon fermentation.

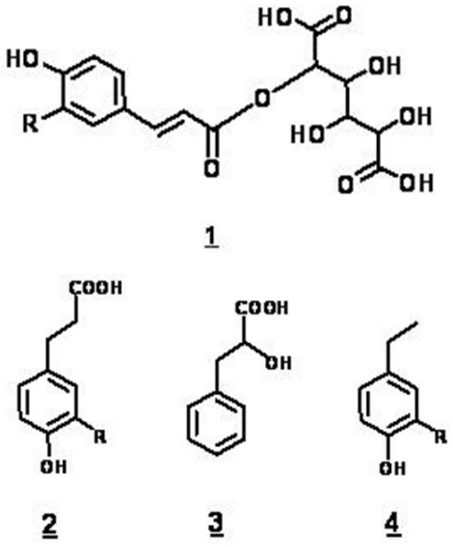

Fig. 1

References: 1. Lorenz P., Hradecky M., Berger M., Bertrams J., Meyer U., Stintzing FC. (2009) Phytochem. Anal. 21:234 – 245.

P375 Stimulation of anthocyanin synthesis in grape (*Vitis vinifera*) suspension cultures by different enzymes

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The use of coloring as additives in foods and drinks is a significant factor for food manufactures and customers alike in determining the acceptability of processed foods. The accumulation of colors and pigments in cell cultures can facilitate by direct selection of high producing cell lines. Anthocyanins are compounds that provide some of the coloring pigment of plants, flowers and fruits. Anthocyanin from grape cell cultures can be used as a natural alternative to synthetic dyes, particularly because of their various health-promoting properties. The present study was concentrated on the production of anthocyanin in suspension culture of Vitis vinifera by exposing them to different enzymes that can act as elicitors. In order to enhance the productivity of anthocyanin from grape (Vitis vinifera) cell cultures, different enzymes such as Indanyl isoleucine, Linolenoyl Glutamine, Malonyl coenzyme A and saliva were applied to the cell cultures. The results indicate that the treatment with Indanyl isoleucine and saliva can give higher concentration of anthocyanin than control ones in the cell culture during 12 days. This shows the effect of enzymes as elicitors which can affect the anthocyanin synthesis in the grape cells. **Keywords:** anthocyanin, plant cell culture, elicitation, enzymes.

P376 Antioxidant activity and total phenolic content of extracts and fractions of cultivated *Leonurus cardiaca* L.

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Motherwort (*Leonurus cardiaca* L), which originally came from central Europe, has spread to all temperate areas of the world. It has been used for a variety of human diseases, specifically in cardiac disorders [1,2]. In Iran, *L. cardiaca* is cultivated for the first time in order to produce industrial formulations. Officinal motherwort is known to have significant amounts of phenolics and flavonoids [2]. Due to the important role of phenolic compounds in prevention of heart disease [3], we evaluated *L. cardiaca* extract for its antioxidant activities and total phenolic contents. After collecting flowering aerial parts of cultivated motherwort, the total phenolic content and antioxidant activities were determined, using the Folin-Ciocalteu assay and *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging respectively. Average phenol content of the total extract was estimated to be about 2.10 ± 0.01 mg gallic acid equivalents (GAE)/g of dried plant. The analysis of fractions showed the highest total phenolic content in the 50:50 metanolic- aqueous fraction (70.79 ± 4.41 GAE/g of fraction). This fraction also exhibited significant antioxidant activity and strongly scavenged DPPH radical, with an IC_{50} value of 53.79 µg/ml. **Acknowledgements:** This research has been supported by Tehran University of Medical Sciences and health services grant number 9289 – 33 – 03 – 88. **References:** 1. Fleming T., et al. (2000) PDR for herbal medicine. Medical Economics Company. Montvale. 2. Barnes, J., Anderson, LA. Phillipson JD (2007) Herbal Medicines. Pharmaceutical Press. London. 3. Hertog, M.G., et al. (1993) Lancet 342:1007 – 1011.

P377 Determination of mutagenic and antimutagenic properties of flavonoid compounds isolated from *Mentha longifolia ssp. longifolia* and *Origanum vulgare ssp. vulgare* by using AMES test system

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One of the important short time test systems, used for the determination of chemically induced mutations at cell level, is the Ames/Salmonella-microsome test system. This system constitutes a great potential in terms of determination of carcinogenic substances and prevention of their risks following the presentation of strong relationship between mutagenic effects of chemical substances and development of cancer. Ames test system is also used for the determination of antimutagens and anticarcinogens, which eliminate the mutagenic or carcinogenic effects of chemicals and prevent the interaction of these chemicals with DNA. In this study, mutagenic and antimutagenic activities of flavonoid compounds obtained from traditionally used Mentha and Origanum plants, are investigated. Mentha longifolia ssp. longifolia (Lamiaceae) was collected from Palandoken Mount and Origanum vulgare ssp. vulgare (Lamiaceae) was collected from Erzurum-Oltu. Apigenin-7-O-glucoside, Luteolin-7-O-glucoside, Luteolin-7-O-rutinoside, Apigenin-7-O-rutinoside, Luteolin-7-O-glucuronide, Apigenin-7-O-glucuronide were isolated from Mentha, and Luteolin 7-O-xyloside and Luteolin 7-O-glucuronide were isolated from Origanum. In the mutagenity and antimutagenity studies conducted by using S. typhimurium TA1535-TA1537 strains, results were statistically evaluated by applying the active compounds at different doses. When the findings were obtained from the

studies, it was determined that the examined compounds at the applied doses do not exhibit any mutagenic effect. On the other hand, it was determined that at 20 mM dose application, Apigenin-7-O-glucoside and Apigenin-7-O-rutinoside exhibited respectively 91% and 88,55% antimutagenic activity. It was seen that the remaining compounds exhibited strong antimutagenic activities, which are considered statistically important, at all applied dosage levels. **Acknowledgements:** This study was supported by grants from the Scientific and Technological Research Council of Turkey (TUBITAK). (Project No: 107T203).

P378 Levels of some trace elements in some Macedonian edible wild Tricholomataceae mushrooms

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The Chemical composition and nutritive value of some Macedonian edible Boletaceae mushrooms Bauer Petrovska B1, Kirovska Cigulevska O 2, Ugrinova L1 1Faculty of Pharmacy, Vodnjanska 17, P.O. Box 36, 1000 Skopje, R. Macedonia; 2JZO ZZZ Skopje, Treta Mak. Brigada 18, P.O. Box 809; 1000 Skopje, R. Macedonia; Over the last few years there has been a great interest in the collecting of wild mushrooms in Macedonia. Except for some cultivated mushrooms, little is known about the nutritional quality of the wild – spread, edible mushroom species in Macedonia. Their great number enable the selection of that which are characterized with great nutritional quality. Therefore in the present study the nutritionally important components were determined with various contemporary nutritients analyses in 15 wild species of Boletaceae mushrooms from various Macedonian areas. Macedonian Boletaceae mushrooms, contained relatively high total dietary fibre content (up to 11.73%, dry basis), protein content (av. 30.98%, dry basis) with high biological values (up to 78.94 BV, 2.60 PER) and were low in fats (av. 4.77%, dry basis). 100 g dry mushrooms comprised low energetic value of 1670.64 kJ on the average. The contents of minerals showed great variation (5.15 – 11.15%, dry mass) in agreement with literature data [1]. P, Mg, Cu, Fe and Zn were found in higher concentrations than other ions. Cd and Pb have lower values than the maximum concentrations imposed by low [2]. Compared with other provisions [3], Macedonian Boletaceae mushrooms proved to be a good source of protein and mineral matter for the human diet. They are deficient in fat, posse low energy but are nutritionally valuable food. Table 1. Content of protein, fat, ash and energy value of Macedonian Boletaceae mushrooms and other provisions [3] expressed in dry mass provisions Protein% Fat% Ash% Energy value kJ/100 g Macedonian Boletaceae mushrooms 30.98 4.77 8.18 1670.64 Milk 27.1 29.0 6.0 2186.6 Veal 70.7 23.9 4.4 2085.8 Soya 36.9 19.8 5.1 1475.4 Beans 24.6 2.5 7.4 1429.3 Wheat bread 13.3 1.7 2.7 1447.1 Beef 56.4 44.4 3.1 2600.9 **Acknowledgements:** Faculty of Natural Science and Mathematics, Karadelev M. **References:** 1. Falandysz, J., Lipka, K. (2006) Rocz Panstw Zakl Hig. 57(3): 217 – 33. 2. Pravilnik za opshti baranja za bezbednost na hrana, Sl. Vesnik RM 118/05. 3. Souci, S., Faschmann, W., Kraut, H. (1994) Food composition and tables. Medpharm. Stuttgart.

P379 Isolation of bioactive aporphinoid alkaloids in *Oxandra asbecki* (Annonaceae)

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Since prehistoric times, humans beneficially used natural resources for their daylife needs. Plants are able to synthesize complex molecules and consequently have a unique chemical diversity. This is a source of inspiration for new drugs discovery. Dyrk1A kinase is a target used in research on Alzheimer's disease. Inhibition of this kinase is associated with treating symptoms of this disease [1,2]. In France, the annual number of new cases is 230 000. The prevalence is expected to double in industrialized countries and quadruple in developing countries in the coming decades. The development of a better diagnosis and treatment is essential. The Annonaceae is a large family of tropical plants that have been investigated intensively. There exist 38 species in *Oxandra* genus and *Oxandra asbecki* species in Venezuela and in primary forests of French Guiana. To the aim of discovering new bioactive plants from French Guiana, *Oxandra asbecki* was selected for phytochemical study because of its potent inhibition of DyrK1A kinase. Bioassay-directed fractionation of the ethyl acetate extract provided three bioactive alkaloids. We isolated three aporphinoid alkaloids and show for the first time an strong activity (with micromolar IC50) of Velutamin under two kinase: CDK1 and DyrK1A, and activity of Aristolactam AII on DyrK1A kinase. **References:** 1. Lee, V.M-Y., et al. (2004). Trends in neurosciences 27. 2. Nam Doo Kim, N.D., et al. (2006) Biol. Med. Chem. Lett. 16, 3772 – 3776.

P380 Secondary metabolites from endemic *Iranecio* species from Iran

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The genus *Iranecio* belongs to the Asteraceae and contains four species, whose main habitat is Iran. Except for botanical information in the Flora Iranica [1], no information about the chemistry of the plants is given in the literature. Since many members of the Asteraceae contain secondary metabolites which display cytotoxic effects [2,3], extracts from *Iranecio elbruzensis* Bioss (endemic to Iran) and *I. othonae* were tested for cytotoxic activity, following the method previously reported by Azizi et al. [4], IC_{50} values were found at 1 mg/ml and 0.75 mg/ml, respectively. Aerial parts of *I. elbruzensis* and *I. othonae* were extracted with dichloromethane in a Soxhlet apparatus and the obtained extracts purified by column chromatography using methanol and Sephadex LH-20. Purification of two fractions by repeated column chromatography, flash chromatography or preparative TLC afforded the steroids β-sitosterol [5] and its glycoside daucosterol [6], as well as the sesquiterpene spathulenol [7] from *I. elbruzensis*. *Iranecio othonae* also contained daucosterol and a rarely found palmitic acid ester derivate of calenduladiol (see fig.) [8].

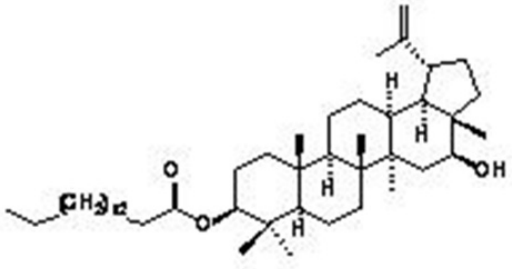

Fig. 1

All structures were elucidated by means of their mass and NMR spectra. Calenduladiol palmitate, which was found in larger amounts, will be tested on its cytotoxicity in the near future. **Acknowledgements:** Institute of Pharmaceutical Biology and Biotechnology of Heinrich Heine University of Dusseldorf, Mrs Eva Müller **References:** 1. Ditrich, M et al. (1982) Flora Iranica Compositae IV. Rechinger, KH (ed). Akademische Druck und Verlagsanstalt, Graz, Austria. 2. Woerdenbag, HJ et al. (1994) Planta Med, 60:434 – 437. 3. Francois, G. et al. (1996) Planta Med. 62:126 – 129. 4. Azizi, E. et al (2008) J. Biol. Sci. 8:380 – 385. 5. Seo, S. et al. (1988) J. Chem Soc. Perkin Trans I 2407 – 2414. 6. Gu, J-Q et al (2004) Z. Naturforsch. 59c: 797 – 802. 7. Brochini, CB and Roque, NF (2000) Braz.Chem.Soc. 11:361 – 364. 8. Schmidt, T. et al (2004) Planta Med. 70:967 – 977.

P381 *Tussilago farfara* L.: History of its therapeutical use and illustration in the 19th century

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Historical pharmaceutical and medical sources are celebrating a common renaissance not only due to their information content about old formulations but also due to their remarkable attraction of rich and skilful illustrations. In the present study there have been selected with the help of the Index Londinensis [1] almost 40 original historical sources of the 19th century which have been analysed in regard to their content of their texts as well as of their illustrations. Although text and

illustrations served physicians, pharmacists and botanists amongst others for a doubtless identification of species it could be shown that approximately one third of the illustrations show mistakes in plant anatomy. Furthermore it could also be shown, that copying was a frequent feature. However the findings suggest that a substantial change occurred in the mode of copying. The until then wildly common part copies [2] have been replaced extensively through entire copies, which actually seldom reached the quality of the original. The analysed texts contain a mixture of traditional medical applications going back on the ancient authors like Plinius, Dioscurides and Galen and new insights of the upcoming sciences in the 19th century. *Tussilago farfara* (coltsfoot) was prevalently used as a remedy against cough, catarrh and mucous obstruction of the lung but also against phthisis. External use was also recommended to cure dermatitis and erythemas. Moreover in the 19th century the leaves of coltsfoot were still a widespread used vegetable. **References:** 1. Stapf, O. (1931) Index Londinensis. Clarendon Press. Oxford. 2. Nickelsen, K. (2006) Botanists, Draughtsmen and Nature: The Construction of Eighteenth-Century Botanical Illustrations. Springer, Berlin

P382 Acylated triterpene saponins from *Atroxima liberica* Stapf

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The genus *Atroxima* (Polygalaceae), represented by trees, shrubs, herbs, or lianas (rarely) comprises 6 species distributed in the forests of Western and Central Africa [1]. We previously reported the isolation and structural elucidation of seven pairs of preatroxigenin [(2β,3β,4α,22β)-2,3,22,27-tetrahydroxyolean-12-ene-23,28-dioic acid] glycosides called atroximasaponins A1/A2-G1/G2 from roots of *A. congolana* [2,3]. However, there is no previous study on *A. liberica*. In the course of our studies on the saponins of the Polygalaceae family to find the chimiotaxonomic markers, we have investigated the saponin fraction of the roots of *A. liberica*. We report here the isolation and identification of eight new acylated triterpene saponins (**1** – **8**) isolated as pairs of epimers named libericosides A1/A2 – D1/D2, one pair of epimer the (Z)-epimer being new: libericoside E (**9**), one new sucrose ester: atroximoside (**10**), and six known compounds have been isolated from the roots of *Atroxima liberica* by repeated MPLC over silica gel. Their structures were elucidated on the basis of extensive 1D and 2D NMR spectroscopic studies and mass spectrometry. The new saponins (**1** - **9**) were evaluated for their cytotoxicity against HCT 116 and HT-29 human colon cancer cells, respectively. **Acknowledgements:** The authors are grateful to the Conseil Régional de Bourgogne, France for financial support. **References:** 1. Bila, B. et al. (1982) Bull. Soc. Chim. Belg. 91: 321 – 331. 2. Elbandy, M. et al. (2003) J. Nat. Prod. 66: 1154 – 1158. 3. Elbandy, M. et al. (2003) Helv. Chim. Acta 86: 522 – 531.

P383 Chemical constituents from needles of *Torreya nucifera*

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Torreya nucifera (Linn.) Sieb. et Zucc. (Taxaceae), a species mainly distributed in Japan, Korea and China and it has long been extensively used in traditional medicines for the treatment of various diseases relating to oxidation and inflammation [1 – 3]. In this study, needles of T. nucifera were extracted with acetone-H2O (7:3, v/v), fractionated with hexane, chloroform and ethylacetate (EtOAc), and freeze dried to give some dark brown powders. The resulting EtOAc soluble fraction mixture was then repeatedly chromatographed on a Sephadex LH-20 open column using a series of aqueous methanol and ethanol-hexane mixtures as eluents. Most of the needle extractives were flavan and their methyl ether derivatives, such as (+)-catechin (1), (−)-epicatechin (2), (+)-gallocatechin (3), (−)-epigallocatechin (4), 3-O-methyl-(+)-catechin (5) and 3-O-methyl-(−)-epicatechin (6), as well as protocatechuic acid (7), one of benzoid acid. The structure elucidation and determination of the isolated compounds were based on physiochemical and spectroscopic methods, including 1 H and 13C NMR, NOE and EI-MS analysis. Compounds 1, 3, 4, 5 and 7 were isolated from the needles of this species for the first time. Antioxidant potential of the isolates was assessed by DPPH free radical scavenging assay. Results indicated that EtOAc soluble fraction (IC 50 13.8 g/ml), compounds 1 – 4 and 7 (IC 50 9.6, 9.4, 12.7, 9.5, and 12.9 g/ml, respectively) exhibited significant scavenging effects, compared with positive control of curcumin (IC 50 13.8 g/ml. This indicates that T. nucifera needles could be a promising source for antioxidant-related-medicine exploitation. **Acknowledgements:** This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009 – 0094074), Natural Science Foundation of Tianjin City (09JCYBJC 15800) and Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616). **References:** 1. Cao, CM. et al. (2009) Nat. Prod. Res. Dev. 21:737739. 2. Harrison, LJ. et al. (1987) Phytochemistry. 26:12111212. 3. Yoon, WJ. et al. (2009) Int. J. Pharm. 5:3743.

P384 Diversity of popular names of the main medicinal plants (MP) used by the local people in the central region of South America

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The diversity of the main "medicinal plants names"-(MP) most used by the local people in the central region of South America (21 municipalities of the Southwest of Mato Grosso, Brazil) were investigated in 2005. Firstly, the popular names of the main MP were obtained. Three "reference" people in each of the 21 municipalities were indicated as informants by their pairs (21 x 3 = 63 informants). In every region, the informants (63) listed 503 MP. The list presented from 42 to 137 different MP names by municipality. Two presented more than 100 MP names while one presented less than 50, and the remaining 18 municipalities between 50 – 100 MP. One way to measure the diversity of MP "within and between municipalities" was through "recurrent indications" of same MP. To this RI index was used (RI = Sum of the number of MP names cited by the three informants of each municipality divided by the number of different MP names cited in this municipality). The lowest RI value was 1.085 and the highest 1.693, indicating that the list of MP names was more distinct in the municipalities with lower RI and more similar in the municipalities with higher RI. The average number of MP listed per county (municipality) was 107. Only one MP(urtigão) was mentioned unanimously by the three informants of a same municipality. In general, the recurrence of indications (RI) was low. It's revealing a great informative, cultural and ethnoknowledge diversity in respect of medicinal plants used by these communities in central region of South America. **Acknowledgements:** UNEMAT, FAPEMAT and EMPAER-M "by institutional support offered" -; PLAMED Project team for their support and participation in various construction stages: Bonila MGO, Carniello M, Ramos A PR et al.

P385 Volatile components of *Juglans mandshurica* root shell

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Juglans mandshurica Max., a fast growing hardword species in family of Juglandaceae, has an extensive distribution ranging from China, Siberia to Korean peninsula. The tree has been used as a folk medicinal plant for treatment of esophageal, gastric, cardiac and lung cancer [1 – 3]. By

comparison of mass fragmentation pattern of each component with two modern MS libaries namely Wiely 6 and NIST, the volatile compounds from root shell of *J. mandshurica* were determined as three naphthoquinone derivatives such as 1,4-naphthoquinone (**I**), 5-hydroxy-1,4-naphthoquinone (Juglone, **II**) and 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin, **III**), and one aliphatic alcohol, 3-ethyl-2-methyl-1-pentene-3-ol (**IV**). Evaluated by quantitative investigation with authentic compounds of the three corresponding naphthoquinone derivatives, compounds **I** and **II** were the major volatile components in J. mandshurica root shell and their yields were around 54.4 μg/g and 21.3 μg/g, respectively. **Acknowledgements:** This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009 – 0094074), Natural Science Foundation of Tianjin City (09JCYBJC 15800) and Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616). **References:** 1. Kim, TW. (1994) The Woody Plants of Korea in Color. Kyohak Press, Seoul, pp. 58 – 59. 2. Kim, JK. et al. (2006) J. Kor. Wood Sci. Technol. 34(6): 51 – 60. 3. Min, BS. et al. (2003) Biol. Pharm. Bull. 26(7):1042 – 1044.

P386 Comparative effects of plant-derived smoke and potassium nitrate on germination and post-germination parameters of four medicinal species

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The effect of smoke water and Potassium nitrate on germination and post germination parameters of four medicinal species of the Rosaceae (*Sanguisorba minor* Scop.), Apiaceae (*Pimpinella anisum* L.), Lamiaceae (*Melissa officinalis* L.) and Ranunculaceae (*Nigella sativa* L.) was tested. Dry seeds were exposed to smoke water (1:500) and KNO_3 (150 mM) for 1 h. Smoke enhanced some seed germination parameters of the study species. In species of *S. minor*, germination percentage (GP) and germination speed (GS) was stimulated by 1 h of smoke water exposure while in species of *P. anisum* only GS was enhanced by smoke water. In addition, 1 h of smoke water exposure enhanced the post-germination parameters including shoot length (SL), root length (RL), total seedling length (TSL), seedling fresh mass (SFM), vigour index (VI), and germination value (GV) in *S. minor* and SL, VI, and GV in species of *P. anisum*. In species of *M. officinalis*, smoke water enhanced parameters of RL, root/shoot ratio, and SFM. The species of *N. sativa* no had response to smoke. Potassium nitrate enhanced GS, SL, VI, and GV in *P. anisum* species, GS, SL, RL, TSL, SFM, VI, and GV in species of *M. officinalis* and SL in *N. sativa*. Pearson's correlation coefficients revealed significant relation between response to smoke and Potassium nitrate for germination percentage (0.577, $p < 0.01$), germination speed (0.606, $p < 0.01$), root/shoot ratio (cm) ($p < 0.01$) and germination value (0.429, $p < 0.05$) in the examined species.

P387 Effects of salinity and temperature on germination and seedling growth of nine medicinal plants

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Salinity stress is a major environmental constraint in arid and semi-arid regions such as Iran. Excessive amounts of salt in soil severely reduce seed germination and seedling growth of crops in agricultural systems. The purpose of this research was to study the effect of temperature, salinity and their interaction on the germination and seedling growth of nine medicinal plant species including *Salvia nemorosa* L., *Marrubium vulgare* L., *Hyssopus officinalis* L., *Origanum majorana* L., *Ocimum basilicum* L., *Nepeta racemosa* Lam., *Oenothera biennis* L., *Silybum marianum* L. and *Cnicus benedictus* L. Treatments included three temperatures (15, 25 and 35 °C) and four NaCl concentrations (0, 5.3, 8.48 and 10.6 g.l-1). A completely randomized design with three replications was used. Results showed that salinity treatments had significant effect on germination percentage, germination rate, seedling growth and seedling vigor in all nine medicinal plant species. Germination percentage and germination rate of all medicinal plant species gradually declined as the concentration of NaCl increased. Significant decrease in germination percentage and germination rate was observed at higher levels of salt concentration. Ocimum basilicum L. and Salvia nemorosa L. were the only two among nine medicinal plants in this study that germinated in salinity concentration higher than 5.3 $g.l^{-1}$. Germination rate and germination percentage of all species, except Ocimum basilicum were adversely affected by increasing temperature to 35 °C. The highest seedling vigor in most species was observed in a temperature range of 15 – 25 °C and increasing temperature up to 35 °C, strongly decreased it. The interaction effect of temperature and NaCl concentration on final germination in all species was significant, indicating that germination response to salinity depended on temperature. The inhibitory effect of high salinity on final germination, germination rate, seedling growth and seedling vigor was greater at 35 °C than at 15 °C. **References:** 1. Auld, D.Let al.(1998). Planting date and temperature effects on germination, emergence and seed yield of Chickpea. Agron. J. 80: 909 – 914. Almansouri, M.et al. (2001). Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum Desf). Plant and Soil. 231:243 – 254.

P388 Study on the effects of prepriming seed with salicylic acid in salinity stress condition, on germination and growth characteristics of chamomile (*Matricaria recutita L.*)

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In order to study the effects of prepriming seed with salicylic acid in salinity stress condition, on germination and growth characteristics of chamomile, an experiment was conducted in 2009 in the seed laboratory of Zabol University, Iran, as a factorial arrangement based on completely randomized design with three replications. The experimental treatments by salicylic acid were in 5 levels (0, 0.5, 1, 1.5 and 2.5 mM) and salinity in 4 levels (0, 0.5, 1 and 1.5%). Results indicated that salt stress had an negative effect on all factors ($p < 0.01$). So that the highest percentage and speed of germination, length of radicle and hypocotyle, endosperm weight and the lowest length of time of 50% germination obtained was with 0% salinity treatment. With respect to salicylic acid treatments, the best effect except speed of germination and length of hypocotyle was achieved with 2.5 mM salicylic acid treatment. **Key words:** Germination, Salicylic acid, Salt stress, Chamomile

P389 Effect of different levels of organic and inorganic fertilizer on yield and main composition of essential oil of chamomile (*Matricaria recutita* L.)

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In order to study the effect of different rates of fertilizer, manure, micronutrients and mixtures of them, on flower and seed yields and the composition of essential oil of chamomile, a field experiment was conducted in 2006 – 2007 in a farm located 5 kilometer west of Shirvan city. The experimental design was randomized complete block with three replications and eight treatments including: unfertilized (F1), 100% fertilizer (F2), 100% manure (F3), micronutrients foliar (F4), 50% fertilizer +50% manure (F5), 100% fertilizer + micronutrients foliar (F6), 100% manure + micronutrients foliar (F7), and 50% fertilizer + 50% manure + micronutrients foliar (F8). The results showed that the highest seed, fresh and dry flower yield was obtained from 100% fertilizer + micronutrients treatment, and there wasn't significant difference between this treatment with 100% fertilizer treatment. The highest essential oil and chamazulen content was obtained with 50% fertilizer + 50% manure and 100% manure treatments. The highest essential oil and chamazulen yield achieved from 50% fertilizer + 50% manure + micronutrients treatment and there wasn't significantly different from this treatment and 50% fertilizer + 50% manure treatment. **Key words:** Chamomile, Fertilizer, Manure, Micronutrients, Essential oil, Yield

P390 Influence of planting date and nitrogen on yield and components of medicinal flax

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The effect of sowing date and different nitrogen (N) levels on the quality of medicinal flax was investigated in an experiment performed in split plot in form of Randomized Complete Block Design with 4 replications in 2005 – 2006 and 2006 – 2007 in the Yasooj Agriculture Research Station. The 5 sowing dates included 4, 6,8,10, and 12 °C on the basis of temperature in 5-cm soil depth in the main plots and 4-fertilizer levels in sub-plots with no fertilizer, 50, 100 and 150 kg/ha pure N (urea as source) of which 50% was used at the time of sowing and 50% in the way of top-dressing. The results of complex 2-year analysis of data indicate that with delayed sowing the plant height, the number of branches, the number of fruits, the grain yield, the 1000-seed weight, the leaf area index, the dry matter, the crop growth rate and the oil percentage were reduced significantly. The use of 100 kg/ha pure N significantly increased plant height, number of branches, number of fruits, grain yield, leaf area index, dry matter, crop growth [1]. First sowing date with 1801.12 kg/ha resulted inhighest yields and fifth sowing date with 760.48 in the lowest one. **References:** 1. Beighi, O.R. (2005). Production and processing medicinal plants. Vol. 1 Astane-Ghodse Rezavi Publication p. 347. 2. Zarghari, Gh. (2004). Medicinal plants. Tehran: Tehran University Press. P.342.

P391 Comparative study of rosmarinic acid content in *Satureja* species

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Satureja species are used in traditional medicine and phytotherapy mostly in gastrointestinal disorders. This genus is rich in essential oil and phenolic compounds. Rosmarinic acid which is found in this genus exhibits important biological activities such as antiviral, antioxidant, anti-inflammatory and anti HIV properties [1 – 3]. In this investigation, rosmarinic acid content of different *Satureja* species which grow in various parts of Iran was determined by using HPLC method. Aerial parts of six *Satureja* species named *S. hortensis, S. khuzestanica, S. bakhtiarica, S. atropa, S. mutica* and *S. macranta* were collected, identified, dried and milled. 200 mg of each plant was extracted by using water, methanol and 2- propranol in a ratio of 80:10:10 as solvent. O-phosphoric acid (0.085%) was added to the solvent subsequently. The HPLC was performed by using C_{18} column. The mobile phase was the same as extraction solvent with gradient mode. UV spectra were collected across the range of 200 – 900 nm, extracting 330 nm for the chromatograms. The results showed that the HPLC method could separate rosmarinic acid from other components of the plants efficiently. By using above-mentioned method, rosmarinic acid content in *Satureja* species was found 0.12 – 1.90% in dried plants. The highest amount of the rosmarinic acid was found in *S. mutica*, whereas *S. khuzestanica* contained the lowest content of rosmarinic acid. **References:** 1. Toth, J. et al. (2003) Acta Facultatis Pharmaceuticae Universitatis Comenianae 139 – 145. 2. Petersen, M. et al. (2003) Phytochemistry 62: 121 – 125. 3. Swarup, V. et al. (2007) Antimicrob. Agents Chemother. 3367 – 3370.

P392 Assessment of fluoride content and daily intake from different brands of tea bags in Iran

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Tea is the second most commonly consumed beverage world wide. Tea (*Camellia sinensis*) is a naturally rich source of fluoride. The quantity of fluoride intake is important in optimizing its dental caries-preventive role. Intense concentration of fluoride in tea, can lead to excessive fluoride intake which may cause health problems in turn. The measurement of fluoride intake usually requires information on the fluoride concentration in food and beverages. The main objective of this study was to investigate the fluoride content of various commercial brands of tea bag available on the market in Iran. Furthermore, daily fluoride intake from these brands is assessed. The results of this study showed that among 15 brands of tea bag assessed in this study, Nemooneh™ had the highest fluoride concentration (0.41 ± 0.01 mg/100 ml/3 min), whereas Ahmad™ had the lowest level (0.10 ± 0.01 mg/100 ml/3 min). The average fluoride concentration was 0.23 ± 0.01 mg/100 ml/3 min. Thus, it seems that daily consumption of four cups of tea could provide up to 73% of recommended daily dose of fluoride in children 2 – 5 years old and up to 50% recommended daily dose for adults. It seems that existing tea bag in Iranian market contain proper amounts of fluoride and there is no toxicity with their regular consumption.

P393 Antioxidant and anticancer activity and composition of the essential oil of *Dracocephalum multicaule* from Iran

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The genus Dracocephalum L. (Lamiaceae) is represented in flora of Iran by eight annual and perennial species [1]. Infusion of the aerial flowering parts of the Dracocephalum multicaule Montbar. & Auch. are used locally in the treatment of colds and gastrointestinal disorders. The goals of the present study were to determine: (1) the chemical composition of the essential oil of the plant, (2) the antioxidant activity of the essential oil and its major compounds using the 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the?-carotene-linoleic acid blenching assays and (3) the protective effect of essential oil in oxidative stress damage of K562 cells induced by hydrogen peroxide. Chemical composition of the hydrodistilled essential oil of Dracocephalum multicaule was characterized by GC and GC-MS. Monoterpenoids including oxygenated and hydrocarbons ones with 71.5 and 28.3%, respectively, were the principal groups. Perilla aldehyde (71.5%) and limonene (28.1%) were identified as the main components. Radical scavenging capacity and β-carotene bleaching assay was carried out to determine the antioxidant activity of samples according to Blois' [2] and Miller's [3] procedures. Antioxidant studies based on DPPH assay indicated that the essential oil of D. multicaule possesses a marked antioxidant and radical scavenging activity with an IC50 value of 438.2 μg/ml. In addition, pre-treatment with essential oil and main constituents protected K562 cells 49.5% against H2O2 induced oxidative damage throughout increasing the activities of antioxidant enzymes and glutathione content in K562 cells. **References:** 1. Rechinger KH. (1982) Flora Iranica, Vol. 150, Graz, Akademische Druck-u. Verlagsanstalt. 2. Blois MS. (1958) Nature, 181: 1199 – 1200. 3. Miller HE. (1971). J. Am. Chem. Soc 48: 91 – 94.

P394 Phenolic compounds from *Lespdesza cuneata*

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Phenolic compounds, especially flavonids and its derivatives play an important role in medicine, food addictives and cosmetic materials fabrication and development [1]. As one chain of our continually exploring active and functional flavonoids from plants, aerial parts of Lespdesza cuneata G. Don (Leguminosae) was studied in this work. *L. cuneata*

naturally grows in fields of East and South Asian countries, such as Korea, China, Japan and India [2]. In folk medicines, the aerial parts of *L. cuneata* have long been used to cure various diseases [3]. However, its chemical constituents are far from fully investigated. The aerial parts of *L. cuneata* were collected, air-dired and extracted with 95% aqueous ethanol. Then was successively partitioned with n-hexane, chloroform, ethyl acetate and H_2O, and freeze dried. Repeat Sephadex LH-20 open column chromatography on a portion of ethyl acetate and aqueous soluble powders resulted in the isolation of four flavone glycosides, and their structures were elucidated as desmodin, homoadonivernith, kaempferol and quercetin based on extensive spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR and Mass spectrum. **Acknowledgements:** This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009 – 0094074), Natural Science Foundation of Tianjin City (09JCYBJC 15800) and Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616). **References:** 1. Si, CL. et al. (2009) Planta Med. 75:1165 – 1167. 2. Lee CH. (1994) The pharmacology of Chinese Herbs, CRC Press, China. 3. Ding, JL. et al. (2006) Korean J. Biotechnol. Bioeng. 21:414 – 419.

P395 Inhibition of mediator release from RBL-2H3 cells and human granulocytes by the *Pelargonium sidoides* root extract EPs®7630

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The incidence of allergic disorders is increasing worldwide. This applies especially to type-I hypersensitivity reactions which are caused by immunoglobulin E (IgE) antibodies against normally innocuous environmental substances. Cross-linking of IgE bound to high-affinity receptors (FcεRI) on mast cells and basophiles by the triggering allergen leads subsequently to the release of inflammatory mediators (e.g. histamine, eicosanoids and cytokines). EPs®7630, a proprietary aquous-ethanolic extract from *P. sidoides* roots, is an effective treatment for upper respiratory tract infections. Besides other pharmacological effects, EPs®7630 has been shown to possess immunomodulatory properties. Thus, it was considered worthwhile to investigate if EPs®7630 also disposes of anti-allergic activities. Experiments were performed with RBL-2H3 rat basophilic leukemia cells. Dinitrophenyl (DNP)-IgE-sensitized cells were treated with the extract for 30 min and then stimulated by DNP-BSA to induce degranulation. Released histamine was determined using a commercial ELISA. EPs®7630 concentration-dependently inhibited release of histamine with an IC_{50} of about 39 µg/ml. In order to gain insight into the contribution of different extract constituents to this effect, EPs®7630 was fractionated by Sephadex LH20 column chromatography using eluents of different polarity. Whereas a water and an ethanol eluate exerted only a very weak effect, a strong inhibition of histamine release was observed for the methanol (IC_{50} 21 µg/ml) and acetone fraction (IC_{50} 19 µg/ml) indicating that mainly polymeric proanthocyanidins are responsible for this activity. Similarly, the extract suppressed the synthesis of eicosanoids in calcium ionophore-stimulated human granulocytes. The results suggest that EPs®7630 may have a therapeutic potential for the treatment of allergies, such as hay fever or asthma.

P396 The effect of corm weight on saffron (*Crocus sativus* L.) production in Saudi Arabia

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The kingdom of Saudi Arabia (KSA) is one of the highest consumer countries for saffron spice. Last year in the local market the price for one kg of saffron spice reached 18,000 SR (~US$ 5,000). Here we report for the first time the cultivation of saffron in the KSA in particularly at Alkharj Governorate. The effect of corm weight on saffron production was investigated under Alkharj governorate cultivation conditions, KSA. Corms of *Crocus sativus* L. (Iridaceae) of Spanish origin (accession #: BCU001584 from Minaya, Albacete, Spain) were provided by Professor J.A. Fernández (Biotechnology IDR, University of CastillaLa Mancha, Albacete, Spain). Three different corm weights as CW1: > 10 g, CW2: ≥ 5 g – ≤ 10 g and CW3: < 5 g were studied. The higher weight of saffron corms increased the number of leaves per corm. The maximum mean values of leave length were obtained as a result of lesser weight of saffron corms weighting < 5 g. The highest number of sprouts was observed with the use of saffron corms weighting > 10 g. None of the three corm weights produced saffron flowers, that might because of planting date was too late in December, while the flowering period is mainly in November. Daughter corms have been produced by the three different corm weights. The higher weight of saffron corm increased the number of daughter corms, up to three daughter corms per mother corm were produced at the end of May. **Acknowledgements:** The authors gratefully acknowledge Mr. Abdullah L. Al-Onazi and Mr. Ali Ibrahim for help in professional photographing and field work, respectfully. **References:** 1. Sharaf-Eldin, M. et al. (2008) Planta Med. 74: 1316 – 1320.

P397 Effect of nitrogen fertilizer and plant density on growth, yield and essential oil content of newly introduced plant species in Egypt: 1- *Monarda citriodora*

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Here we are reporting for the first time in the recent agricultural history of the Egypt the cultivation of monarda (*Monarda citriodora*) in Egypt. The effect of nitrogen fertilizer and plant spacing on growth, yield, and essential oil content on monarda production was investigated under Giza governorate cultivation conditions. Seeds were obtained from Jelitto seed company, Germany on October 2008 and were sown in the nursery on October 14th and the seedlings were cultivated in a clay-loamy soil in December. Three nitrogen doses were applied (33.5, 50.25 or 67 kg N/fedden = 4200 m^2) and four plant spacing (20, 25, 30, or 35 cm between plants) were studied. The highest nitrogen dose result in the highest growth parameters and essential oil% obtained, while plant spacing of 30 cm seems to be the optimum for monarda production.

P398 Antioxidant activities of methanolic extract of *Sapium ellipticum*

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The stem bark extract of *S. ellipticum* (Hochst) Pax was investigated for its antioxidant properties in this study. The extract was evaluated for antioxidant activity in vitro in terms of its ability to inhibit lipid peroxidation and its free radical scavenging reducing, metal chelation powers [1,2,3]. The total amount phenolic compounds in the extract were also determined in terms of gallic acid equivalent. The extract produced effective free radical scavenging and reducing activities in a dose dependent fashion. The extract exhibited noticeable inhibition of lipid peroxidation of linoleic acid emulsion. These activities were less that of ascorbic acid and BHT used as positive controls. The extract however demonstrated poor iron chelating ability compared to EDTA. The total phenolic content of the extract was 50.61 ± 0.08 mg/g in terms of gallic acid. This study showed that the stem bark extract of *S. ellipticum* exhibits significant antioxidant activity and is a good source of natural antioxidants. **References:** 1. Chang, LW et al. (2002). Food Chem., 78: 347 – 354. 2. Lai, LS et al. (2001). J. Agric. Food Chem., 49: 963 – 968. 3. Willcox, JK et al. (2004). Crit. Rev, Food Sci., 44: 275 – 295.

Natural products for the treatment of infectious diseases

P399 Protein kinase inhibiting anthraquinones and NF-κB inhibiting naphthopyrones from the Phillippine echinoderm *Comanthus parvicirrus*

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A bioassay-guided investigation of a Philippine specimen of the echinoderm *Comanthus parvicirrus* yielded ten compounds evenly divided into anthraquinones and naphthopyrones. Four of the five anthraquinones were identified as 1′-deoxyrhodoptilometrin (**2**) and rhodoptilometrin (**3**) along with their respective 6-*O*-sulfate derivatives (**4** and **5**) in addition to a new natural anthraquinone congener (**1**). The isolated naphthpyrones were comaparvin (**6**), 6-methoxycomaparvin (**7**), 6-hydroxycomaparvin-8-*O*-sulfate (**10**), 6-methoxycomaparvin-5-methylether (**8**), and its 8-*O*-sulfate derivative (**9**). The structures of the isolated compounds were unambiguously assigned on the basis of spectroscopic methods. The absolute configuration of **3** and **5** was determined as (*S*)-(−) isomer. The isolated compounds were evaluated for their antibacterial, antifungal, antiviral and cytotoxic activities. In the cytotoxicity (MTT) assay against mouse lymphoma L5178Y cells, **2** and the unseparable mixture of **6** and **7** exhibited pronounced activity (IC_{50} values of 7.71 and 16.5 μM, respectively) compared to kahalalide F (IC_{50}= 4.3 μM) which was tested and proven to be mainly through induction of apoptosis. Moreover amongst the tested compounds for the protein kinase inhibitory activity against 24 different enzymes, anthraquinone congeners (**2-5**) and the unseparable naphthopyrone mixture (**6/7**) exhibited the most potent and selective behaviour with IC_{50} values between 1.8 and 33 μM. Compounds (**1-3**) and (**6-8**) were also tested for their potential to inhibit the activation of the transcription factor NF-κB, which plays an important role in cancer development and inflammation. Only naphthopyrones (**6-8**) significantly inhibited NF-κB activity (IC_{50}'s of 50 – 100 μM) and their mechanism of action was investigated.

P400 New alkaloids from the leaves of *Tecoma stans* L. Bignoniaceae grown in UAE

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Tecoma stans L. (Bignoniaceae) known as Yellow Elder, grown in United Arab Emirates was investigated for the first time, since it is used in Mexico as herbal medicine for the control of diabetes.(1). The leaves were collected from the gardens of UAE in June and July. The dried leaves were soaked in distilled water, ethanol and dichloromethane for 72hr at 25 °C with occasionally stirring. The alkaloids in the plant extracts were separated by thin layer chromatography and detected by Dragendorff's reagent. In addition purification and isolation was performed by column chromatography and gradient elution with dichloromethane and ethanol. The detection of the major alkaloids tecomine, boschniakine and 5- hydroxyskitanthine as well as of two new alkaloids was confirmed by gas chromatography-mass spectrometry (GC-MS) analysis and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Bacteriological assays for different aqueous, ethanolic and dichloromethane extracts of plant material were analysed to evaluate the antibacterial effect against gram negative and gram positive bacteria. **Acknowledgements:** Dean College of Pharmaceutical Sciences Ras Al Khaimah, Ras Al Khaimah Medical &Health Sciences University, Ras Al Khaimah, UAE. Microbiology department, Ras Al Khaimah Medical &Health Sciences University, Ras Al Khaimah, UAE. Professor Tim flower University Of Sussex School of Life Science Department Biology, John Maynard Smith Building, UK **References:** 1. Costantino, L.; Laura, R. (2003) Il Farmaco58: 781 – 785.

P401 New antibiotic metabolites from the fungal endophyte *Stemphylium globuliferum* isolated from *Mentha pulegium*

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During our ongoing search for new bioactive metabolites from endophytic fungi with focus on the discovery of new antimicrobial compounds, we investigated the fungal strain *Stemphylium globuliferum* isolated from the medicinal plant *Mentha pulegium* (Lamiaceae). The EtOAc extract of the poorly investigated *S. globuliferum* fungal strain afforded six new bisanthraquinones, together with four known related compounds. All compounds were tested for their antimicrobial activity (including antibacterial, antifungal and antiviral activities). All dimers were found to be highly active at minimal inhibitory concentrations (MIC) of 7 – 125 μg/mL. Of specific interest is the fact that the alterporriol-type dimers, alterporriol E and its atropisomer D, exhibited different activity patterns against similar pathogenic microorganisms.

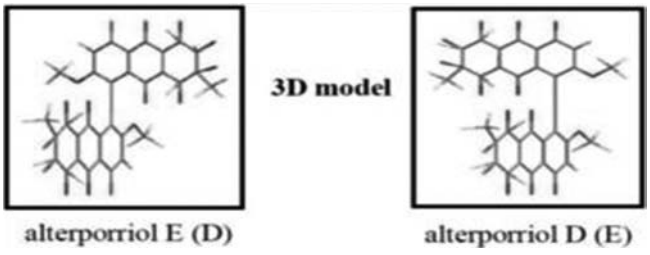

Fig. 1

P402 Antitrypanosomal metabolites from an endophytic *Penicillium* sp. isolated from *Limonium tubiflorum*

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Trypanosomiasis (also known as sleeping sickness), an important parasitic disease caused by *Trypanosoma brucei*, is considered as a neglected disease that reemerged over the last few decades. This fact necessitates the search and development of new drugs to target this disease. In our continuous search for new and biologically active natural products for medicinal applications or as leads for the development of new therapies, fungal endophytes have proven to be a promising vast resource of unique metabolites with great pharmacological potential. In this study we investigated an endophytic *Penicillium* sp. which was isolated from stem tissues of *Limonium tubiflorum* (Rutaceae) growing in Egypt. Chemical investigation of the fungal extract yielded four new metabolites, including a new bianthrone (**1**), two new curvularin derivatives (**2** and **3**), as well as a new sulfinylchromone (**4**), in addition to 13 known metabolites (**5-17**). All structures were assigned by comprehensive spectral analysis (1D and 2D NMR) and mass spectrometry. Some of the isolated consti-

tuents proved active against *T. brucei* with MIC values ranging from 4.9 to 9.7 µM.

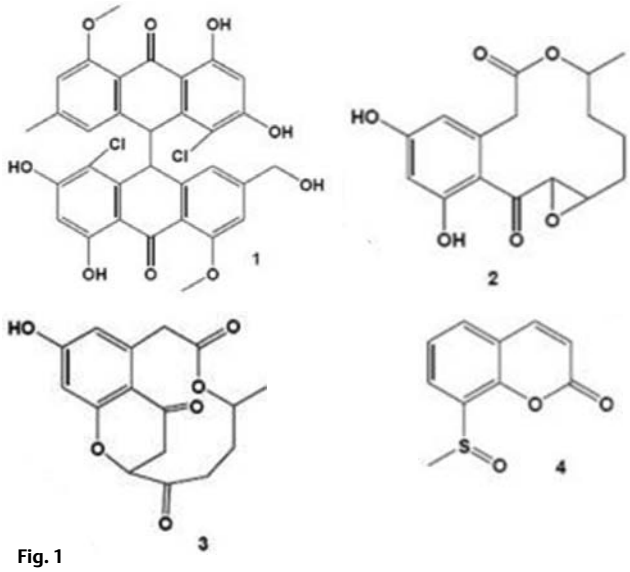

Fig. 1

P403 Bio-inspiration in the discovery of active natural products: an example with the search of antifungal agents inspired from long-lasting woods

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Plant natural products have been perfected through evolution with respect to their specific biological roles (defense, elicitor, and so on) and are, therefore, an excellent starting point in the search for new biologically active chemicals. Hence, despite the progressive abandonment of the exploration of naturally sourced bioactive substances by the pharmaceutical industry, more than half of the drugs approved in the United States between 2005 and 2007 are natural products or natural product-derived drugs, five of which constituted the first members of new drug classes [1]. Clearly, chemical research into natural substances still has an important role to play in improving quality of life, and can play an important role by inventing innovative strategies to discover new bioactive compounds [1,2]. In the present work, we demonstrated that a bio-inspired approach for the identification of novel bioactive natural products represents a promising biotechnological tool for the development of new drugs. We have studied how natural defenses within decay-resistant wood can generate a large number of positive hits in the search for antimycotic agents. In addition, it was found from bioguided fractionation that ethyl acetate extracts of Sextonia rubra wood contain a relatively large proportion of antifungal metabolites rubrenolide (1) and rubrynolide (2), 1 being slightly more active than 2. The therapeutic potential of the above compounds will be discussed through the evaluation of their antifungal activities against 16 pathogenic fungi strains and their cytotoxicities towards KB cells.

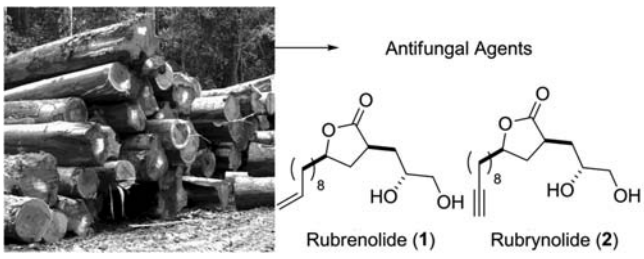

Fig. 1

Acknowledgements: The authors gratefully acknowledge the Programme Amazonie du CNRS (France), CAPES, CNPq and FAPDF (Brazil) for financial support. **References:** 1. Li, J. W.-H. et al. (2009) Science 325: 161 – 165. 2. Dobson, C. M. (2004) Nature 432: 824 – 828.

P404 Chemistry and biology of *Malassezia* metabolites related to skin diseases

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Malassezia yeasts are symbiotic to human skin and can become pathogenic under currently insufficiently understood conditions. During the last years several works have contributed to the description of the metabolome of Malassezia yeasts. Previously [1,2] we compared *Malassezia furfur* isolates from healthy and seborrhoic dermatitis skin for the production of indole derivatives and identified the preferential biosynthesis of malassezin, indolo[3,2-b]carbazol (ICZ) and indirubin by *M. furfur* strains isolated from diseased skin. Ongoing study revealed that tryptanthrin is also a yet undescribed Malassezia metabolite. The simultaneous production of three metabolites (ICZ, indirubin and tryptanthrin) which are among the most active known Aryl hydrocarbon receptor (AhR) inducers is a fascinating finding which boosts the interest about this yeast and strengthens its relation with serious skin diseases. Interestingly, a previously reported by us [3] biomimetic synthesis of indirubin by one step oxidation of indol-3-carboxaldehyde (I3A) using hydrogen peroxide, leads also simultaneously to one step formation of tryptanthrin. This evident reveals the common biosynthetic origin of indirubin and tryptanthrin from I3A which is a common tryptophan metabolite found in all Malassezia studied species. Although, AhR is an orphan receptor, there is increasing data about its relation with skin homeostasis and skin nosology. Ongoing and previous work on the activation of AhR in HaCaT cells by Malassezia extracts [3] point to a significant role of Malassezia synthesized potent AhR ligands in the development of skin diseases. **References:** 1. Gaitanis, G. et al. (2008) J. Invest. Dermatol. 128:1620 – 1625. 2. Giakoumaki, D. et al. (2008) Planta Med. 74: 1081. 3. Magiatis, P. et al (2009) Planta Med. 75: 912.

P405 A crude extract of *Loxostylus alata* is as effective in treating aspergillosis in poultry as a commercial drug

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Leaf extracts of several South African tree species with activity against *Cryptococcus neoformans* were evaluated for activity against *Aspergillus fumigatus* the causal agent of aspergillosis, an economically important disease in birds. The *Loxostylis alata* acetone leaf extract had a good inhibitory activity with an MIC of 50 ug/mL [1]. The main antifungal agent present in the extract, lupeol had a lower activity than the crude extract against *A. fumigatus* (MIC of 92 ug/mL) indicating strong synergistic effects. The toxicity and antifungal activity of the crude *L. alata* acetone leaf extract was evaluated in broiler chicks. Experimental aspergillosis was induced by intraperitoneal infection with *A. fumigatus*. Because the extract had some toxicity at a dose of 300 mg/kg, lower doses were used. Antifungal activity was assessed by comparing degree and severity of clinical signs, lesion scores, fungal re-isolation and a series of biochemical and haematological indices observed between chicks treated with the extract and not-treated chicks. The extract at the doses of 100 and 200 mg/kg significantly reduced the lesions induced by the aspergillosis, as well as the number of fungal colonies isolated from infected chick lungs ($p \leq 0.05$) in a dose dependent manner. The crude extract proved to be as effective as the positive control ketoconazole

dosed at 60 mg/kg, the highest non-toxic dose. The results indicate that a crude extract of *L. alata* leaves has potential as an antifungal agent to protect poultry against avian aspergillosis. **Acknowledgements:** The National Research Foundation of South Africa provided funding. **References:** 1. Suleiman MM. et al. (2010) S. Afr, Jl Botany 76:64 – 71.

P406 Investigation of the tolerability and safety of film-coated tablets containing ivy extracts (Prospan® Cough Tablets) in the treatment of colds accompanied by coughing

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The only saponin drug currently prescribed in any significant amount in monotherapy medicines is ivy. This post-marketing surveillance study (PMSS) aimed at investigating the safety of film-coated tablets containing ivy extract (extracting medium: ethanol 30%, DER 5 – 7.5:1 [Prospan® Cough Tablets]) under practice conditions. The study included adults and children aged 12 and above suffering from colds accompanied by coughing or from chronic, inflammatory bronchial diseases but not taking any other preparations containing dried ivy leaf extracts. They were, however, allowed to take other medicines. The patients were scheduled to undergo treatment for a period of at least seven days, take at least two film-coated tablets twice daily, and have a final medical examination seven days after starting the tablets at the earliest. The tolerability of the tablets was rated by means of questionnaires. A total of 331 patients aged between 11 and 85 years (mean age: 42; median: 43) of both sexes were included in the PMSS. The patients took the tablets for nine to ten days on average. 310 patients were treated for at least seven days. No serious or unexpected adverse drug reactions occurred during the observation period. The results of this recognised PMSS confirm once more the good to very good tolerability of the tablets in its global assessment of Prospan® Cough Tablets by both, the physician (98.48%) and the patient (96.36%) thus emphasizing the high acceptance and compliance (rated as "good" in 98.8% of all cases) of this product.

P407 Antimycobacterial activity of traditional medicinal plants used in Mozambique

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Mycobacterium tuberculosis infects about one-third of the world's population, and causes almost 2 million deaths annually. In 2007, there were 9.27 million new TB cases. Despite more than 40 years of anti-TB chemotherapy, tuberculosis remains one of the leading infectious diseases worldwide. The association with HIV epidemic, the increasing emergence of multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) make TB virtually untreatable with available drugs [1 – 3]. From this point, there is evidently an urgent need to develop new and more effective TB drugs. Natural products, well defined as providing novel examples of anti-infective drug leads [4], play key roles in the modern day chemotherapy of tuberculosis. n Mozambique, where has been reported to possess around 500 plant species using as traditional medicine [5], medicinal plants are an important part for peoples' basic health care, particularly in rural areas. Due to limited scientific evidence concerning the uses of these plants, it is crucial to establish the safety and efficacy for these medicinal plants. This study evaluated seven plants used in Mozambique traditional medicine to treat tuberculosis and other respiratory diseases. The antimycobacterial activity for different species of mycobacteria such as *M. smegmatis*, *M. bovis* BCG, *M. avium*, and *M. tuberculosis* was assessed using a rapid screening method (Broth Dilution MIC Method). Five crude extracts of five plants showed promising activity against one or more mycobacterial species with a MIC ranging from 15 μg/mL to 250 μg/mL. The data support traditional uses of these medicinal plants in Mozambique. **Acknowledgements:** This study was supported by a fellowship from FCT, Portugal (reference number SFRH/BPD/37179/2007). **References:** 1. Corbett E. L. et al. (2003) Arch. Intern. Med. 163:1009 – 1021. 2. Gomez, J. E. et al. (2004) Tuberculosis (Edinb) 84:29 – 44. 3. Smith, C. V. et al. (2003)J. Biol. Chem. 278:1735 – 1743. 4. Newman D.J. et al. (2000) Nat. Prod. Rep. 17:215. 5. Bandeira S. O. et al. Pharm. Biology 2001, 39, 70 – 73.

P408 Antibacterial activity of ellagic acid-rich pomegranate rind extracts

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Pomegranate fruit peel extracts have recently attracted interest because of their potential use as natural food preservatives and nutraceuticals. Antibacterial activities of standardized ellagic acid-rich pomegranate rind extracts were therefore studied *in vitro*. The extracts were prepared and standardized to contain 13% w/w ellagic acid by previously described methods [1]. The antibacterial activity of the ellagic acid-rich pomegranate rind extracts was evaluated against *Staphylococcus aureus*, *S. epidermidis*, *Propionibacterium acnes*, *Salmonella typhimurium*, *S. typhi*, *Shigella sonnei* and *Escherichia coli* using the disc diffusion and broth microdilution methods [2]. The extracts exhibited potent bacteriostatic effect against Gram-positive facultative anaerobic bacteria, *S. aureus* and *S. epidermidis*, with the same MIC values of 7.8 μg/ml, and *P. acnes*, a Gram-positive anaerobe, with an MIC value of 15.6 μg/ml. However, the extract was not active against any of the four Gram negative bacteria studied. The results from this study support the potential use of the standardized ellagic acid-rich pomegranate rind extracts as nutraceuticals for antibacterial proposes, especially against acne involved bacteria. However, the use of the extract as natural preservatives is not recommended due to its narrow spectrum antibacterial activity. **Acknowledgements:** 1. National Research Council of Thailand 2. Prince of Songkla University **References:** 1. Panichayupakaranant, P. et al. (2010) Pharm. Biol. 48: 201 – 205. 2. NCCLS (2008) NCCLS document M100-S9. National Committee for Clinical Laboratory Standard. Wayne.

P409 Taxonomy, occurrence, phytochemical evaluation and biological activity of selected basidiomycetes from Yemen

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The first scientific collection of basidiomycetes in Yemen was carried out in the time from 1880 till 1889. After this date there has been no further scientific collection of basidiomycetes in Yemen. We collected more than 40 species between 1999 and 2007 and determined their taxonomic classification [1,2,3]. In total, 39 taxa of larger fungi (macromycetes: 36 basidiomycetes and 3 ascomycetes), including former records from literature, were enumerated and annotated from the territory of Yemen. 22 taxa have been collected in recent times; from which 16 were new records for Yemen. Dichloromethane, methanol and aqueous extracts of 23 selected basidiomycetes species fruiting bodies were screened in vitro for their antibacterial activities against three Gram-positive bacteria (*Staphyloccocus aureus*, *Bacillus subtilis*, *Micrococcus flavus*) and against two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), as well as for their cytotoxic and antioxidant activity [4,5]. The highest antibacterial activity was shown by extracts from *Agaricus* sp. (type 1), *Coriolopsis caperata*, *Ganoderma colossus*, *Ganoderma resinaceum*, *Phellorinia herculea* and *Tulostoma obesum* [4]. The results confirm the potential of Yemeni mushrooms as source of biologically active compounds. **References:** 1. Al-Fatimi, M. (2001) Isolierung und Charakterisierung antibiotisch wirksamer Verbindungen aus Ganoderma pfeifferi Bres. und aus Podaxis pistillaris (L.: Pers.) Morse. – Dissertation Universität Greifswald. 2. Kreisel H., Al-Fatimi M. (2004) Feddes Repertorium 115: 7 – 8. 3. Kreisel H., Al-Fatimi M. (2008) Feddes Repertorium

119: 5 – 6. 4. Al-Fatimi, M. et al. (2005). Pharmazie 60: 776 – 780. 5. Al-Fatimi, M. et al. (2006) Evidence-based Complementary and Aalternative Medicine 3 (1), 87 – 92.

P410 Lipid composition and biological activity of extracts from *Zygophyllum oxianum*

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Zygophyllum species (*Zygophyllaceae*) are widely distributed in Uzbekistan and used in folk medicine as antiinfectious, antirheumatic and antidiabetic means. Continuing our research on the chemical constituents of this genus [1,2], we have studied the lipid composition of the aerial parts of *Zygophyllum oxianum* Boriss. The yields of the total lipids were: leaves – 0.75%, stems – 0.33% and fruits – 0.49% from fresh weight of organ. Phospholipids (PL) are presented by 9 classes, from them phosphatidylcholine is dominating (leaves – 55.8%, stems – 56.4%, fruits – 63.7% from sum of PL). The study of antimicrobial activity against several bacterial strains and fungi showed that the n-butanol extract from all aerial parts exhibits significant activity against *Candida* sp. but only weak antibacterial activity. In cytotoxicity assays against 5637 human urinary bladder cells the n-butanol extract showed only weak activity. Otherwise the chloroform-methanol (2:1) extract of steams and leaves has a remarkable cytotoxicity against 5637 cells with an IC50 value of 6.2 μg/ml, but only weak antibacterial and antifungal activity. The chloroform-methanol extract (2:1) of the fruits was less toxic (IC50 value: 37.6 μg/ml). **References:** 1. Sasmakov SA et al. (2003) Pharmazie, Vol.58 (8): 602 – 603. 2. Sasmakov SA et al. (2007) Pharmazie, Vol.62 (12): 957 – 959.

P411 Antibacterial from *Hypericum acmosepalum* showing inhibition of ATP dependent MurE ligase from *Mycobacterium tuberculosis*

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In a project to characterise new antibacterial chemotypes from plants, hyperenone A and hypercalin B were isolated from the hexane and chloroform extracts of the aerial parts of *H. acmosepalum*. The structure of compounds were characterised by extensive 1- and 2D NMR spectroscopy and confirmed by MS spectrometry. Both hyperenone A and hypercalin B exhibited anti-bacterial activity against multidrug-resistant strains of Staphylococcus aureus, with MIC values ranging from 2 – 128 μg/mL to 0.5 – 128 μg/mL, respectively. Hyperenone A also showed growth inhibition against *Mycobacterium tuberculosis* H37Rv and M. bovis BCG at 50 μg/mL (H37Rv) and 75 μg/mL (BCG). The hyperenone A did not inhibit growth of *Escherichia coli* and was not toxic to cultured mammalian macrophages cells at the concentration at which anti-mycobacterial activity was determined. Both the compounds were also tested for their ability to inhibit MurE and MurF enzymes of *M. tuberculosis* which are crucial enzymes in the cytoplasmic steps of peptidoglycan biosynthesis. Hyperenone A inhibited MurE selectively whereas hypercalin B did not have any effect on both the enzymes. This is first report of a natural product from plant source showing inhibition of ATP dependent MurE ligase from *M. tuberculosis*. These studies represent promising starting points for further development. These highly active antibacterial compounds encourage further studies on the *Hypericum* genus. **References:** 1. Decosterd, L. et al. (1989) Photo S Helvetica Acta 70 (8): 1833 – 1845. 2. Kikuche, T. et al. (1985) Chem. Pharm. Bull. 33(2): 557 – 564.

P412 Phytochemical and antibacterial studies on *Ocimum kilimandscharicum*

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The development of resistance by bacteria to currently available antibiotics is a major problem in the treatment of infectious diseases. To exemplify this, there has been an increase in the number of deaths by MRSA world wide. As part of on-going efforts to characterize new antibacterials with activity against multidrug-resistant (MDR) strains of Staphylococcus aureus, an extract of the aerial parts of *Ocimum kilimandscharicum* (Lamiaceae), have been investigated. This species is commonly known as camphor Tulsi. Ocimum has been used in the Ayurvedic System of Medicine for bronchitis, bronchial asthma, skin diseases, arthritis, as an astringent, in inflammation and for fever. The plant material was extracted with chloroform, methanol and acetone successively by cold maceration. Further sub-fractionation by SPE and purification by P-TLC led to the isolation of six compounds eugenol, quercitin, cadinol, thymol, β-sitosterol and stigmasterol. The structures of the compounds were characterized by extensive 1 and 2D NMR spectroscopy. The isolated constituents were evaluated for their antibacterial activity by the broth microtitre MIC assay against a panel of multidrug-resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains (ATCC-25923, SA-1199B, XU-212, RN-4220, EMRSA-15 and EMRSA-16) and minimum inhibitory concentrations (MICs) of eugenol and cadinol were found to be in the range of 2 – 128 μg/ml. This study corroborates the traditional claims of *Ocimum kilimandscharicum* as a topical antibacterial. **Acknowledgements:** Kamlesh Shinde thanks the Association of Commonwealth Universities for a Split Site Ph.D scholarship. **References:** 1. Prakash, P., Gupta, N. (2005) J. Physiol. Pharmacol. 49: 125 – 131. 2. Smith, ECJ. et al. (2008) Phytochem. Lett. 1:49 – 53.

P413 Screening of South African medicinal plants and HPLC based profiling for the identification of leads with antiprotozoal activities

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A library of 300 extracts from one hundred and seven plant species, used in South African folk medicine to treat malaria were collected and screened in vitro against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Leishmania donovani*. Of these 102 (34.0%) exhibited more than 50% growth inhibition of one of the parasites at the screen concentration of 9.7 μg/mL and were thus considered active. *P. falciparum* against which seventy-two plant extracts (24.0%) showed activity was the most susceptible parasite, followed by *L. donovani* (forty-nine, 16.3%) and *T. b. rhodesiense* (thirty-six, 12.0%), with *T. cruzi* (zero) being the least susceptible. Twenty plants (6.6%) were selected for further investigation based on activity and specificity criteria as well as on chemotaxonomic considerations. Just 350 μg of the selected extracts were fractionated by analytical scale HPLC into 32 one-minute fractions in a fully automated 96 well microfractionation scheme [1]. After drying the microfractions were subsequently tested against the parasite for which the extract had shown activity, to identify the peaks responsible for the overall activity of the extracts. HPLC hyphenated methods (MS, UV, ELSD, HRMS and offline LC-NMR) helped to identify many active substances online. Active compounds were isolated and tested against the parasites as well as human cancer cell lines to determine their cytotoxicity. Screening results as well as selected examples of activity profiling and active compounds will be shown. **References:** 1. Adams et al. (2009) Nat Prod Comm. 10:1377 – 81.

P414 Alkaloids and phytosterols from *Cakile maritima* present in UAE

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Cakile maritima is a halophyte exhibiting potential for secondary metabolite production. Plants grown in the United Arab Emirates (UAE) were investigated for the first time for its phytochemical constituents and antibacterial activity. Dried leaves and stems collected from the gardens in Ras Al-Khiama, UAE were (soxhlet) extracted using equal amounts of ethanol and water for three hours. Alkaloids were detected by thin layer chromatography with dichloromethane:ethanol:ammonia as the mobile phase and Dragendorff's spraying reagent. Isolation and purification of alkaloids and phytosterols was performed by column chromatography using dichloromethane and ethanol as the mobile phase in gradient elution technique. The detection of alkaloids and phytosterols was confirmed by gas chromatography-mass spectrometry (GC-MS) analysis and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The bacteriological assays for different plant extracts (aqueous, ethanolic and dichloromethane) were conducted to evaluate the antibacterial effect against gram negative and gram positive bacteria. **Acknowledgements:** Dean College of Pharmaceutical Sciences Ras Al Khaimah, Ras Al Khaimah Medical &Health Sciences University, Ras Al Khaimah, UAE. Microbiology department, Ras Al Khaimah Medical &Health Sciences University, Ras Al Khaimah, UAE. Professor Tim flower University Of Sussex School of Life Science Department Biology, John Maynard Smith Building, UK

P415 Antifungal activity against *Candida albicans* and effect on mitochondrial NADH oxidation of galangin

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Galangin is a flavonoid found in the rhizomes of *Alpinia officinarum* [1]. In Asia, this plant has long been used as traditional medicine for gastrointestinal ailments and infectious diseases. Galangin has been shown to possess several biological and pharmacological properties, including antimicrobial activity [2]. *Candida albicans* resides in a diversity of organ surfaces of a healthy host, such as oral cavity, digestive tract, skin and vagina. As an opportunistic pathogen, it can cause life-threatening systemic infection in immunocompromised hosts [3], especially in HIV-infected patients. Nowadays, the number of antifungals against candidiasis is quite limited. In the present study, we found that galangin exhibited antifungal activity against *Candida albicans*. The MIC value was 25 μg/ml (92.5 μM). Galangin has been reported to strongly interact with rat liver mitochondrial membranes by acting as an uncoupling agent [4]. In mitochondria isolated from *Candida albicans*, galangin was found to inhibit NADH oxidation and CCCP-stimulated respiration, suggesting that galangin interfered with the electron transport chain in *Candida albicans* mitochondria. This mitochondrial effect may contribute to the antifungal activity of galangin against *Candida albicans*. **References:** 1. Mutsuda, H. et al. (2006) Bioorg. Med. Chem. 95:138 – 142. 2. Cushnie, TPT. et al. (2003) Microbiol. Res. 158:281 – 289. 3. Calderone, RA., Fonzi, WA. (2001) Trends Microbiol. 9:327 – 335. 4. Dorta, DJ. et al. (2005) Chem. Biol. Interact. 152: 67 – 78.

P416 Anti-plaque activity of *Piper betle* leaf extracts

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Leaves of Piper betle L. (Piperaceae) have been shown to possess antimicrobial, gastroprotective, wound healing, hepatoprotective and antioxidant properties [1]. The antimicrobial activity of betel leaves relevant in oral care lead to the investigation of its anti-plaque activity. Five varieties of Piper betle leaves (Banarasi, Calcutta, Desi, Maghai and Puna) were subjected to successive extractions at room temperature with various solvents (hexane, diethyl ether, ethyl acetate, ethanol, hydro-ethanol and water). Two varieties (Banarasi and Calcutta) were selected on the basis of their in vitro activities. Of the extracts, hexane extract was found to be active. Banarasi and Calcutta leaves were also subjected to super critical extraction and steam distillation. TLC was carried on hexane extracts of all varieties, super critical extracts and steam distillation of Banarasi and Calcutta. A solvent system was developed and plates were derivatized with Vanillin sulphuric acid. Since eugenol is present in all varieties this compound was used as marker compound. Anti-plaque activity was evaluated using a microbial assay (Agar Streak Plate method). Leaf extracts were tested for antimicrobial activity against Streptococcus mutans, Streptococcus sobrinus, and Actinomyces viscosus (early colonizers of dental plaque) [2]. Antimicrobial activity of Piper betle leaf extracts are summarized in the table below.

Table 1: Antimicrobial activity of Piper betle – Direct extracts

		Observed MIC (μg/ml)		
Extract	**Diluent**	**S. mutans**	**S. sanguis**	**A. viscosus**
Hexane (Banarasi)	Ethyl acetate	50	75	50
Hexane (Banarasi)	Methanol + Tween-20	> 125	> 125	> 125
Hexane (Calcutta)	Ethyl acetate	100	500	500
Hexane (Maghai)	Ethyl Acetate	50	300	> 600
Hexane (Pune)	Ethyl acetate	200	> 500	> 500
Hexane (Desi)	Ethyl Acetate	200	> 500	> 500
Di ethyl ether (Calcutta)	Ethyl acetate	100	400	> 500
Di ethyl ether (Pune)	Ethyl acetate	250	500	1000
Ethanol (Maghai)	DMSO	> 1000	> 1000	> 1000

Antimicrobial activity was observed for the hexane extract of Piper betle leaves (Banarasi) and could be attributed to the presence of allypyrocatechol [3] obtained in the chromatographic data of these extracts. **References:** 1. Arambewala L.S.R et al. (2005), Journal of Ethnopharmacology, 102:239 – 245. 2. Svensäter G et al. Caries Research, 2003; 37,395. 3. Ramji N. et al. (2002) Journal of Ethnopharmacology, 83: 149 – 152.

P417 Antiprotozoal and cytotoxic potential of British and Irish red algae

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Marine algae are a prolific source of diverse natural products, but their biomedical potential has barely been explored. As part of our continuing research on seaweeds [1], we have screened crude extracts of 23 marine red algae (Rhodophyta) collected from Britain and Ireland. Algal material was extracted with $CHCl_3$: MeOH mixtures (3:1 and 1:1) at room temperature and evaporated to dryness in vacuo before use in bioassays. The clinically important blood-stage life forms of *Trypanosoma brucei rhodesiense, T. cruzi* and *Leishmania donovani* were used as test organisms in the in vitro assays. The selective toxicity of the extracts was determined towards mammalian skeletal myoblast (L6) cells. All algal extracts showed activity against *T. brucei rhodesiense*, with *Corallina officinalis* (Corallinaceae) and *Ceramium virgatum* (Ceramiaceae) being the most potent (IC_{50} values 4.8 and 5.4 μg/ml, respectively), whilst none of the algal extracts inhibited the growth of *T. cruzi.* With the exception of a *Porphyra leucosticta* (Bangiaceae) extract, all seaweed extracts also displayed leishmanicidal activity with IC_{50} values ranging from 16.5 to 85.6 μg/ml. *Corallina officinalis* was the only seaweed that showed some marginal cytotoxicity (IC_{50} value 88.6 μg/ml), whereas all remaining extracts were non-toxic towards L6 cells at 90 μg/ml concentration. To our

knowledge, this is the first study reporting antiprotozoal activity of British and Irish red algae. **References:** 1. Orhan, I. et al. (2006) Phytomedicine 13:388 – 393.

P418 Phytochemical study of plants used in traditional medicine in the treatment of malaria in the Comoros islands

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An ethnobotanical survey was carried out in the Comoros Islands about plant species used traditionally for the treatment of various diseases including malaria. The in vitro antiplasmodial activity of 76 vegetal extracts obtained from 17 species traditionaly used to treat malaria symptomes, was evaluated using *Plasmodium falciparum* choloroquine-resistant strain (W2). The results showed that 10 plant extracts had a moderate activity ($5 < IC50 \leq 10\,\mu g/ml$), and 6 showed particularly interesting in vitro activity with IC50 value $\leq 5\,\mu g/ml$ [1]. The phytochemical study realized on three selected plants, *Flueggea virosa* (Roxb. Ex Willd.) *Voigt* subsp. *Virosa* (Euphorbiaceae), *Piper capense* L.f. (Piperaceae), and *Flacourtia indica* (Burm. F.) Merr. (Flacourtiaceae) allowed us to isolate compounds from the most active extracts. The structures of isolated compounds were elucidated by spectrometric methods and their antiplasmodial activity was evaluated in vitro against the chloroquine-resistant strain W2 of *Plasmodium falciparum*, according to Azas, 2002 [2]. From F. virosa, rutine, gallic acid, methyl gallate, bergenin, (−)-norsecurinine, securinol A, N-methyltetrahydro-β-carboline and N-methyltryptamine were isolated. The highest antiplasmodial activity was found for methyl gallate (IC50 = 14.1 μM). Together with apigenine dimethylether and piperchabamide A, a new amide alkaloid, Kaousine and the Z form of antiepilepsirine were isolated from the aerial part of *P. capense*. Lower activity was observed for kaousine and apigenine dimethylether, whereas antiepilepsirine demonstrated the same activity that the chloromethylenic extract of P. capense (IC50 = 7 μg/ml) [3]. Pyrocatechol, homaloside D and poliothrysoside were isolated from the decoction of the aerial parts of F. indica. The poliothrysoside presented a strong antiplasmodial activity (IC50 = 7.4 μM) and a good selectivity index (> 28) similar to chloroquine. **References:** 1. Mohamed Kaou, A. et al. (2008) J. Ethnopharmacol 116: 74 – 83. 2. Azas, N et al. (2002) Parasitol Res. Ss: 165 – 171. 3. Mohamed Kaou, A. et al. (2010) Fitoterapia (In press).

P419 Marine algal extracts inhibit growth of the human pathogens *P. falciparum*, *T. brucei* and *L. donovani*

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Marine macrophytes have been highlighted as resources containing a variety of biologically active compounds and particularly showing *in vitro* antiprotozoal activities[1,2]. As a part of a screening program to search new natural antiprotozoal products, we screened 20 seaweed species from the Normandy's coast belonging to Rhodophyta, Pheophyta and Chlorophyta against protozoa responsible for 4 major endemic parasitic diseases *in vitro*. Thus, brown, red and green seaweeds extracts were screened *in vitro* against *P. falciparum*, *T. brucei*, *T. cruzi* and *L. donovani*, respectively responsible of malaria, African trypanosomiasis, Chaga's disease and visceral leishmaniasis. No or weak activity was shown against *T. cruzi* and *L. donovani*. In contrast, more than half of the species tested showed good to strong activity against *P. falciparum* and *T. brucei* ($0.5 < IC_{50} < 5\,\mu g/ml$). Interestingly, the active species are brown or red algae, all sharing phyllogenetic origins with *P. falciparum*. Green algae were inactive. Bio-guided fractionation of the most active extracts ($IC_{50} < 5\,\mu g/ml$) is under process. **Acknowledgements:** Agence Universitaire de la Francophonie, S. Sritharan. **References:** 1. Lin et al. 2010 J. Nat. Prod 73:275 – 8. 2. Afolayan et al. 2009 Phytochemistry 70:597 – 600.

P420 Antiplasmodial evaluation and pharmacomodulation of lanaroflavone, a biflavonoid isolated from *Campnosperma panamense* Standl. (Anacardiaceae)

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Activity-guided fractionation of the leaves from *Campnosperma panamense* Standl. (Anacardiaceae) was undertaken as a part of a screening program to search new antiprotozoal drugs[1]. The biflavonoid lanaroflavone was isolated and showed high in vitro antiplasmodial activity against *Plasmodium falciparum* erythrocytic stages and mild activity against *Leishmania donovani* amastigotes, but was inactive against Chaga's disease vector, *Trypanosoma cruzi*[2,3]. A possible machanism underlying antiplasmodial activity implicating inhibition of beta-hematin formation by this compound was supported by in vitro tests. The screening of eight natural biflavonoids structurally related to lanaroflavone allowed us to identify the nature and the length of the interflavonoid linkage as of high relevance regarding antiplasmodial activity. Thus, a serie of new flavone derivatives was synthetized and tested against *P. falciparum* resistant strains (K1, 7G8). They were also tested on their cytotoxicity effects upon mouse hepatocyte and L6 cells. One new aminated flavone derivative showed significant activity in the nanomolar range and very satisfying selectivity indexes (IC_{50}= 80nM, SI> 100), as well as absence of hemolytic effect indicating selective antiplasmodial activity. The importance of the hydrophylic framework attached at the C8 of the flavone is presently explored. The most promising derivatives will be evaluated in vivo in *P. berghei* infected mice. **References:** 1. Weniger et al. 2001 J. Ethnopharmacol. 78:193 – 200. 2. Weniger et al 2004 Fitoterapia 75:764 – 7. 3. Weniger et al 2006 Phytomedicine 13:176 – 180.

P421 Characterization of immunostimulatory activities of fractions obtained from *Taraxacum officinale*

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Plants of the genus *Taraxacum officinale* have a history of use in Korean, Chinese, Arabian and Native American traditional medicine to treat a variety of infectious diseases and cancers [1]. In Traditional Medicine, it is also known as choleretic, diuretic, anti-rheumatic and anti-inflammatory properties [2]. In the current study, in vitro immunostimulatroy effects of *Taraxacum officinale* have been investigated. The crude methanolic extract of the whole plant was sequentially fractionated into three subextracts; explicitly, ethyl acetate (EtOAc), n-butanol, and remaining water extracts. Further studies were carried out on the most active subextract, i.e. the EtOAc subextract, was further subjected to fractionation through successive column chromatographic applications on Silica gel 60. For the activity assessment, each extract or fraction was submitted to bioassay systems; in vitro neutrophil migration, spleen lymphocyte proliferation, nitric oxide (NO) production and phagocytosis for immunostimulatory activity assessment. Among the fractions, the ethyl acetate fraction was identified to be most effective in the migration activity of neutrophils, spleen cell proliferation, phagocytosis and NO production, significantly greater than media control. Sequential chromatographic separation of the ethyl acetate fraction on Silica gel column resulted in a more purified preparation that retained the immunostimulatory activity. From the EtOAc subextract, a major component was isolated and its structure was determined as taraxinic acid β-D-glucoside by means of spectral techniques. This study provides a new scientific data on *Taraxacum officinale* extracts or individual components present in the extracts may be of value as novel immunostimulatory agents.

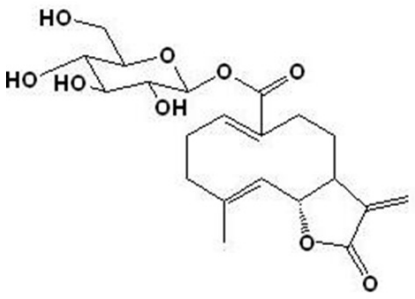

Fig. 1

References: 1. Koo, H.N et al. (2004) Life Sci 74, 1149 – 1157. 2. Choi, U. Ket al. (2010) Int J Mol Sci 11, 67 – 78.

P422 *In vitro* effect of an aqueous extract of *Artocarpus lakoocha* on the intestinal parasites in cattle

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The crude extract of *Artocarpus lakoocha* has been used as traditional medicine for treating tapeworm infections [1 – 2]. Reportedly, the anthelmintic effect of an aqueous extract of *A. lakoocha* inhibited the motility of *Fasciola gigantica* [3]. However, the effects of a crude extract of *A. lakoocha* on other trematodes and nematodes in cattle have not been studied. Therefore, this work aimed to investigate the anthelmintic effect of an aqueous extract of *A. lakoocha* on trematodes (*Fasciola gigantica, Paramphistomum cervi, Eurytrema pancreaticum, Gigantocotyle explanatum, Cotylophoron cotylophorum, Fishoederius cobboldi)* and nematodes (*Seteria labiato papillosa* and *Haemonchus placei*). The activity was evaluated after incubating parasite in M-199 medium containing 250 and 500 μg/ml of crude extract, for 3, 6, 12 and 24 h, using the relative motility assay and scanning electron microscope (SEM). It was found that the aqueous extract reduced the motility of *S. labiato papillosa*, and revealed complete immobilization after 3 h of incubation with the crude extract at the concentrations 250 and 500 μg/ml, respectively. The motility of trematodes was progressively decreased upon 3 to 12 h exposure, except for *C. cotylophorum, P. cervi* and *G. explanatum,* which remained in active movement. SEM observation showed the numerous blebs, erosion and desquamation of tegument of trematodes while *H. placei* showed the furrowed appearance. These results suggest that *S. labiato papillosa* is susceptible, whereas *G. explanatum, C. cotylophorum* and *P. cervi* are resistant to the aqueous extract of *A. lakoocha.* The crude extract of *A. lakoocha* could be used as motility inhibitor of intestinal trematodes and nematodes in the cattle. **Acknowledgements:** This work was supported by the Thailand Research Fund (Senior Research Scholar Fellowship to Prof. Prasert Sobhon), Postgraduate Education and Research Program in Chemistry (PERCH), Mahidol University and Suranaree University of Technology **References:** 1. Charoenlarp P, Radomyos P, Harinasuta T. (1981) Southeast Asian J Trop Med Public Health. 12: 568 – 570. 2. Charoenlarp P, Radomyos P, Bunnag D. (1989) J Med Assoc Thai. 2: 71 – 73. 3. Saowakon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V, Sobhon P. (2009) Exp Parasitol. 122(4):289 – 98.

P423 Antiplasmodial activity of amides and amines from *Withania aristata*, an endemic species of the Canary islands

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Malaria continues to be a major health challenge in most tropical and subtropical regions. The most severe form of human malaria is caused by *Plasmodium falciparum*, responsible for more than one million deaths per year. Due to the emergence and spread of drug resistance to the available antiplasmodial drugs, its treatment has become a serious problem. Therefore, new molecules with novel mechanisms of action are required [1]. In this context, natural products can deliver new lead compounds for more effective drugs than those currently in clinical use [2]. As part of our research for bioactive metabolites from natural sources, *Withania aristata* (Aiton) Pauquy (*Solanaceae*), an endemic species of the Canary Islands used in folk medicine, was studied. The phytochemical analysis of the dichloromethane extract from the leaves of this plant led to the isolation of 3 amide and 6 amine type metabolites, two of them, 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-oxetanamine and *N*-4-(3-furoylamine)-1-butanol, not previously reported. Their structures were determined by means of spectroscopic studies, including 1D and 2D NMR experiments. The antiplasmodial activity of the compounds was evaluated against a strain of *Plasmodium falciparum* (F-32 Tanzania). *N-cis*-feruloyltyramine and 4-*O*-methyldopamine showed moderate activity (IC_{50} 4.2 and 3.0 μg/mL, respectively), whereas the activity of the most potent compound, *N-cis-p*-coumaroyltyramine, was comparable to that of the control chloroquine (IC_{50} 0.7 and 0.1 μg/mL, respectively). A preliminary structure-activity relationship study indicated that the configuration of the double bond in the amides and the regiosubstitution of the aromatic ring in the amines play an important role in the activity. **Acknowledgements:** We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) project for financial support. LGG thanks the Gobierno Autónomo de Canarias and CO the CajaCanarias for the fellowships. **References:** 1. Timothy, N. C. et al. (2009), Nature Rev. Drug Discov. 8, 879 – 891. 2. Cruz SJ (2007) Más de 100 Plantas Medicinales. Medicina Popular Canaria Monografías. Imprenta Pérez Galdós. Las Palmas de Gran Canaria.

P424 Chemical composition of essential oil of *Pinus peuce* (Pinaceae) from Macedonian flora and its antimicrobial activity

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Pinus peuce (Pinaceae) or Macedonian pine is a conifer which grows in the southern and western parts of the Macedonian territory, mainly on the Pelister and Nidze mountains, as well as the Shara Mountain where it can be found in small populations. The essential oil from the needles was obtained by hydrodestillation with a Klevenger apparatus. The yields obtained were from 0.2 to 1.2%. The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry. The most abundant components were alpha-pinene (9.84 – 30.27%), germacrene D (5.73 – 22.06), bornyl acetate (2.92 – 13.93%), beta-pinene (3.45 – 13.13%), D-limonene (1.74 – 10.04%), trans-caryophyllene (2.40 – 8.12%), camphene (1.32 – 7.42%), alpha-terpenyl acetate (0.57 – 5.05%), delta-cadinene (1.20 – 4.29) and alpha-cadinol (0.54 – 2.04%). The essential oil was screened for antimicrobial activities against seven type strains of Gram positive and Gram negative bacteria and one fungal type strain using agar diffusion assays. The essential oil had significant antimicrobial activity, especially against Gram positive bacteria such as *Staphylococcus aureus* ATCC 29213 and the yeast *Candida albicans* ATCC 10231. The obtained results indicate that the essential oil from the needles of the Macedonian pine can be used as an antimicrobial and disinfectant.

P425 Antiparasitic activity of flavonoids from *Piper* species

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Parasitic diseases, including leishmaniasis, chagas and malaria, are serious problems for public health throughout the world, especially in tropical and subtropical regions [1]. Due to the absence of effective vaccines, inadequate chemotherapy and the emergence of drug resistance, currently, there is an urgent need to search for novel, effective and safe drugs for the treatment of these diseases [2]. Several *Piper* species have been used in the traditional medicine in Latin America, including for the treatment of parasitic diseases [3]. As part of our research aiming to uncover antiparasitic compounds from Bolivian *Piper* species, we studied the dichloromethane extract from the leaves of *Piper aduncum*, *P. acutifolium*, *P. glabratum*, *P. heterophyllum*, *P. pilliraneum* and *P. rusbyi*. This study has led to the isolation of eight known flavonoids and a new compound, 5,5′-dihydroxy-7,3′-dimethoxy-flavanone. Their structures were elucidated on the basis of spectroscopic data, including homo- and heteronuclear correlation NMR experiments (COSY, ROESY, HSQC and HMBC). In the search for new antiparasitic agents, these compounds were evaluated *in vitro* against three strains of *Leishmania* (*L. amazonensis*, *L. braziliensis* and *L. donovani*), *Trypanosoma cruzi* and *Plasmodium falciparum*. 4′,7-Dimethoxy-5-hydroxy-flavanone (IC_{50} 4.0 μg/mL) showed a moderate activity against *P. falciparum*. The most active compound against promastigote forms of the three *Leishmania* strains was flavokavain B (IC_{50} 5.4 μg/mL), with twice the activity of the control pentamidine (IC_{50} 10.0 μg/mL). These results support the use of *Piper* species as a traditional remedy in the treatment of parasitic diseases. **Acknowledgements:** We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) project for financial support. TJC is grateful to MAEC-AECID for a fellowship. **References:** 1. Kerboeuf, D. et al. (2008) Mini Rev. Med. Chem. 8:116 – 128. 2. Loset, J. R. (2008) Curr. Org. Chem. 12:643 – 666. 3. Townson, S. (2001) Adv. Parasitol. 50:199 – 295.

P426 Anti-adhesive activity of extracts from edible and medicinal mushrooms against *Campylobacter jejuni*

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Campylobacter jejuni is one of the most common bacterial causes of diarrhoea in the industrialised world [1], being associated with the occurrence of Guillain-Barré Syndrome (GBS) [2] and induces diseases partially through intestinal adherence [3]. With increasing reports of *C. jejuni* drug resistance against standard antibiotics [4] investigations into anti-adhesive agents for the prevention of bacterial infection [5] are highly significant, particularly given the possibility of avoiding resistance [6]. Different studies have shown anti-adhesive activity of different plant compounds against a variety of bacteria including *C. jejuni* [5]. Although two studies report on anti-adhesive effects of fungal compounds/extracts against yeast [7] and virus [8], this is the first study that describes anti-adhesive effects of basidiomycetes against any bacteria. Nineteen extracts from 9 edible and medicinal mushroom species (*Calvatia gigantea, Flammulina velutipes, Inonotus obliquus, Inonotus hispidus, Inonotus nodulosus, Inonotus dryadeus, Ganoderma lucidum, Ganoderma pfeifferi, Piptoporus betulinus*) were screened for anti-adhesive activity against *C. jejuni* using modifications of previously published anti-adhesion assays [9, 10]. The 2 ethanolic extracts derived from the fruiting bodies of *Inonotus nodulosus* and *Inonotus obliquus* showed high anti-adhesive activity (IC 50 0.017 mg/mL and IC 50 0.021 mg/mL, respectively). Moreover, the methanolic extracts of the fruiting bodies of *Calvatia gigantea* and *Piptoporus betulinus* showed, although not dose dependent, high anti-adhesive activity (81 %± 13 % and 71 %± 10 %, respectively) at 0.002 mg/mL. Further investigations need to be conducted to evaluate whether isolated compounds would be candidates for the development of novel drugs against bacterial adhesion. **References:** 1. Young KT et al. Nat Rev Microbiol, 2007. 5(9): p. 665 – 79. 2. Mishu, B. et al. Clin Infect Dis, 1993. 17(1): p. 104 – 8. 3. Park, S.F., Int J Food Microbiol, 2002. 74(3): p. 177 – 88. 4. Allos, B.M., Clin Infect Dis, 2001. 32(8): p. 1201 – 6. 5. Wittschier, N., et al., J Pharm Pharmacol, 2007. 59(6): p. 777 – 86. 6. Brown, E.D. et al., Chem Rev, 2005. 105(2): p. 759 – 74. 7. Falkensammer, B., et al., Mycoses, 2008. 51(6): p. 505 – 514. 8. Eo, S.K., et al., J. Ethnopharmacol., 2000. 72(3): p. 475 – 481. 9. Beil, W. et al. Phytomedicine, 2007. 14 Suppl 6: p. 5 – 8. 10. O'Mahony, R., et al., World J Gastroenterol, 2005. 11(47): p. 7499 – 507.

P427 Antibacterial metabolites from Australian macrofungi from the genus *Cortinarius*

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Mushrooms have demonstrated significant pharmacological activity including antimicrobial, cytotoxic, anti-inflammatory, hypoglycaemic, immunomodulatory and hallucinogenic properties [1]. The fungi of Australia are diverse, largely endemic and, in contrast to their floral counterparts, have not undergone intensive taxonomic, chemical or pharmacological evaluation [2]. Furthermore, some Australian indigenous macrofungi are currently considered to be conspecific with Northern Hemisphere species, might be described as separate species once taxonomic revisions are carried out [3]. Consequently Australian mushrooms represent an under-explored resource of potentially novel metabolites. In this study, ethyl acetate and aqueous fractions from 117 collections of Australian macrofungi belonging to the genus *Cortinarius* were screened for antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Overall, the lipophilic fractions were more active than the aqueous fractions. The ethyl acetate fractions of most or all collections of 13 described *Cortinarius* species and 47 collections of un-described *Cortinarius* species exhibited IC 50 values of ≤ 0.09 mg/mL against *S. aureus*. In contrast, most or all collections of only 4 described *Cortinarius* species and only 11 un-described *Cortinarius* collections exhibited similar effects against *P. aeruginosa* (IC 50 ≤ 0.09 mg/mL). The fungal octaketides austrocortilutein, austrocortirubin, torosachrysone, isolated from *C. basirubescens*, together with physcion and emodin were found to strongly inhibit the growth of *S. aureus* (IC 50 0.7 – 12 μg/mL) whereas only physcion and emodin exhibited potency against *P. aeruginosa* (IC 50 1.5 and 2.0 μg/mL, respectively) [4]. Australian mushrooms from the genus *Cortinarius* are promising sources of natural products for further drug development research, due to the high biological diversity and unique evolutionary lineages found only in the region. This is coupled with the large proportion of bioactive species and high diversity of chemical constituents. **References:** 1. Lindequist, U., et al. Evid Based Complement Alternat Med, 2005. 2(3): p. 285 – 99. 2. Lepp, H., 2007. The study of Australian fungi. http://ww.anbg.gov.au/fungi/history-today.html (accessed Jun 18, 2009). 3. May, T.W. et al. Australian Systematic Botany. Vol. 14. 2001. 329 – 356. 4. Beattie, KD et al. Phytochemistry, accepted 18.03.2010.

P428 Antitubercular resorcinol analogs and benzenoid C-glucoside from the roots of *Ardisia cornudentata*

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Ardisia cornudentata Mez (Myrsinaceae) is a small shrub, endemic to Taiwan, and distributed at low altitudes in the North and South of the island [1]. The whole plant of this species has been used as folk medicine to eliminate blood stasis, disperse swelling, improve blood circulation, and also as an analgesic [2]. The methanolic extract of the root of this species showed antitubercular activity against *Mycobacterium tu-*

berculosis H37R_V *in vitro*. The extract was partitioned into *n*-hexane, EtOAc, and H_2O-soluble layers. The EtOAc-soluble layer showed potent antitubercular activity against *M. tuberculosis* H37R_V *in vitro*. Bioassay-guided fractionation of the active EtOAc-soluble layer of the roots of *A. cornudentata* led to the isolation of three new compounds, 3-methoxy-2-methyl-5-pentylphenol (**1**), 3-methoxy-2-methyl-5-(1′-ketopentyl)-phenol (**2**), and cornudoside (**3**), together with twenty-six known compounds. Their structures were elucidated by analysis of spectroscopic data. Thirteen of these isolates, **1**, **2**, **4-6**, **9-15** and **21** showed antitubercular activities against *M. tuberculosis* H37R_V *in vitro*, with MIC values of 2.5 – 60 μg/mL.

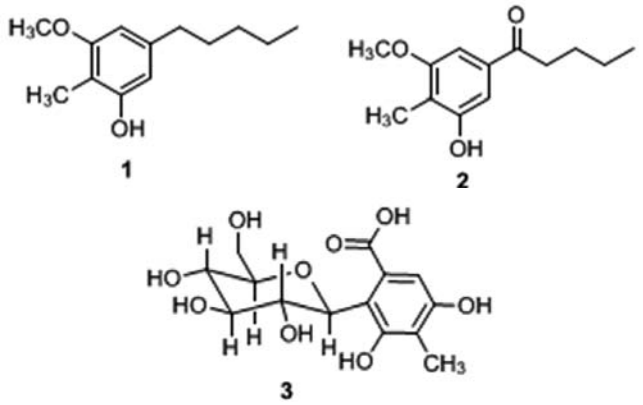

Fig. 1: Structures of compounds **1**-**3**.

References: 1. Lu SY, Yang YP (1998) Flora of Taiwan, Editorial Committee of the Flora of Taiwan, Taipei. 2. Committee on Chinese Medicine and Pharmacy (2003) The Catalogue of Medicinal Plant Resources in Taiwan, Committee on Chinese Medicine and Pharmacy, Department of Health Executive Yuan, Taipei.

P429 New bioactive altersolanol derivatives from the endophytic fungus *Stemphylium globuliferum* isolated from *Mentha pulegium*

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Extracts of the fungus *Stemphylium globuliferum*, an endophyte of the Moroccan medicinal plant *Mentha pulegium*, exhibited considerable cytotoxic and antimicrobial activities when tested *in vitro*. Chemical investigation of the extracts yielded four new secondary metabolites, including altersolanol K (**1**), altersolanol L (**2**), altersolanol M (**3**), and altersolanol N (**4**), together with the known compounds 6-O-methylalaternin (**5**), macrosporin (**6**), altersolanol A (**7**), tetrahydroaltersolanol B (**8**), altersolanol C (**9**), altersolanol J (**10**), stemphypyrone (**11**) and indole-3-carboxaldehyde (**12**). The structures of all compounds were determined on the basis of one- and two-dimensional NMR spectroscopy and mass spectrometry. Among the altersolanol derivatives tested, compound (**3**) was the most active congener against L5178Y cell line with an EC_{50} value of 1.14 μM. Moreover, all anthraquinones exhibited antibiotic activity against several pathogenic microbes. Interestingly, the new altersolanol N together with altersolanol C showed considerable antiviral activity against HRV39, whereas tetrahydroaltersolanol B was very active against HRV2, HRV8 and HRV16.

P430 Antimicrobial acylphloroglucinol derivatives from *Hypericum linarioides* (Hypericaceae)

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Hypericum linarioides Bosse (taxonomic section *Taeniocarpium*) has one center of distribution in western and southern Transcaucasia, adjacent parts of Russia and northern Iran and Turkey, and a second center in southern Serbia, northern Greece and western Bulgaria. Naphthodianthrones, simple anthrones, flavonoids, flavonol glycosides, caffeic acid derivatives and the xanthone-C-glycoside mangiferin have been previously reported from this species [1, 2]. TLC and HPLC screening of an extract of this species collected in western Bulgaria revealed the presence of a rich diversity of acylphloroglucinol derivatives. Correspondingly, bioassay-guided fractionation of the dichloromethane extract of dried, ground aerial parts of *H. linarioides* was performed, testing extracts against a range of bacterial and fungal pathogens. Upon observation of promising activity against *Candida glabrata* (IC_{50}= 16.91 – 21.86 μg/mL), *Cryptococcus neoformans* (IC_{50}= 2.48 – 8.43 μg/mL), *Staphylococcus aureus* (IC_{50}= 2.98 – 24.90 μg/mL) and MRSA (IC_{50}= 2.72 – 18.99 μg/mL), extracts were further fractionated, resulting in the isolation of several structurally related acylphloroglucinol derivatives. Extracts were analyzed by chromatographic means (TLC, OC, HPLC) and structure elucidation performed using data from NMR and MS. This work resulted in the isolation of 3-geranyl-1-(2′-methylbutanoyl)phloroglucinol (**1**) and 3-geranyl-1-(2′-methylpropanoyl)phloroglucinol (**2**) as main constituents, as well as several minor derivatives. **1** has been previously reported from *H. empetrifolium* (sect. *Coridium*) [3] and **2** has been previously reported from *H. empetrifolium, H. jovis* (sect. *Coridium*) [4] and *H. styphelioides* (sect. *Brathys*) [5]. Implications of this chemical convergence are discussed.

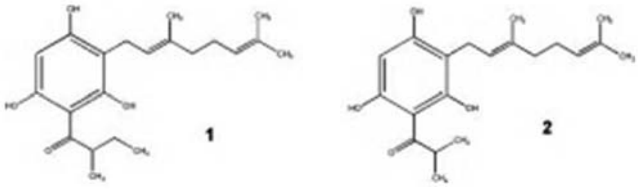

Fig. 1: Major phloroglucinol derivatives of *Hypericum linarioides*

Acknowledgements: This research was supported in part by a grant from the Austrian Science Foundation (FWF, Project T345). **References:** 1. Šmelcerovic *et al.* (2006) *Phytochemistry* 67: 171 – 77. 2. Ayan and (Ccedil))irak (2008) *Pharmaceutical Biology* **46**: 288 – 91. 3. Crockett *et al.* (2008) *Phytochemical Letters* **1**: 37 – 43. 4. Athanas *et al.* (2004) *Journal of Natural Products* **67**: 973 – 77. 5. Gamiotea-Turro *et al.* (2004) *Journal of Natural Products* **67**: 869 – 71.

P431 Research and development of new dosage form of isoniazid with low adverse reaction

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Isoniazid (INH), a widely used drug in the prophylaxis and treatment of tuberculosis, has been found to be associated with 1 to 2% risk of severe and potentially fatal hepatotoxicity. Studies suggest that cytochrome P450 2E1 (CYP2E1) could play an important role in the pathogenesis of INH-induced hepatotoxicity. Recently, many studies presented flavonoids or ingredients of herbal medicines may regulate liver P450 isozymes. HUCHE010 is a flavonoid compound found in many vegetables, fruits, seeds or roots of plants, and herbal medicines. Purpose of this study is using CYP2E1 inhibitor, HUCHE010 to prove the concept that CYP2E1 inhibitor can reduce or even eradicate INH combine Rifampicin (RIF), CYP2E1 stimulator, induced hepatotoxicity. Three groups of *129/sv* mice were studies with 3 weeks dosing period: Hepatotoxic group was given INH/RIF 50/100 mg/kg/day, Hepato-protective group was given INH/RIF combined with HUCHE010, and Vehicle group was given vehicle only. To assess the hepatotoxicity, we employed galactose single point

(GSP), a quantitative measurement of liver function, histopathologic features of the liver tissues, and general liver function assement of AST and ALT. The INH/RIF-induced hepatotoxicity were significantly associated in AST (from 90 ± 15 to 571 ± 295 U/L, $p < 0.001$), ALT (40 ± 5 to 364 ± 192 U/L, $p < 0.001$), and GSP (from 184 ± 24 to 866 ± 339 mg/L, $p < 0.001$). Hepato-protective pretreatment of HUCHE010 could reduced INH/RIF-induced hepatotoxicity (significantly reduced GSP to 401 ± 178 mg/L). These results show that INH/RIF-induced hepatotoxicity can be reduced by CYP2E1 inhibitor, HUCHE010.

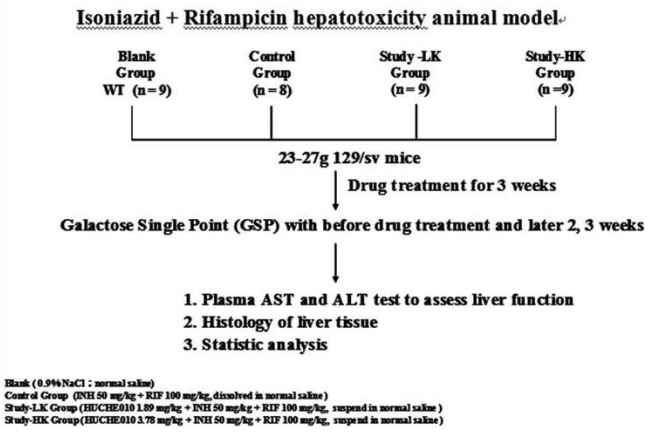

Fig. 1: Experimental Procedure

P432 Antifungal screening of six *Ficus* species native to Zambia

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The genus *Ficus* (Moraceae) is well known for antioxidant, anticancer, antidiarrhoeal, antimicrobial, antiplasmodial, antiulcer and gastroprotective activities [1]. In Zambia, the milky latex of some *Ficus* species is traditionally used against ringworms, while other plant parts are used to treat wounds, chest infections, stomach problems and fevers [2,3]. The folk medicinal knowledge in Zambia is poorly documented and the potential of the medicinal plants has remained unexplored. In this study, we collected the milky latex, as well as the leaves and stem barks of six *Ficus* species (*F. sycomorus*, *F. sansibarica, F. ovata, F. wakefieldii, F. lutea* and *F. natalensis*) that are native to the Zambezi valley of Zambia. The *in vitro* antifungal activity of the latex (used directly) and the crude MeOH extracts of the leaves and stem barks were assayed against clinical cultures of two fungi causing ringworm infections (*Trichophyton tonsurans* and *T. interdigitale*), as well as a yeast (*Candida albicans*) and a mould (*Aspergillus fumigatus*). The agar plate disc and well diffusion techniques were employed by using miconazole and MeOH as positive and negative controls, respectively. Except for the milky latex of *F. sansibarica*, all other latices and the crude extracts were inactive at 100 µg/ml concentration. Previous studies have however, reported significant activity for the crude MeOH extract of *F. ovata* against a clinical isolate of *C. albicans* [1], and the activity was attributed to the presence of terpenoids, isoflavonoids and phenolic acids. This is the first antifungal screening study evaluating the antifungal activity of native Zambian *Ficus* species against various fungi. **Acknowledgements:** The Commonwealth Scholarship Commission and the Rick-Cannell Travel Fund of the School of Pharmacy are acknowledged for funding. **References:** 1. Kuete, V. *et al.* (2009). J Ethopharmacol. 124:556 – 561. 2. Fowler, D.G. (2007). Zambian Plants: Their vernacular names and uses. Royal Botanical Gardens. Kew, UK. 3. Burrows J., Burrows S. (2003). Figs of Southern and South-Central Africa. Umdaus Publishers. Hartfield, South Africa.

P433 Effects of demethyl fruticulin A and fruticulin A from *Salvia corrugata* Vahl. (Lamiaceae) on biofilm production in vitro by multiresistant strains of *Staphylococcus aureus* and *S. epidermidis*

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In this study we assessed whether demethyl fruticulin A (dfA) and fruticulin A (fA) two quinones that represent the mayor diterpenoid components of the exudate produced by the aerial parts of *Salvia corrugata* Vahl., were able to inhibit the synthesis of biofilm produced in vitro by multiresistant *S. aureus* and *S. epidermidis*. Five clinical strains of *S. aureus* -3 methicillin resistant (MRSA) and 2 methicillin susceptible (MSSA) – and five clinical strains of *S. epidermidis* (4 MRSE and 1 MSSE) were used. FA decreased by at least twofold the hydrophobic properties of *S. aureus* cell membrane, evaluated by standard methods [1]. Biofilm formation on polystyrene plates was quantified spectrophotometrically by established methodologies [2] and was also confirmed by the Congo red plate assay [3]. DfA and fA were more effective against *S. aureus* strains (> 70% effect at sub-MIC concentrations) than against *S. epidermidis* in inhibiting slime synthesis. Moreover, the two compounds were shown to posses chelating activity on divalent and trivalent metal cations by the batochromic shifts of their UV spectra in presence of Al3+ and Mg2+ ions [4, 5]. Our results indicate that fA and dfA interaction with bacteria could be very complex, being possibly species-specific, and could depend not only on EPS synthesis inhibition but also on their chelating activity and on changes in the microorganism surface, including cell hydrophobicity. **References:** 1. Rosenberg, M. Bacterial adherence to hydrocarbons: A useful technique for studying cell surface hydrophobicity. FEMS Microbiol. Lett.1984, 22, 289 – 295). 2. Cramton SE, Gerke C, Götz F. In vitro methods to study staphylococcal biofilm formation. Methods Enzymol. 2001;336:239 – 55. 3. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989 Aug;42(8):872 – 4. 4. Mabry T.J., Markham K.R., Thomas M.B. 1970. The Systematic identification of Flavonoids. Springer, Berlin, 35 – 39. 5. European Pharmacopoeia. Fourth Edition. Council of Europe, Strasbourg. 1227.

P434 *In vitro* antimycobacterial activity of synthetic 1-methyl-2-alkenyl-4(1*H*)-quinolones against *Mycobacterium smegmatis*

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Since long quinolones are widely used in the treatment of many types of infectious diseases caused by bacteria, including tuberculosis. Previously we have disclosed the antimycobacterial properties of natural 1-methyl-2-alkyl-4-(1 H)-quinolone alkaloids isolated from Evodia rutaecarpa (Hook f. and Thomson. (Rutaceae) [1]. In our attempt to optimize the antimycobacterial potential of these new class of alkaloids, we have synthesized a wide range of derivatives having aliphatic side chain at position-2 with varying chain length and double bond position.

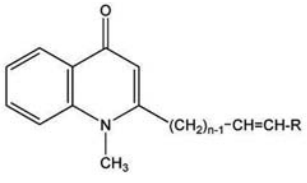

Fig. 1

Tested against *M. smegmatis* ATCC 14468 using the microbroth dilution assay [2], the synthetic derivatives containing C10-C17 aliphatic side

chain showed more potency (MIC's values 0.5 – 16 μg/mL) compared to the natural products and even the standard drugs ethambutol and isoniazid. The structure-activity relationship study revealed that the size of the aliphatic side chain is essential for the observed antimycobacterial effects. **Acknowledgements:** This work is financially supported by Austrian Science Fund (FWF) Project No. P21152-B18. **References:** 1. Adams, M. et al (2004) Planta Med. 70; 904 – 908. 2. Wube, AA. Et al. (2005) Phytochemistry 66; 2309 – 2315.

P435 Isolation of curcacycline A from latex of *Jatropha curcas* and its antibiotic and cytotoxic effect

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Euphorbiaceae are known as a rich source of cyclic peptides from higher plants. One of cyclic peptides was isolated from the latex of *Jatropha curcas*, namely curcacycline A (C 37H66N8O9) [1]. In the past different techniques have been applied to determine the structure of curcacycline A. In a new approach the structure of the isolated curcacycline A confirmed by more sophisticated bioanalytical techniques (HPLC-MS, 2D-NMR, COSY, APT, HSQC, IR). For the first time the structure was confirmed by a full synthetic approach and first biological assays have been carried out to shed light on the pharmacological activity of curcacycline A as representative of a rather unknown group of cylicpeptides. The analytical data indicate that curcacycline A consists of 8 amino acids, while ESI-MS data showed complete sequencing of amino acid c(Gly-Leu-Leu-Leu-Gly-Thr-Val-Leu-Leu-Gly). Screening of antibiotic activity of curcacycline A were done against several bacteria (*Bacillus subtilis*, *Staphyloccus aureus*, *Eschericia coli*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans*) using the agar diffussion method. Curcacycline A inhibits the growth of *B. subtillis* and *P. aeruginosa*. Cytotoxic effect was tested to two cell lines, human ovarian cancer cell-line OVCAR-3 (ATCC®) and human colon cancer cell-line (Colo205). Curcacycline A decreased the level of OVCAR 3 at a concentration of 1 mg/mL, while no effect was found on Colo205 (ATCC®). Curcacycline A had no mutagenic activity in the non-activated and activated AMES test. **References:** 1. van den Berg, A. J., S. F. Horsten, et al. (1995). FEBS Lett 358(3): 215 – 8.

P436 Secondary metabolites from the root of *Ehretia longiflora* and their antitubercular activity

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Ehretia longiflora Champ. ex Benth. (Boraginaceae) is a medium sized deciduous tree, distributed in south China, Indochina, and in forests from low elevations to 750 m of Taiwan [1]. The methanolic extract of the root of this plant showed antitubercular activity against *Mycobacterium tuberculosis* H37Rv *in vitro*. The aim of this study is the isolation of chemical constituents and antitubercular activity. Bioassay-guided fractionation of the ethyl acetate-soluble layer from the root of this species led to the isolation of four new compounds, including one quinonoid: ehretiquinone (1); one triterpenoid: ehretiolide (2); one alkaloid: ehretilonine (3); one acetamide: ehretilonamide (4), together with nineteen known compounds, of which three compounds, comprising of 1-(4-methoxyphenyl)-4-phenylbutane-2,3-diol (5), ehretiquinonone (6), (2S,3S)-1,4-bis(4-methoxyphenyl)butan2,3-diol (7), were first isolated from nature. The structures of these isolates were elucidated by spectral analysis. Among these isolates, 1, 5 and 8 exhibited antitubercular activity against *M. tuberculosis* H37Rv *in vitro*, showing MIC values of 25.0, 30.0 and 50.0 μg/mL, respectively. The clinically use antitubercular agent, ethambutol, was used as a positive control. Antitubercular activity assay of the isolates are still in progress.

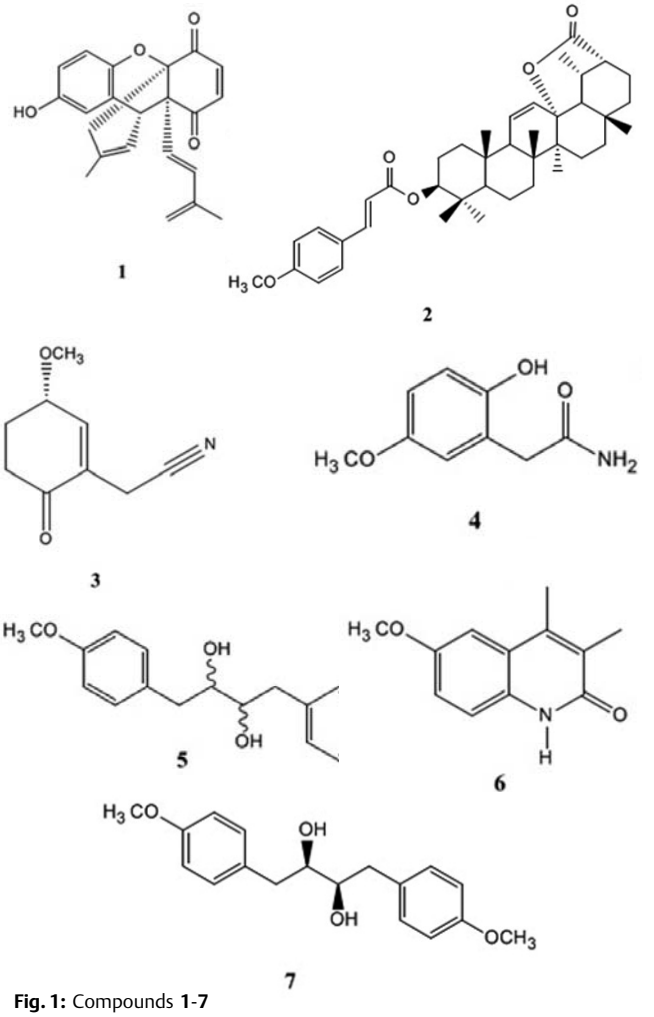

Fig. 1: Compounds **1-7**

References: 1. Hsiao JY, Liu HY (1998) Flora of Taiwan, Editorial Committee of the Flora of Taiwan, Taipei.

P437 Kaempferol rhamnosides from *Bryophyllum pinnatum*, a medicinal plant used against infectious diseases and as analgesic in Mbouda, Cameroon

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An ethnobotanical survey was made on plants used in the treatment of infectious diseases in Mbouda subdivision, Cameroon. According to our survey, one of the most important medicinal plants in that area is *Bryophyllum pinnatum* (Lank.) Oken (Crassulaceae), a succulent plant native to Africa. *B. pinnatum* (leaves or whole plant) was found to be well known and was used against blennorrhoea, syphilis, jaundice, candidiasis and for the treatment of others ailments such as dysmenorrhoea, external ulcers, burns, convulsions and as analgesic. In order to identify the biologically active compounds of the plant and to confirm or infirm its ethnopharmacological claims, phytochemical study of ethyl acetate extract of the whole plant was carried out. As a result of this kaempferol rhamnosides, kaempferol 3,7-O-bis-α-L-rhamnopyranoside (kaempferitrin) [1], kaempferol 3-O-α-L-(3-acetyl)rhamnoside-7-O-α-L-rhamnopyranoside [2], kaempferol 3-O-α-L-rhamnoside (afzelin) [1] and kaempferol 7-O-α-L-rhamnoside (α-rhamnoisorobin) [1] were isolated and identified by extensive NMR and MS studies. All compounds were described for the first time in this species. These isolates are reported in other plant species to possess antioxidant, antinociceptive and anti-inflammatory activities [3, 4], therefore the kaempferol rhamnosides of B.

pinnatum may account for the medicinal use of the plant against pain and inflammatory disorders. **Acknowledgements:** AUF (Agence Universitaire de la Francophonie) **References:** 1. Nakano, A. et al. (1983) Phytochemistry 22:2831 – 2833. 2. Pérez-Castorena, A-L. et al. (1997) Phytochemistry 46:297 – 1299. 3. De Melo, G.O. et al. (2009)J Ethnopharmacol 124:228 – 232. 4. Fang, S.H. et al. (2005) Bioorg Med Chem 13:2381 – 2388.

P438 Anti-leishmania activity of isochromenes from an unidentified endophytic fungus isolated from *Spermacoce verticillata* L.

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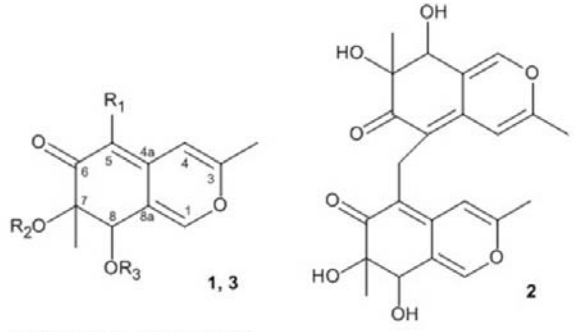
Endophytes could be defined as microorganisms (fungi or bacteria) that can be detected at a given moment within the tissues of an apparently healthy host plant. The metabolic interactions of an endophyte with its host may also favour the biosynthesis of different bioactive natural products that includes polyketides, shikimate derivatives, terpenes, as well as steroids, alkaloids, and peptides. In fact, a huge number of bioactive compounds has been isolated from endophytes [1,2]. The aim of the present work was to isolate and identify bioactive secondary metabolites from an endophytic fungus strain isolated from the medicinal plant *Spermacoce verticillata* L. (Rubiaceae), native to South America. The fungus was grown in solid medium for 30 days at 30 ôC. The ethanol crude extract of the culture was submitted to different chromatographic methods to afford austidiol (**1**) and a novel natural product, austidiol dimer (**2**). The structures of the compounds were elucidated by NMR spectroscopy (^{1}H, ^{13}C, HMQC, HMBC and COSY) and HRMS data. Also austidiol diacetate (**3**) was obtained by austidiol acetylation [Fig.1]. These compounds were tested for anti-leishmania activity *in vitro* against *Leishmania major* to determine the lethal dose (LD_{50}). Compounds **1-3** displayed promising LD_{50} results: 0.52, 0.13 and 0.28 µM, respectively, in comparison with the positive control geneticin (G418) (LD_{50} 3.43 µM). Therefore, it is suggested that the isochromenes isolated could be used as lead compounds for the development of new anti-leishmania agents.



1. Austidiol: R_1= CHO, R_2,R_3= H.
2. Austidiol dimer.
3. Austidiol diacetate: R_1= CHO, R_2,R_3= -$COCH_3$.

Fig. 1

Acknowledgements: Capes, CNPq and FAPESP, INCT-INBEQUIMeDI. **References:** 1. Gunatilaka, A.A.L. (2006). J. Nat. Prod. 69, 509 – 526. 2. Borges, W.S., Borges, K.B., Bonato, P.S., Said, S., Pupo, M.T. (2009) Curr. Org. Chem. 13, 1137 – 1163.

P439 Benzophenone synthase from *Hypericum calycinum* cell cultures: cDNA cloning and functional expression

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Hypericum is a medicinally important genus (Clusiaceae). *H. perforatum* (St. John's wort) is the best-known member of the genus and widely used as an antidepressant agent. Cell suspension cultures of the related species, *H. calycinum*, form 1,3,6,7-tetrahydroxy-8-prenylxanthone upon elicitation with yeast extract. Xanthones thus appear to serve as phytoalexins in *Hypericum* species, as reported previously. In addition, they exhibit antitumour, anti-HIV, and antimicrobial activities [1].

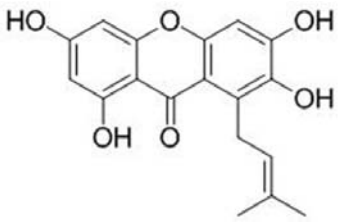

Fig. 1: 1,3,6,7-tetrahydroxy-8-prenylxanthone

The carbon skeleton of xanthones is formed by benzophenone synthase (BPS), which catalyses the condensation of benzoyl-CoA and three molecules of malonyl-CoA followed by intramolecular cyclization. Time-course changes in BPS activity and xanthone formation were studied. Maximum product formation and enzyme activity were found at 12 and 9 h, respectively, after addition of the elicitor. The BPS cDNA was cloned using primers derived from *H. androsaemum* cDNA [2] and the open-reading frame was functionally expressed in E. coli as 6xHis-tagged protein. The enzymatic product after incubation with benzoyl-CoA and malonyl-CoA was identified as 2,4,6-trihydroxybenzophenone. Characterization of the recombinant enzyme is underway. **References:** 1. Beerhues L, Liu B (2009) Phytochemistry 70: 1719 – 1727. 2. Liu B et al. (2003) Plant J. 34: 847 – 855.

P440 Research of *Nonea rossica* bioactive compounds and estimation of an antibacterial activity of extracts made from it

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Nonea rossica Steven. (Boraginaceae) is used in folk medicine as an anti-inflammatory and an antibacterial remedy. The aim of this work was to investigate bioactive compounds of aerial parts of *N. rossica* gathered in the flowering stage and also to estimate the antibacterial activity of its total extract. The qualitative composition and quantitative content of the following chemical constituents was determined by TLC, spectrophotometry and chromatography-mass spectrometry with standard samples: tannins – 9,7%, anthocyanidins – 1,1%, water-soluble polysaccharides and pectin – 7,1%, steroid saponins – 0,12%. The *in vitro* antimicrobial activity of the extract was evaluated. The extract from aerial parts of the plant was prepared using 40% ethanol as the extractive solvent (ratio of raw material: extractant – 1: 50). The antibacterial properties of these extracts were investigated against *Staphylococcus aureus*. First the plant extracts were added to the nutrient medium, followed by bacterial inoculation (*S. aureus* strain MR, ATCC 35591). It could be established that the initial extract showed antibacterial activity (MIC) against this strain until a dilution of 1:10 with water. Most probably, steroid saponins are responsible for the established antibacterial activity of the extract.

P441 Leishmanicide activity of oleanolic acid against promastigotes of *Leishmania braziliensis* and *Leishmania chagasi*

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Leishmaniasis is a major worldwide health problem, with around 12 million people infected and 600 thousand new cases appearing each year. In Brazil, 30 thousand new cases appear annually only in the Northeast region. Pentavalent antimonals are the first line treatment for leishmaniasis. Disadvantages such as costs, long-term treatment, side effects and low efficacy against many strains have been reported. Although great efforts had done along the last century to develop new drugs for leishmaniasis treatment, a drug with high efficacy and low side effects is still need. Among all strategies used to develop new agents against leishmaniasis, the research of natural products produced good results. This work investigated the leishmanicidal activity of oleanolic acid (OA), a natural triterpene found in a great variety of plants against promastigotes forms of *L. braziliensis* and *L. chagasi*. Promastigotes were incubated in the presence of different concentrations of OA, which was added only once to the cultures. After 3 days at 26 °C, parasite survival was estimated by counting viable or motile forms. OA exhibited a good anti-promastigote activity both for *L. braziliensis* and *L. chagasi*, reducing parasite survival in 75 and 94%, respectively, when we used 50 μg/ml of the drug. DMSO 1% was used as solvent control. These results suggest that *L. braziliensis* is less susceptive than *L. chagasi*. No toxicity to the macrophages was observed after the treatment with OA, as measured by their spreading and adherence to glass surface. These results reinforce that OA could be a strong candidate for an antileishmanial drug. **Acknowledgements:** Hebron Farmacêutica, MackPesquisa, PIBIC/Mackenzie, CNPq, FAPERJ

P442 Screening of *Thamnolia vermicularis* var. *subuliformis* for antimicrobial, antioxidant and cytotoxic activities

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Thamnolia vermicularis (Icmadophilaceae) has commonly been used as a tea with the local name of "snow tea" in some part of China, where this species of lichens is also used as food [1] and for the treatment of psychic disorders, high blood pressure and inflammatory conditions of the respiratory tract [2]. In this study, *T. vermicularis* var. *subuliformis* is collected in Serbia and chloroform, ethyl acetate and methanol extracts of this lichen were studied for the antioxidant, antimicrobial and cytotoxic activities. The antimicrobial activity of the extracts were tested against twelve human and plant pathogenic bacteria using a disc-diffusion method, and the minimum inhibitory concentration (MIC) values of each active extract were determined. The inhibition zones and minimal inhibitory concentration (MIC) values of bacterial strains were in the range of 10 – 42 mm and 50 – 300 μg/ml, respectively. Furthermore, the extracts scavenged 1,1-diphenyl-2-picrylhydrazyl radical (DPPH.), resulting in IC50 > 50 μg/ml. All the extracts showed moderate antioxidant activity comparable with BHT. Viability HeLa cells treated with the ethyl acetate and chloroform extracts of *T. vermicularis* var. *subuliformis* decreased with increasing concentrations of these extracts. IC50 value after 24 hours for the ethyl acetate extract was 162.50 ± 5.80 μg/ml, while for the chloroform extract this value was 159.32 ± 5.16 μg/ml. All the tested concentrations of the ethyl acetate and chloroform extracts after 72 hours of treatment of HeLa cells showed a cytotoxic effect. The methanolic extract containing the lowest content of baeomycesic and squamatic acid showed the lowest activity. **Acknowledgements:** Ministry of Science and Environment of the Republic of Serbia (Grant No. 142025). **References:** 1. Hanssen HP, Schädler M (1985). Pflanzen in der tradi tionellen chinesischen Medizin. Dtsch Apoth Ztg. 125:1239 – 1243. 2. Wang LS, et al. (2001) Bryologist 104:345 – 349.

P443 Anti-MRSA and anti-VRE of some traditional Chinese medicinal (TCM) plants

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Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE) are the most important resistant pathogens which cause nosocomial infections [1 – 2]. Antimicrobial activity of methanol and dichloromethane extracts of 84 Traditional Chinese Medicine (TCM) plants was investigated against 10 strains of MRSA and 5 strains of VRE (references strains and clinical isolates). *Eucalyptus globulus* (Myrtaceae) and *Glycyrrhiza glabra* (Fabaceae) exerted a substantial activity against all strains of MRSA and VRE. All MRSA strains were inhibited by dichloromethane extracts of *G. glabra* (MIC range 16 – 32 μg/ml) and *E. globulus* (MIC range 8 – 16 μg/ml). Anti-VRE activity was also observed for dichloromethane extracts of *G. glabra* and *E. globulus* with MIC values range 32 – 250 and 8 – 16 μg/ml, respectively. In addition, methanol extracts of *E. globulus* also exhibited a strong inhibition against MRSA and VRE with MIC values range 16 – 32 μg/ml. The dichloromethane extracts were generally more active than methanol extracts against the bacteria tested. In this study, we have demonstrated that some TCM plants exerted a promising activity as anti-MRSA and anti-VRE agents. **References:** 1. Jones RN. (2001) Chest 119:397S-404S. 2. Ma XX, et al. (2002) Antimicrob Agents Chemother 46:1147 – 1152.

P444 Antifungal activity in *Juglans nigra* green husks

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Juglans spp. (Juglandaceae) are known to be used in folk medicine to treat a wide range of health disorders. In this work, the activity against yeasts and dermatophyte strains was assessed from fresh green husks of *J. nigra*. The material was extracted with 70% aqueous ethanol using an Ultra-Turrax homogeniser. Subsequently, volatile (VF) and no volatile (NVF) fractions were obtained by extract distillation under vacuum and evaluated for their antifungal activity. Six yeasts (five *Candida* spp. and *Cryptococcus neoformans*) and seven dermatophytes (four *Trichophyton* spp., two *Microsporum* spp. and *Epidermophyton floccosum*) were assayed by broth macrodilution methods [1] to determine minimum inhibitory concentrations (MIC), and for a reference antifungal compound (fluconazole). Minimum lethal concentration (MLC) was also evaluated. Phenolic profiles of the fractions were established by HPLC-PDA-ESI/tandem MS. MICs between 48 – 768 μg/mL and 192 – 768 μg/mL were obtained against yeasts and dermatophytes, respectively, for NVF. The VF, the most active fraction, showed MICs between 23.8 – 95.3 μg/mL and 23.8 – 47.6 μg/mL for yeasts and dermatophytes, respectively. An important activity was verified from VF, the MLC values being lower than those of fluconazole for eight fungi: *Candida albicans*, *C. tropicalis*, *C. krusei*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis* and *M. gypseum*. Chromatographic profile of Juglans nigra VF showed that naphthoquinones are the main phenolic compounds. These results suggest that this fraction can constitute an alternative for the treatment of *Candida krusei*, a recurrent strain of vulvovaginal candidosis and dermatophytes of nails and skin. **Acknowledgements:** FCT and POCTI/FEDER for financial support and LEM/UC integrated in RNEM of Portugal for the HPLC/MS analyses. **References:** 1. Clinical and Laboratory Standards Institute (CLSI) (2008). Wayne, Pa, USA.

P445 Bioassay-guided isolation of antiplasmodial strychnos alkaloids from the stem bark of *Strychnos icaja* Baill.

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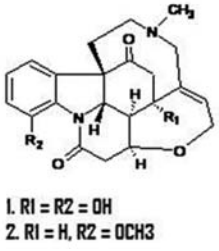
Strychnos icaja Baill. is a 20 – 100 m long liana distributed in all central Africa. The roots have been reported to be used by pygmies tribes in Cameroon to cure malaria [1]. Several alkaloids have been previously isolated from the roots, some of which, especially dimers have shown good in vitro antiplasmodial activities [2 – 6]. In the search of new antiplasmodial compounds from *Strychnos* species, the *in vitro* bioassay guided fractionation of the total alkaloid fraction of the stem bark using the chloroquine-sensitive 3D7 strain of Plasmodium falciparum was carried out and resulted in the isolation of two new tertiary alkaloids monomers, 15-hydroxyvomicine **1**, 12-methoxyicajine **2** and a new dimer, Nb-oxyde sungucine **3** along with the known monomers, icajine **4**, vomicine **5**, 15-hydroxy-19,20a-epoxynovacine **6**, 12-methoxy-19,20α-epoxyicajine 7, strychnine **8** and dimers sungucine **9**, isosungucine **10**, strychnogucine C **11** and bisnorhydroxytoxiferine **12**. The compounds were isolated by a combination of silica gel chromatography columns, sephadex LH-20 and preparative TLC and their structures determined based on the analysis of their spectral data (UV, NMR and MS). The most active fractions were those containing dimers with IC_{50} ranging from 0.32 to 4.31 µg/ml. The antiplasmodial activities of the known dimers were comparable to those previously reported against other strains of *P. falciparum* [3 – 6]. Compound **3** (IC_{50} 3.4 µg/ml) was about 3 times more active than sungucine **9** whereas the monomers **1** and **2** were inactive. Besides the new derivatives **1-3**, compounds **4-7** and **9-12** are isolated for the first time from the stem bark.



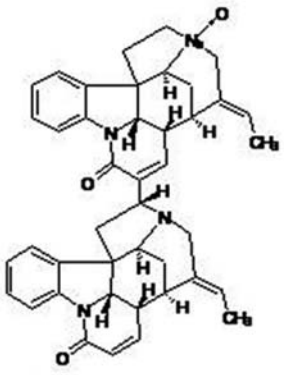


Fig. 1

Acknowledgements: This research was supported by the 'Subside federal de la recherche' of the University of Liège, Belgium through a postdoctoral fellowship to A.T.T. and by the Belgian National Fund for Scientific Research (FNRS – grant N. 3.4533.10). M.F. is a research associate from the FNRS. The authors wish to thank Mr. J.-C. Van Heugen, ATC, Belgium for ESI-MS measurements. **References:** 1. Neuwinger, H.D. (1996). African Ethnobotany: Poisons and Drugs, Chemistry, Pharmacology, Toxicology. Chapman & Hall, London. 2. Delaude, C. and Delaude, L. (1997) Bull. Soc. Roy. Liege. 66: 183 – 286. 3. Frédérich, M. et al. (2000) Planta med. 66: 262 – 269. 4. Philippe, G. et al. (2003) Phytochemistry 62: 623 – 629. 5. Frederich, M. et al. (2002). J. Nat. Prod. 65: 1381 – 1386. 6. Frédérich et al. (1999). Antimicrob. Ag. Chemother. 43: 2328 – 2331.

P446 New steroidal saponins from *Agave angustifolia*

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Extraction of the dried leaves of Agave angustifolia with aqueous methanol gave a crude material that was purified by Diaion HP-20 and silica gel column chromatography to yield 1 and 2. The structure of 1 was proposed by positive ion FABMS, 1D and 2D NMR techniques and acid hydrolysis as a steroidal saponin with new pentasaccharide chain. The structure of the aglycone bearing 27 carbons was determined 25R 5 α spirostan-12 -one 3 β -ol 3 -O [β – D- xylopyranosyl (1 –> 4) β -D- galactopyranosyl (1 –> 3) β -D- xylopyranosyl (1 –> 3) β -D- glucopyranosyl (1 –> 2) β -D- glucopyranoside]Acid hydrolysis as well as1 H and 13C NMR data of 1 revealed the presence of five monosaccharide units, two β -D- glucopyranose, one β -D- galactopyranose and two β -D-xylopyranose assigned by 1 H- 1 H COSY, HMQC and HMBC spectra. Compound 2 showed similar NMR signals except the absence of those due to the terminal β -D- xylopyranose attached to the outer β-D- glucopyranose, it was characterized as Cpd 2: 25R 5 α spirostan-12 -one 3 β -ol 3 -O [β -D- xylofuranosyl (1 –> 4) β -D- galactopyranosyl (1 –> 3) β -D- glucopyranosyl (1 –> 2) β -D- glucopyranoside] Further two known saponins were isolated from Agave angustifolia. The methanol extract and the saponin fraction of this plant proved to exhibit marked anti-inflammatory, analgesic, schistosomicidal, cercaricidal & miracidicidal activities.

P447 Secondary metabolites from the root of *Helicia rengetiensis*

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Helicia rengetiensis Masamune (Proteaceae) is an endemic evergreen trees, growing in thickets at lower elevations in the central and southern parts of Taiwan. Flavonol glycosides, phenolic glycosides, benzenoid glycosides, and their derivatives distributed in plant of genus *Helicia*. In our studies on the antitubercular constituents of Formosan plants, 1200 species have been screened for *in vitro* antitubercular activity, and the *H. rengetiensis* has been found to be an active species. However, the chemical constituents and biological activities of this plant have never been studied. Bioassay-guided fractionation of active EtOAc-soluble fraction of the root of this species has led to the isolation of two new compounds, including helicinol A (**1**) and helicinol B (**2**), together with six known compounds, including one cyclophane, kermadecin H (**3**), one fatty acid, stearic acid (**4**), one triterpene, squalene (**5**), one chroman, α-tocopherol (**6**), and a mixture of β-sitosterol (**7**) and stigmasterol (**8**). The structures of these new compounds were determined through spectroscopic analyses including 2D-NMR data. The structural elucidation of these new compounds and the antitubercular activities of the isolates will be discussed in this symposium. The successive isolation and antitubercular assay are in progress.

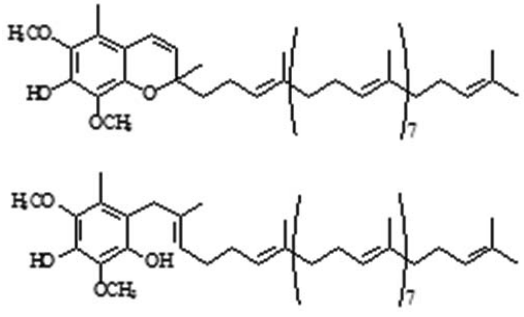

Fig. 1: Compounds **1** and **2**

P448 Analysis of anthraquinone contents and antimicrobial evaluation of the pods of *Senna alata* (Linn.) Roxb. and *Senna podocarpa* (Guill. & Perr.) Lock. (Fabaceae)

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Senna alata and *Senna podocarpa* are two medicinal plants that have long been used as laxative and treatment of skin diseases. The pods have not been investigated for antimicrobial activity. This study was aimed at evaluating the antimicrobial properties and analysis of the anthraquinone contents of the pods. The antimicrobial activity of the ethanolic extract of the two *Senna* species pods were evaluated by agar diffusion method using these organisms: bacteria – *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*; and fungi – *Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*. The ethanolic extract of *S. alata* exhibited antibacterial activity against *B. subtilis* - zone of inhibition at 250 mg/ml was 19 mm while the zones of inhibition for *S. podocarpa* against *B. subtilis* and *E. coli* at 125 mg/ml was 17 mm and 20 mm respectively. The two *Senna* species inhibited only one fungus, *T. rubrum* - the zone of inhibition at 125 mg/ml was 14 mm for *S. alata* and 23 mm for *S. podocarpa*. The thin layer chromatography (TLC) analysis of the anthraquinone contents of the *Senna* species pods using solvent system – Benzene: ethyl acetate: glacial acetic acid (7:2:1) and aluminum coated silica gel plates showed the presence of both free and combined anthraquinone while UV analysis showed that the percentage of combined anthraquinone for *S. alata* was 0.004% w/w and 0.0303% w/w for *S. podocarpa*. Official *Senna* pod used as standard had a value of 0.133% w/w. **Key words:** *Senna alata, Senna podocarpa*, antimicrobial activity, anthraquinone contents

P449 Secondary metabolites from the unripe fruits of *Persea americana* and their anti-tubercular activity

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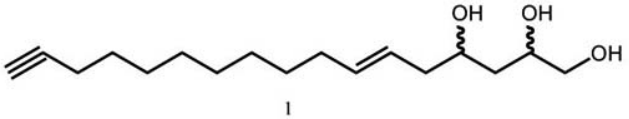
Persea americana Mill. (Lauraceae) is an evergreen tree, distributed in tropical and subtropical regions around the world. Until now, the bark, leaves, fruits and seeds of *P. americana* have been extensively studied. Previous studies on this plant identified various classes of chemical constituents, such as monoterpenoids, sesquiterpenoids, triterpenoids, aliphatics, lignans, flavonoids and alkaloids. From the previous investigations, this plant showed numerous bioactivities, including cytotoxicity, antifungal, antibacterial, acetyl-CoA carboxylase inhibition, nitric oxide and superoxide generation inhibition, and liver injury suppressing. Approximately 1200 species of Formosan plants have been screened for antitubercular activity against *Mycobacterium tuberculosis* H37Rv *in vitro*, and the methanolic extract of the unripe fruits of *P. americana* was shown with antitubercular activity. The aims of this study are the isolation of chemical constituents and their antitubercular activity. The methanolic extract of the unripe fruits of *P. americana* was partitioned into ethyl acetate and water-soluble layers. Bioassay-guided fractionation of the ethyl acetate-soluble layer has led to the isolation of four new aliphatic alcohols: avocadenols A-D (**1-4**), together with three known compounds: 1,2,4-trihdroxyheptadec-16-ene (**5**), 1,2,4-trihydroxyheptadec-16-yne (**6**), and 1,2,4-trihydroxynonadecane (**7**). The structures of these isolates were elucidated by spectroscopic analysis. 1,2,4-Trihdroxyheptadec-16-ene (**5**) and 1,2,4-trihydroxyheptadec-16-yne (**6**) have showed antitubercular activity against *M. tuberculosis* H37Rv *in vitro* with MIC values of 35.7 and 60.5 μg/mL, respectively. The isolation of the unripe fruits and the antitubercular assay of the isolates are still in progress.



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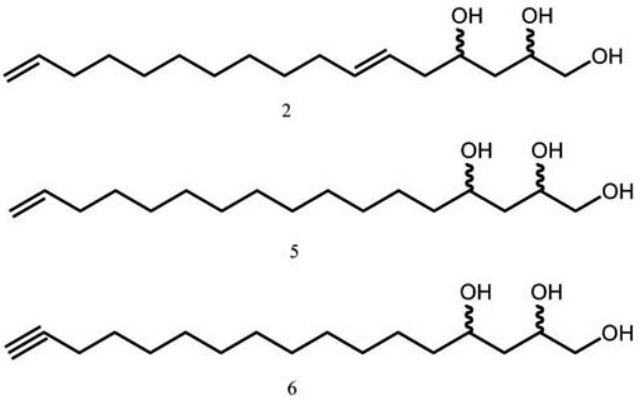

Fig. 1: Compounds **1**-**2** and **5**-**6**

P450 Antiplasmodial *in vivo* activity of *Carica papaya* leaf decoction

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Within the framework of a larger research project [1] aiming at evaluating a variety of widespread natural products for their possible plasmodicidal activities we now report the *in vivo* evaluation of a decoction of papaya leaf. The antimalarial activity of papaya is mostly anecdotal but we have recently reported the *in vitro* antiplasmodial properties of a dried decoction of papaya leaf. In the present study the antiplasmodial activity was investigated in the murine malaria model of *P. berghei*. BALB/c female mice weighing about 20 g, maintained in cages and kept in standard conditions at the ISS animal facilities, were used for experimental infections with *P. berghei* NK 65 strain, stored in liquid nitrogen in our laboratory. By analyzing the reduction of parasitemia, it was found that the papaya extracts at the two higher doses tested had low plasmodicidal activity until the seventh day but the plasmodicidal activity became markedly positive in the following days. In particular, in the D 10 the reduction of parasitemia was 76.7% in the papaya 500 group and 100% in the papaya 750 group; the reduction of parasitemia by D 13 was 100% for both doses. These preliminary results obtained so far in our laboratory are definitely encouraging and suggest us to plan a new series of experiments to provide further evidences and eventually confirm the antiplasmodial activity of *Carica papaya* leaf extracts. **Acknowledgements:** The Ente Cassa di Risparmio di Firenze and Toscana Life sciences are gratefully acknowleged for generous financial supports. **References:** 1. Sannella, AR. et al. (2007) Biochem Biophys Res Commun 353: 177 – 181. 2. Berkelar, D. (2002) Echo Tech Notes 71:1 – 4.

P451 Evaluation of stability of constituents of herbal drug preparations from *Artemisia annua* L.

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Artemisia annua L. (Asteraceae) is an annual herb that is native of China, but naturalized throughout the world, especially in Europe, America and Africa continents. This herb is a traditional Chinese medicine, used for more than 2000 years for treating many fever disorders including malaria. The active antimalarial constituent, artemisinin, a unique sesquiterpene lactone, has been largely used in therapy. Chemical composition of *A. annua* consists of volatile and non-volatile constituents (sesquiterpenoids, and polyphenols including flavonoids and coumarins). In a recent publication the need for evaluating both the active artemisinin and

the polymethoxyflavonoids in the quality assesment of the herbal drugs are reported [1]. A number of simple preparations (decoctions, infusions) and other herbal drug preparations (oil suspensions) obtained from *A. annua* leaves are being developed in many poor countries (especially Africa) for therapeutic applications because of their low cost [2]. In this report *A. annua* raw materials, including sachets of aluminium packages and oily formulations, were investigated for their stability during storage at 30 °C, 60% RH for six months. The content of artemisinin and flavonoids was then evaluated by HPLC/DAD/ESI-MS. The stability results, after six months, showed that artemisinin is stable in the sachets of aluminium with a degradation of about 16%, while the main polymethoxylated flavonoids, ranged between 6.8 and 15.8. The artemisinin in the oil suspension showed a degradation of about 22% and the polymethoxylated flavonoids, ranged between 0 and about 18.75%. **Acknowledgements:** Hanze University Groningen, Institute for Life Sciences and Technology, Groningen (NE) in combination with Erasmus European Comission Education & Training for the fellowship to Cristiaan Wessel. **References:** 1. Bilia et al. (2006) Phytomedicine. 13: 487 – 493. 2. Baraldi et al. (2008) Biochem. Syst. Ecol. 36: 340 – 348.

P452 Antibacterial and antifungal activity of *Mentha cervina* essential oils and their main components

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Mentha cervina L. is an aromatic plant traditionally used in Portugal to flavour food and for its medicinal properties. Native of the Iberian Peninsula and North Africa, it can be found in river banks, damp and wet places, being representative of the priority habitat Natura 3170[1]. In this work, the chemical composition of the essential oil (EO) of *M. cervina*, grown in Portugal, was characterized by GC and GC-MS, and its antimicrobial activity assessed against 23 bacteria including Gram-positive and Gram-negative multiresistant strains and the yeast *Candida albicans*. The main aromatic constituents of the EO were pulegone, menthone and isomenthone, which occurred in different relative amounts depending on the origin of the population studied. To understand the importance of the chemical composition of the EO, these as well as pure standards of pulegone, menthone and isomenthone, were also tested for antimicrobial activity. The minimum inhibitory concentration (MIC) ranged from 2 to 63 mg/mL for almost all microorganisms tested including the multiresistant strains. The most effective was expressed by the EO, and not by any of the components alone. These results support the view that the EO bioactivity is a function of the synergistic effect of the different oil constituents. **Acknowledgements:** L. Rodrigues is grateful to the FCT for the grant SFRH/BD/38143/2008. **References:** 1. Rodrigues L., Monteiro P., Póvoa O., Teixeira G., Moldão M., Figueiredo A. & Monteiro A. (2008) Morphology of secretory structures and essential oil composition in *Mentha cervina* L. from Portugal. *Flavour Fragr.* J. 23: 340 – 347.

P453 Screening of selected essential oils for their *in vitro* antileishmanial activity against *Leishmania amazonensis*

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Leishmaniasis is a group of tropical diseases caused by a number of species of protozoan parasites belonging to the genus *Leishmania* (1). In this work we have investigated the *in vitro* leishmanicidal activity of seven essential oils (EO) from herbaceous plant species against promastigote forms of *Leishmania amazonensis*. The EOs were obtained from leaves by hydrodistillation in a Clevenger-type apparatus for 3 h. Samples of EOs were dissolved in dimethylsulfoxide (DMSO) and evaluated in concentrations of 8, 32 and 128 µg/mL for 24 h using the MTT colorimetric method (MTT) (2). The essential oil of *Artemisia camphorata* Vill (Asteraceae) showed significant activity against *L. amazonensis*, with 52.89% and 84.55% of lysis at concentrations of 32 µg/mL and 128 µg/mL, respectively. The EOs of *Ageratum conyzoides* L. (Asteraceae) and *Plectranthus neochilus* Schltr. (Lamiaceae) were also active, causing 74.90% and 76.44% of lysis at 128 µg/mL. Amphothericin B (32 µg/mL), used as positive control, caused 75.85% of lysis. GC-MS analysis of the active EOs revealed that 1,8-cineole (monoterpene), (E)-caryophyllene (sesquiterpene) and precocene I (chromene) are the major compounds of the EO of *A. camphorata*, *P. neochilus* and *A. conyzoides*, respectively. Our results indicated that the investigated EOs exhibited *in vitro* leishmanicidal activity against promastigote forms of *Leishmania amazonensis*. Further biological studies are in progress regarding the activity of their major compounds identified in the active EOs. **Acknowledgements:** FAPESP (Proc. 07/54241 – 8) **References:** 1. Da Silva Filho et al. (2008). Phytotherapy Res. 22: 1307 – 1310. 2. Muelas-Serrano, S et al. (2000). Parasitol. Res. 86: 999 – 1002.

P454 Antimicrobial activity of *Diospyros villosa* root

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Diospyros villosa L. (de Winter) root is used as toothbrush and to treat oral infections in Mozambique. This medicinal plant is known as "mulala" a vernacular name also common to *Euclea natalensis* A.DC. root, another Ebenaceae species with the same traditional uses. The antimicrobial activity of *E. natalensis* root against diverse microorganisms is already determined, and naftoquinones were identified in this plant. [1,2,3,4] Hereby we present the results of the antimicrobial activity of a *D. villosa* root hydroethanol extract (70% ethanol, Dvr) and corresponding liquid-liquid fractions: n-hexane (Dvrh), ethyl acetate (Dvre), n-buthanol (Dvrb) and water (Dvrw). A preliminary chemical characterization of this extract and fractions was also done. The MIC of each extract and fraction was determined against *Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 435628, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 10240 and *Staphylococcus aureus* ATCC 25923. Dvr extract shows antimicrobial activity against all tested strains (MIC between 62.5 and 312.5 µg/ml) except *P. aeruginosa*, as well as the fractions Dvrh (MIC between 31.2 and 62.5 µg/ml) and Dvre (MIC between 15.6 and 62.5 µg/ml). Triterpenes and hydrolysable tannins were identified as main compounds of this extract and fractions. Naftoquinones were vestigial compounds of the active extract and fractions. As far as we know, this is the first study concerning the chemical and biological characterization of *D. villosa* root. **References:** 1. Lall, N. et al. (2000) J. Ethnopharmacol. 72:313 – 316. 2. Lall, N. et al. (2006). S. Afr. J. Bot. 72:579 – 583. 3. van der Kooy F. et al. (2006) S. Afr J. Bot. 72:349 – 352. 4. Lall N. et al. (2001) J. Ethnopharmacol. 78:213 – 216.

P455 Antifungal activity of *Lavandula x intermedia* Emeric ex Loisel. 'Budrovka'

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Lavandula x intermedia Emeric ex Loisel. 'Budrovka' is an indigenous cultivar of lavandin which has been widely cultivated in Croatia as an essential oil crop. Our previous studies revealed strong antioxidant and antibacterial activities of its various extracts [1, 2]. Continuing our evaluation of biomedical potential of lavandin 'Budrovka', the present work focuses on the investigation of antifungal activity. For this purpose, liquid ethanolic extracts were prepared from different plant parts: flowers, inflorescence stalks and leaves. Employing the agar-well diffusion method, the extracts were screened for antifungal activity against eight yeast strains (*Candida albicans*, *C. glabrata*, *C. krusei*, *C. kefyr*, *C. tropicalis*, *Cryptococcus neoformans*, *Blastoschizomyces capitatus* and *Hansenula anomala*) and five molds (*Aspergillus fumigatus*, *A. niger*, *Fusarium oxysporum*, *Penicillium citrinum* and *Trichoderma viride*). Overall, the tested extracts were more effective against yeasts than molds. The measured zones of inhibition of fungal growth strongly indicated that the antifungal constituents reside primarily in the flower (ZI: 10 – 21 mm), whereas the inflorescence stalk and leaves extracts exhibited considerably weaker activity (ZI ≤ 10). Minimal inhibitory concentrations (MIC, vol%) and minimal fungicidal concentrations (MFC, vol%) for each strain were de-

termined by the broth dilution assay. Results obtained for flower, leaf and inflorescence stalk extracts revealed that *Candida krusei* was the most sensitive tested strain (MIC: 0.05, 8 and 9%, respectively), while *Aspergillus niger* was the most resistant (MIC: 4, 30 and 35%, respectively). In conclusion, the present study highlighted the antifungal potential of *Lavandula x intermedia* 'Budrovka' flowers. **References:** 1. Blaekovic, B., Vladimir-Kneevic, S. (2008) Planta Med. 74:951. 2. Blaekovic, B. et al. (2005) Book of Abstracts of 53 rd Annual Congress of the Society for Medicinal Plant Research. Firenca. Societa italiana di fitochimica, 145.

P456 Investigation of essential oils of *Conyza canadensis* herb and root

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Canadian horseweed [*Conyza canadensis* (L.) Cronq.] is an Asteraceae species indigenous to America but it is distributed widely in Hungary. The aerial parts and the root of this plant have been used all over the word as a herbal medicine for gastrointestinal and rheumatic symptoms. Moreover, the volatile oil of horseweed have been applied for bronchitis and cystitis [1,2]. In order to evaluate chemical constituents and antimicrobial activities of horseweed, essential oils obtained from herbs and roots were investigated. The essential oils were analysed by combination of GC and GC/MS. The identification of the constituents was achieved from their retention indices and comparison of their MS data with computer library database and with literature data [3]. Both oils showed different chemical composition. The essential oil of the herbs contained more components than the essential oil of the roots. The major constituent of the oil of the aerial part of horseweed was limonene (78%), while main components of root oil were acetylenes. The antimicrobial activities of the oils were tested on Gram positive (*Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*), Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, reference fungal strains and fungal strains isolated from patients (*Candida*, *Cryptococcus*, *Trichophyton*, *Rhodotorula*, *Aspergillus*). No substantial differences were found between the activities of the essential oils, none of them showed any activity against the bacterial strains tested, but exhibited moderate to strong activity against all fungi with the only exception of *A. fumigatus*. The highest zone of inhibition was observed against *Cryptococcus neoformans*. **Acknowledgements:** Our investigation was supported by the Hungarian Scientific Research Fund (OTKA 72771). **References:** 1. Grünwald, J., Brendler, T., Jänicke, C. (Eds.) (2000) PDR for Herbal Medicines. Thomson. 2. Khare, C. P. (Ed.) (2007). Indian Medicinal Plants – An Illustrated Dictionary. Springer-Verlag. Berlin/Heidelberg. 3. Adams, RP. (1995) Identification of Essential Oil Components by GC/MS., Allured Publishing Co. Carol Stream, Illinois USA.

P457 Seasonal variation, chemical composition, and analgesic and antimicrobial activities of the essential oil from leaves of *Tetradenia riparia*

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Seasonal variations of the essential oil from fresh leaves of Tetradenia riparia (1,2) cultivated in southern Brazil were analyzed by CG-MS, and the analgesic and antimicrobial activities of the oil were assayed. The yield of essential oil varied from 0.17% to 0.26%, being highest in winter and lowest in spring. The essential oil contained 14-hydroxy-9-epi-caryophyllene as the most abundant component (19.3 – 26.1%), followed by calyculone (12.1 – 25.7%), cis-muurolol-5-en-4-α-ol (7.4 – 14.9%), fenchone (2.6 – 13.4%), and α-trans-bergamotene (1.1 – 5.2%). Samples collected in summer were richer in oxygenated monoterpene (20.6%), whereas those in spring were higher in oxygenated sesquiterpene (69.3%), and those in winter were higher in oxygenated diterpenes (39.9%). The contents of most chemical constituents varied significantly ($p < 0.05$) with the seasons. The essential oil exhibited good analgesic activity on acetic acid-induced writhing in mice, and this activity was not affected by seasonal variation. The antimicrobial activity of the essential oil against the bacteria Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Morganella morganii, and Enterobacter cloacae, and the pathogenic fungus Candida albicans was assessed by the disc diffusion method and determination as the minimum inhibitory concentration. The results of the antimicrobial assays indicated that all the microorganisms tested were affected significantly ($p < 0.05$) by seasonal variations in the oil. **Acknowledgements:** The authors are grateful to CNPq for providing a research grant and fellowships **References:** 1. Campbell, W.L. et al. (1997) Planta Medica. 63: 270 – 272. 2. Omolo, M.O., et al. (2004) Phytochemistry, 65: 2797 – 2802.

P458 Biotransformation of *ent*-pimara-8(14),15-dien-19-oic acid and anticariogenic evaluation of the obtained derivatives

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In the present work, the microbial transformation of the diterpene ent-pimara-8(14),15-dien-19 oic (PA, 1) (Figure 1) was performed using submerged shaken liquid culture of two filamentous fungi: *Glomerella cingulata* and *Mucor rouxii* (1.5 x 107 spores/mL). The microorganisms were grown by a two-stage fermentation procedure [1]. PA was added as a dimethylsulfoxide solution (0.1 g/L) and incubated for 10 days. The cultures were filtered and their aqueous layer were extracted with ethyl acetate to furnish the fractions codified as GcE (*G. cingulata* extract) and MrE (*M. rouxii* extract). Chemical and NMR studies of these extracts allowed us to isolate and to identify four PA derivatives (Figure 1: Compounds 2 and 3 from EGc; 4 and 5 from EMr). The antimicrobial activity of these metabolites was evaluated against the main microorganisms responsible for dental caries [2]: *Streptococcus sanguinis, S. mutans, S. salivarius, S. sobrinus, S. mitis* and *Lactobacillus casei*. For this purpose, the broth microdilution method was applied and the minimal inhibitory concentration (MIC) values were determined [2]. Diterpenes 1, 2 and 4 displayed significant inhibitory effect on the growth of these pathogens, showing MIC values very promising [3].

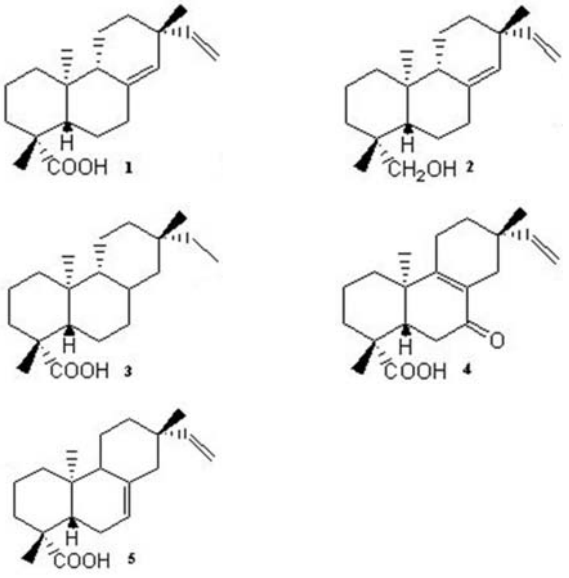

Fig. 1: Chemical structures of PA and their biotransformation metabolites

Acknowledgements: FAPESP (Proc. 2007/54762 – 8) **References:** 1. Bastos, D.Z.L et al. (2007) Phytochemistry 68: 834 – 839. 2. Porto, T.S. et al. (2009) Molecules 14:191 – 199. 3. Gibbons, S. (2008) Planta Med. 74: 594 – 599.

P459 Interactions between pathogenic *Escherichia coli*, porcine intestinal cells, and a phytogenic feed additive and its main active substance

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Plant derived products are claimed to improve animal performance and health, but the knowledge on the mode of action is still limited. The aim was to investigate effects of a phytogenic feed additive and its main active substance *in vitro* regarding 1) their impact on adhesion properties of F4+ *Escherichia coli* to the porcine intestinal epithelial cell line IPEC-J2, 2) their capacity to bind *E. coli*, and 3) their antimicrobial character in context to the pathogenic *E. coli*. The phytogenic additive Fresta® *F* (FF; Delacon, Austria) consists of essential oils, herbs, and spices. The main ingredient of the additive consists of 28% galactomannans (GM). The impact of the test substances was investigated *in vitro* by measuring the fluorescence intensity of CDFA-SE marked bacteria that adhere on the IPEC-J2 cells (trial #1). A flow cytometer was used to quantify the fluorescent cells. *In vitro* trial #2 was performed using a coating method previously published by Becker *et al.* 2007. Antimicrobial effects of FF and GM on the *E. coli* strain were estimated semi-quantitatively from bacterial growth curves. FF and GM significantly reduced the adhesion of *E. coli* to IPEC-J2 ($p < 0.05$); fluorescence intensity was 0.96 ± 0.63, 0.86 ± 0.61, and 2.03 ± 1.13, respectively. FF 0.37 ± 0.20 and GM 0.42 ± 0.16 showed capacity to bind *E. coli* compared with the positive control 0.52 ± 0.05. No direct antimicrobial effects could be detected. It is concluded that the mode of action of Fresta® *F* is not due to antimicrobial effect, but rather due to the anti-adhesion capacity of galactomannans present in the products main component. **References:** 1. Becker, P.M., et al. (2007)J Appl Microbiol 103, 2686 – 2696.

P460 Plants used in popular medicine for treatment of infectious diseases in Central South America

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In communities there are people reference and popular trust, which are sought to guide the needy. This happens also for the treatment of health problems. In the capital (Cuiabá) from Mato Grosso, Brazil (geodesic center of South America) the people who lead and trading medicinal plants are known as healers (raizeiros). They have points of service specific and home-made to meet its demand. To know what are the major medicinal plants listed the treatment of infectious diseases in general, a study was done in the city of Cuiaba, the following species are mentioned for twelve healers: Angico (*Anadenanthera peregrina* (L.) Speg.-Fabaceae), Arnica (*Arnica montana* L.- Asteraceae), Arnica do campo (*Camarea ericoides* A. St.-Hil.- Malpighiaceae), Aroeira (*Astronium urundeuva* (Allemão) Engl.-Anacardiaceae), Bálsamo da mata (*Dicliptera pohliana* Nees – Acanthaceae), Barbatimão (*Stryphnodendron adstringens* (Mart.) Coville -Fabaceae), Cancerosa (*Maytenus ilicifolia* (Schrad.) Planch.- Calastraceae), Copaíba (*Copaifera langsdorffii* Desf.- Fabaceae), Jequitibá (*Cariniana rubra* Gardner ex Miers – Lecythidaceae), Malva branca (*Waltheria americana* L. – Sterculiaceae), Mangava brava (*Lafoensia pacari* A. St.-Hil. – Lythraceae), Marapuama (*Ptychopetalum olacoides* Benth.- Olacaceae), Nó de cachorro (*Heteropterys aphrodisiaca* Mach. – Malpighiaceae), Pau doce (*Vochysia rufa* Mart. -Vochysiaceae), Sucupira (*Bowdichia virgilioides* Kunth – Fabaceae), Vassourinha (*Scoparia dulcis* L. – Plantaginaceae). It was also mentioned other plant species suitable for specific infections: intestinal (2), renal (4), urinary (7), uterus and ovaries (3). It is observed predominance of species rich in tannins, and has antiseptic and disinfectant power, which may partly explain the indication of these plants to combat infectious diseases. **Acknowledgements:** Institutional support- UNEMAT, UFMT and EMPAER-MT; the teacher advisor – Guarim Neto, G; colleagues: Gonçalves MIA, de la Cruz Mota MGF – by participation in data collection and other support.

P461 Dammarenolic acid, a secodammarane triterpenoid from *Aglaia* sp shows potent anti-retroviral activity in vitro

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Screening of a panel of purified compounds isolated from Aglaia spp. (Meliaceae) for inhibition of early steps in the lentiviral replication cycle led to the identification of the 3, 4-secodammarane triterpenoid, ignT1, which inhibited HIV-1 infection potently ($IC50 = 0.48\ \mu g/ml$), while cytotoxic effects and inhibition of cell proliferation were only observed at concentrations exceeding $10.69\ \mu g/ml$. Time of addition experiments revealed similar kinetics to the non-nucleoside RT-inhibitor (NNRTI), Nevirapine, although the latter was significantly less cytotoxic. However, unlike Nevirapine, dammarenolic acid also potently inhibited the in vitro replication of other retroviruses, including Simian immunodeficiency virus and Murine leukemic virus in vector-based antiviral screening studies. Interestingly, the methyl ester analogue of dammarenolic acid, methyl dammarenolate had no anti-HIV-1 activity. Cell cycle analysis revealed that ignT1 arrests HeLa cells at the S and G2/M phase. These results strongly suggest that dammarenolic acid could be a promising lead compound for the development of novel anti-retrovirals. **Acknowledgements:** The authors gratefully acknowledge the Postdoctoral Fellowship Awards to CS Nworu and CO Esimone by the Alexander von Humboldt Foundation **References:** 1. Esimone, CO. et al (2009). Chemotherapy 55:119 – 126. 2. De Clercq, E. (2000). Med Res Rev 20: 323 – 349.

P462 Efficacy of components from leaves of *Calophyllum brasiliense* against *Leishmania amazonensis*

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Leishmanicide potential of *Calophyllum brasiliense* leaves on promastigote and amastigote of *Leishmania (Leishmania) amazonensis* is evaluated. The LD50 of the dichloromethane extract and the hexane fraction for promastigotes was respectively 40 mg/ml and 20 mg/ml. In mouse peritoneal macrophages infected with *Leishmania* amastigotes the Infection Index decreased respectively 100% and 84.2% in 80 mg/ml and 40 mg/ml concentrations of dichloromethane extract. The hexane fraction decreased the Infection Index respectively by 98.7% and 91.3% within the same concentrations. It was found that pretreatment with dichloromethane extract or with hexane fraction of experimentally infected BALB/c mice decrease the volume of the lesions by *L. amazonensis*. Moreover, animals treated topically also revealed healing lesions. Besides, the parasite load in the animals' popliteal lymph nodes was significantly reduced in treated animals, showing that plant components actually control infection. Results show that crude extract and hexane fraction of *C. brasiliense* reveal a significant in vitro and in vivo leishmanicide activity [1]. The amentoflavones, (–)mammea A/BB and mammea B/BB, were identified in the extracts and fractions by comparison with standard samples and by analyses of HPLC-UV. **Acknowledgements:** The authors are grateful to CNPq for providing a research grant and fellowships **References:** 1. Honda A. (2010) Phytomedicine. 17: 333 – 338.

P463 Antimicrobial activities of Longan (*Euphoria longan* L.) skin and seeds

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The methanolic extracts from seeds and skin of Longan (*Euphoria longan* L.) were tested for antimicrobial activity with five strains of pathogenic bacteria including *Staphylococcus aureus, Bacillus cereus, Psuedomonas aeruginosa, Enterococcus faecalis, Escherichia coli* and one strain of pathogenic yeast, *Candida albicans*. The antimicrobial activities of each extract were screened by agar diffusion before conducting with broth macrodilution methods for determining minimal inhibitory concentration (MIC) [1]. The phenolic content of skin and seed extracts was evaluated by analytical high performance liquid chromatography (HPLC) [2]. The results show that the methanolic extract of Longan skin (10 mg/ml) inhibited growth of *S. aureus, P. aeruginosa* and *C. albicans* were 15, 11 and 9 mm of inhibition zone and MIC values were 4.42, 8.84 and 1.11 mg/ml, respectively. The inhibition zones of Longan seed extract (10 mg/ml) were 17, 12 and 11 mm and MIC values were 3.19, 1.59 and 1.59 mg/ml for *S. aureus, P. aeruginosa,* and *C. albicans*, respectively. The phenolic content of skin and seed extracts were 13.38 and 88.51 g/kg of dry weight. It was concluded that the antimicrobial activity was not related to content of phenolic compounds. However, it may be due to types of phenolic compounds presented in the extracts and solubility of extracts [3, 4]. **Acknowledgements:** 1, Faculty of Science and Faculty of Medical Technology, Rangsit University, Phaholyothin Road, Lakhok, Pathumthani 12000, Thailand. 2, Associate Professor Omboon Luanratana, head of Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Sri-Ayuthaya Road, Rajathevi, Bangkok 10400, Thailand. **References:** 1. NCCLS. (1998) Performance standards for Antimicrobial Susceptibility testing: Fifth Informational Supplement M100-S8 18 (1). National Committee for Clinical Laboratory Standards. 2. Owen RW et al. (2000) Eur J Cancer 36: 1235 – 1247. 3. Cowan, MM. (1999) Clin Microbiol Rev 12: 564 – 582. 4. Cushnie, TPT. et. al. (2003) Microbiol Res. 158: 281 – 289.

P464 Protein fraction from *Syzygium cumini* L. (Skeels) seeds active against bacteria isolated from bovine mastitis

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Mastitis, an inflammation of the mammary gland, is the costliest production disease of dairy cattle around the world. The treatment is based on antibiotic therapy, but the therapeutic efficacies of the drugs are decreasing due to the development of antimicrobial resistance. Aiming to evaluated the potential use of herbal compounds on infections of mammary glands, the in vitro activity of *Syzygium cumini* L. (Skeels) seeds protein fraction was tested against different isolated bacteria from bovine mastitis (*Staphylococcus aureus, Staphylococcus intermedius, coagulase-negative Staphylococcus, Staphylococcus hyicus, Streptococcus uberis, α-hemolytic Streptococcus, Streptococcus dysgalactiae, Streptococcus bovis and Enterococcus faecalis*). The tests were carried using protein fraction obtained by sulfate ammonium precipitation, dialysis and lyophilization. The agar diffusion method was used and this fraction showed activity against *S. uberis*, with an inhibition halo of 12 mm, α-hemolytic *Streptococcus* (10,5 mm), *S. intermedius* (15 mm), coagulase-negative *Staphylococcus* (16 mm) and *S. aureus* (18 mm). These results showed that the *S. cumini* seeds protein fraction could represent an interesting alternative method for mastitis control. The formulations based on this natural product will be tested, aiming to development an alternative treatment for antibiotic therapy, with reduced costs and risk of residues on milk.

P465 Antimicrobial activity of *Limonium avei* (De Not.) Brullo & Erben extracts

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Limonium avei (De Not.) Brullo & Erben (Plumbaginaceae) is a rare triploid (2n = 27), annual halophyte, with apomictic reproduction [1], included in the Red List of Endangered Species by the IUCN [2]. The species is endemic to the central Mediterranean coast and in Liguria (Italy) it is present in only one population, with almost 1500 individuals. The increasing urbanization of the Ligurian population natural habitat has prompted the adoption of measures for its conservation. As part of this effort ex situ seed conservation and tissue culture techniques were developed for the species [3]. To the best of our knowledge, no study reporting to biological activity is present in literature. Here we reported for the first time the antimicrobial activity and phytochemical profile of *Limonium avei* ethanol and dichloromethane extracts. Flowering stems collected in the natural site were compared with flowering stems collected in the CRA-FSO greenhouse from micropropagated acclimatised plants. Tissues were air dry at room temperature for 60 days. The antimicrobial activity of extracts was performed by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [4] against Gram-positive, Gram-negative bacteria and mycetes. The extracts were submitted to phytochemical screening by LCMS and HPLC/DAD analyses. The results indicated that the ethanol extracts of both samples displayed higher activity than dichloromethane extracts and this activity was more pronounced against Gram-positive than Gram-negative bacteria and mycetes. In particular, the extracts demonstrated MIC and MBC values ranging from 15.6 to 500 µg/ml and from 500 to 4000 µg/ml respectively. **References:** 1. Brullo S. (1988). Miscellaneous notes on the genus Limonium. Willdenowia 17(1): 17. 2. Conti F. et al. (1997). Liste Rosse Regionali delle Piante d'Italia. WWF & SBI, Camerino: 64. 3. Giovannini A. et al. (2009). Ex situ conservation measures of a threatened Limonium avei (De Not.) Brullo & Erben population. In Book of Abstract "Biodiversity Hotspots in the Mediterranean Area" Cagliari 22 – 24 giugno: 284. 4. CLSI (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically:approved standard, Wayne, Pa 17: 10 – 13.

P466 *In vitro* antimicrobial activity screenning of *Terminalia macroptera* leaf

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Terminalia macroptera Guill. and Perr. (Combretaceae) is a West African species used on traditional medicine to treat infectious diseases.[1] Hereby we present the results of an antimicrobial activity screening performed by the twofold serial microdilution assay against seven reference bacterial strains and against *Candida albicans*, with a leaf hydroethanol extract (**Tml**) and liquid-liquid partition fractions **Tml-1** (hexane), **Tml-2** (diethyl ether), **Tml-3** (ethyl acetate), **Tml-4** (Tml water filtered fraction) and **Tml-5** (Tml water precipitate fraction). Results are displayed on Table 1. In the range of tested concentrations (3200 to 50 µg/ml), the extract was active against all tested microorganisms. The best results were obtained against *Shigella dysenteriae* and *Vibrio cholerae* and the most active fraction was identified as the ethyl acetate one (**Tml-3**). Chemical profile of this fraction includes polyphenols as main compounds.

Table 1: *In vitro* antimicrobial activity of *T. macroptera* leaf extract (Tml) and fractions (Tml-1 to Tml-5)

Microorganisms	Minimum inhibitory concentration (μg/ml)					
	Tml	Tml-1	Tml-2	Tml-3	Tml-4	Tml-5
C. albicans	3200	> 3200	> 3200	800	3200	3200
E. coli	1600	> 3200	> 3200	800	800	3200
E. faecalis	3200	> 3200	> 3200	1600	1600	3200
P. aeruginosa	800	> 3200	> 3200	800	800	3200
S. aureus	3200	> 3200	> 3200	3200	1600	3200
S. dysenteriae	200	> 3200	> 3200	400	800	3200
S. typhimurium	800	> 3200	> 3200	800	800	3200
V. cholerae	200	> 3200	> 3200	200	400	3200

References: 1. Diniz MA. et al. (1996) The Biodiversity of African Plants (In: van der Maesen LJG., Ed. The Biodiversity of African Plants). Kluwer Academic Publishers. Dordrecht.

P467 LC-UV-MS characterization of *Terminalia macroptera* leaf

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Terminalia macroptera Guill. and Perr. (Combretaceae) is a species used to treat infectious diseases in many West African countries. Previously, root and leaf hydro ethanol extracts of this species showed an interesting profile of activity against *Neisseria gonorrhoeae* [1,2]. The root extract of this species also showed to be active against enteropathogenic bacteria [3]. Hereby we present the results of the chemical characterization of *T. macroptera* leaf active extract (Tml) and of the most biological liquid-liquid partition active fractions (diethyl ether and ethyl acetate) by means of liquid chromatography coupled with ultraviolet photodiode array spectroscopy and mass spectrometry (LC-UV-MS). Comparative LC-UV-MS data between leaf and root are also shown. LC-UV-MS results confirmed the polyphenolic profile of Tml, and allowed the identification of flavonoids, phenolic acids and hydrolysable tannins as major compounds. Chemical work made on prosecution allowed the identification of combreglutinin, corilagin, 3,4,5-trimethyl-3′,4′-dioxoloflavellagic acid, 3,3′,4,4′-tetramethylellagic acid, rutin, orientin, vitexin, iso-vitexin, and of a galloylquercetin glucoside and a galloyl-luteolin glucoside on Tml and most active fractions, in addition to chebulagic acid, chebulinic acid, ellagic acid, gallic acid, punicalagin and isoorientin previously identified on this extract [2]. α- and β-terchebulin, the main compounds of *T. macroptera* root [4], were not identified on the leaf of this species. **References:** 1. Silva O. et al. (1997) Pharm Biol 35:323 – 328. 2. Silva O. et al. (2002) FEMS Microbiol Lett 211:203 – 206. 3. Silva O. et al. (1996) J Ethnopharmacol 50:55 – 59. 4. Silva O. et al. (2000) Pharm Res 17:1396 – 1401.

P468 *In vitro* anti-*Neisseria gonorrhoeae* activity of *Terminalia boivinii*, *Terminalia sambesiaca* and *Terminalia spinosa*

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The genus *Terminalia* (Combretaceae) is known as a source of bioactive secondary metabolites. This work aims at the *in vitro* activity of leaf and root hydroethanol extracts of *Terminalia boivinii* Tul., *Terminalia sambesiaca* Engl. and Diels and *Terminalia spinosa* Engl. against nine *N. gonorrhoeae* penicillin and tetracycline sensitive and resistant strains, by means of the agar dilution method. Results showed the activity of all tested samples against the different strains, with minimum inhibitory concentrations (MIC) ranged between 100 and 400 μg/ml (Table 1). The leaf extracts of all species are slightly more active than root ones. The establishment of the metabolomic profile of these extracts is ongoing.

Table 1: Anti-*N. gonorrhoeae* activity of *T. boivinii*, *T. sambesiaca* and *T. spinosa*

N. gonorrhoeae strains	MIC μg/ml)				
	T. boivii	T. sambesiaca		T. spinosa	
	L	L	R	L	R
INSA 195	200	200	200	200	200
INSA 219	200	200	400	200	200
INSA 227	200	200	200	200	400
INSA 232	200	100	200	100	200
INSA 249	200	200	200	200	200
INSA 257	200	100	200	200	200
Bilthoven 7391	200	200	200	200	200
CRA/INSA 7567	200	200	200	200	200
ATCC 49226	200	200	200	200	200

P469 Phytochemical screening and *in vitro* antimicrobial activity of *Calycobolus heudelotii* stem

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Calycobolus heudelotii (Baker ex Oliv.) Heine is a native plant from West Tropical Africa. Stems are used in folk medicine of Guinea-Bissau for inflammations, skin allergies and wounds [1]. First work previously performed was a preliminary botanical analysis of the stem [2]. Here we present results from an antimicrobial activity study of *C. heudelotii* stem methanol extract and a preliminary phytochemical screening. The minimum inhibitory concentration (MIC) was determined by the broth dilution method against *Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 435628, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 10240 and *Staphylococcus aureus* ATCC 25923. The phytochemical screening was made by thin layer chromatography (TLC) and high performance liquid chromatography with ultraviolet diode array detection (HPLC-UV-DAD). In the range of tested concentrations (3200 to 15.62 μg/ml), the extract show antimicrobial activity against all tested strains (MIC between 625 and 156.3 μg/ml) except against *S. aureus*. The highest activity (MIC of 156.3 μg/ml) was demonstrated against *M. luteus*. Phenolic compounds are the major constituents of the extract, as shown by TLC and HPLC-UV-DAD profile, and include phenol acid derivatives like chlorogenic acid. β-sitosterol is also detected. Alkaloids, present in other Convolvulaceae, are absent in this extract. Further chemical, toxicological and bioactivity studies are underway to prove other traditional uses and to check the safety of this medicinal plant. **References:** 1. Catarino L. et al. (2006) Plantas Vasculares e Briófitos da Guiné-Bissau. IICT e IPAD, Lisbon. 2. Martins A.S. et al. (2008) Caracterização botânica da liana medicinal da Guiné-Bissau: Calycobolus heudelotii. Available at http://www2.iict.pt/? idc = 15&idi = 14082. Acessed April 29, 2010.

P470 Screening and bioguided fractionation of Amaryllidaceae species with activity against *Trichomonas vaginalis*

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The Amaryllidaceae family is known by its ornamental and medicinal value and has attracted considerable attention due their content of alkaloids. Taking into account the noteworthy popular use of Amaryllidaceae in sexually transmitted diseases (STD) treatment, the investigation of their anti-*Trichomonas vaginalis* potential is a significant issue. *Trichomonas vaginalis* is a flagellated protozoan that parasitizes the urogenital human tract and causes trichomonosis, the most prevalent non-viral STD worldwide. In this context, this work evaluated Amaryllidaceae plants to anti-*T. vaginalis* activity. Dichloromethane and *n*-butanol extracts from *Hippeastrum* and *Rhodophiala* species and alkaloids isolated from these plants were tested in seven concentrations (12.5 to 0.19 mg/ml and 125 to 1.9 μg/ml, respectively). All alkaloids (lycorine, montanine, pretazettine, tazettine, hippeastrine, and lycosinine) diminished the trophozoites viability, ranging from 15 to 40% citotoxicity. The dichloromethane extract from *R. bifida*, *H. breviflorum*, *H. vittatum*, and *H. psit-*

tacinum reduced the viability of the trophozoites of *T. vaginalis* in about 30 – 60% in the lower concentration. The *H. breviflorum* extracts demonstrated the highest anti-*T. vaginalis* activity (60% of viability reduction) and a bioguided study were realized. Nine alkaloids enriched dichloromethane and *n*-butanol fractions were obtained and tested. Among these fractions the one butanol fraction is the most active against *T. vaginalis* because reduced the viability at 100%. All the active fractions contain lycorine and lycosinine as major components and a synergism can occur considering the higher anti-*T. vaginalis* activity of extracts than alkaloids.

P471 Lapachol and isomeric 5- and 8-hydroxy-2-(1′-hydroxyethyl)naphtho[2,3-b] furan-4,9-diones are effective antileishmanial constituents of *Tabebuia avellanedae*

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The antileishmanial activity of a methanolic extract of Tabebuia avellanedae (Bignoniaceae) and some constituents against transgenic Leishmania major expressing green fluorescent protein (GFP) was examined using parasite retrieval assay and flow cytometry (FACS analysis). Leishmaniasis is a group of diseases caused by flagellate protozoan Leishmania spp. causing high morbidity and mortality in tropical to Mediterranean environments. The methanol extract was sequentially extracted with n-hexane, dichloromethane, ethyl acetate, and n-butanol, yielding finally water-soluble extractives. The highest antileishmanial activity resided in the n-hexane and dichloromethane fractions with IC50 of 64 μg/ml and 41 μg/ml, respectively. Phytochemical analysis of the n-hexane fraction revealed the presence of lapachol which exhibited antileishmanial activity against both extra- and intracellular parasites, with IC50 values of 33 μM and 115 μM, respectively. Lapachol did not show any cytotoxicity on macrophages as host cells of Leishmania parasites (EC50 > 300 μM). From the dichloromethane fraction an inseparable mixture of isomeric 5- and 8-hydroxy-2-(1′-hydroxyethyl)-naphtho[2,3-b] furan-4,9-diones was isolated by a combination of column chromatography and semi-preparative HPLC. This mixture was significantly more effective against both extra- and intracellular L. major parasites (IC50 of 4 μM, ca. 28 fold more active than that of lapachol), while cytotoxic effects on macrophages were not evident at 8 μM. The IC50 of amphotericin B, a reference compound, was 2.5 μM and 0.2 μM against promastigotes and amastigotes, respectively. It appears as if the presence of a furan ring increases the antileishmanial activity of naphthoquinones.

P472 Antileishmanial mode of action of lapachol and plumbagin

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Plumbagin, a naphthoquinone first isolated from species of the genus Plumbago, and lapachol, a prenylated hydroxynaphthoquinone encountered in Tabebuia avellanedae (Bignoniaceae), were reported as antifungal, antibacterial, anticancer and antileishmanial agents [1, 2]. It has been suggested that the antileishmanial activity of these naphthoquinones is due to the generation of reactive oxygen species [3]. The aim of this investigation was to evaluate the antileishmanial activity of lapachol and plumbagin with a closer view on their mode of action. Lapachol exhibited prominent antileishmanial activity against L. major promastigotes with an IC50 value of 8 μg/ml (33 μM) and showed a moderate activity against amastigotes as evident from an IC50 value of 28 μg/ml (115 μM). It did not show any cytotoxicity on macrophages as host cells even at high concentrations (EC50 > 72 μg/ml, 300 μM). On the other hand plumbagin was significantly more effective against both extra- and intracellular L. major parasites (IC50 0.5 μg/ml, 2.6 μM), but was relatively more cytotoxic on macrophages (1.5 μg/ml; 8 μM). Plumbagin and lapachol did not induce NO release in infected macrophages. Both compounds were found to be similarly antileishmanicidal when incubated with or without the iNOS inhibitor L-NMMA. While plumbagin did not show any antileishmanial effects in the presence of glutathione plus ascorbic acid, no difference in activity was found for lapachol. This finding suggests that lapachol and plumbagin mediate their antileishmanial activities by different mode of actions and independent from NO production.

References: 1. Hussain, H et al. (2007), Arkivoc: 145 – 171. 2. Croft S et al. (1985), Annal Trop Med Parasitol 79: 651 – 653. 3. Inbaraj JJ, Chignell CF (2004), Chem ResToxicol. 17: 55 – 62.

P473 Treatment of malaria in Iranian traditional medicine

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The declining efficacy of classical medication in relation to the rapid extension of Plasmodium falciparum chloroquine-resistant strains has led to a need for new efficient anti malarial drugs. It is a real necessity to search for new efficient anti malarial compounds and make them accessible to most of the people. An ethnobotanical study was conducted to find plants traditionally used against malaria in Iran. In this study 35 plants were found on the basis of ethnobotanical investigation and searching in Iranian ancient traditional physician's books (1 – 4). At the same time the anopheles mosquito has developed resistance to many insecticides. There are some plants in Iran that are traditionally used as insect repellent (1 – 4). Also matching the old medicinal plant names with scientific terminology was done (5 – 8). First ethnobotanical studies on these plants were carried out at theTraditional Medicine and Materia Medica Research Center (TMRC). Further assessments are in process.

References: 1. Ibn – sina, Qanoon, Lithography, 1296 A.H. reprinted by Institute of medical history study, Islamic and complementary medicine. Tehran 2004. 2. Jorjani S. Zakhireh Kharazmshahi. Bonyade farhang Iran. Tehran First Ed, 1976 PP: 256 – 273. 3. Davood Ibn-Omar Antaki, Tazkareh olel-Albab. Beirot. 4. Aqili Khorasani, Makhzan ol-Advieh. Enghelab e Eslami publishing and Educational Organization. Tehran 1992. 5. Ghahreman A and Okhovvat A. R. Matching the old medicinal plant names with scientific terminology Vol 1. Tehran University. Tehran. 2004. 6. Dini M. Investigation of various common names of plants used in traditional medicine. Research institute of forests and rangelands. Tehran. 2005. 7. Amin Gh. Popular medicinal plants of Iran. Tehran University. Tehran. 2005. 8. Soltani A. Encyclopedia of traditional medicine (Medicinal plants). Arjmand. Tehran. 2004.

P474 Investigation on pharmacological and antimicrobial activities of *Galanthus transcaucasicus* Fomin growing in Iran

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Galanthus transcaucasicus Fomin (Amaryllidaceae) is an endemic species of the Caucasus region and the Alborz mountains in Iran (1). All species of Galanthus are famous for their bioactive alkaloids such as galanthamine, an acetyl cholinesterase inhibitor, which is used for the treatment of Alzheimer's disease (1). The bulbs of the plant were col-

lected in February 2008 in Alborz mountain area (Rostam abad), Iran. The total extract was prepared by cool percolation method using ethanol and the chloroform fraction was obtained. Preliminary phytochemical screening showed the presence of alkaloids, sterols and cardiac glycosides in bulbs total ethanolic extract. The antimicrobial activity of the ethanolic extract of bulbs and chloroform fraction were evaluated on Staphylococcus aureus, Staphylococcus piogenes, Pseudomonas aeruginosa, Escherichia coli, Helicobacter pylori, Shigella soneii, Salmonella typhi, Bacillus subtilis, Proteus vulgaris and Candida albicans using cup plate diffusion method. The time course of the effects of the ethanolic extract of bulbs, administered intrapritoneally to rats, on the spatial memory retention in the Morris water maze was investigated (2). The results showed that G. transcaucasicus Fomin ethanolic extract of bulbs had antibacterial activity against B. subtilis ATCC 6633 (MIC 9.275 mg/ml) and antifungal activity against C. albicans ATCC 10231 (MIC 150 unit/ml). The chloroform fraction showed activity against S. aureus ATCC 6538 (MIC 1.17 mg/ml). The administered doses of 5, 25 and 100 mg/kg showed a significant reduction in escape latency ($P < 0.05$), and traveled distance ($P < 0.05$) but not swimming speed, compared with control suggesting significant spatial memory retention enhancement by G. transcaucasicus Fomin. **References:** 1. Bastida, J. et al. (2000). The alkaloids. Elsevier Scientific Publishing, Amesterdom 63: 87 – 179. 2. Sharifzadeh, M. et al. (2005). Eur J Pharmacol 511:159 – 66.

P475 Chemical constituents of *Zanthoxylum capense*

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Zanthoxylum capense (Thunb.) Harv (Rutaceae) is a small to medium tree distributed in Zimbabwe, South Africa, and Mozambique. The decoction of its roots is used to treat gallsickness in the Eastern Cape, and its fruits are employed in the treatment of colic and paralysis. Similarly to other *Zanthoxylum* species, such as *Z. chalybeum* Engl. and *Z. davyi* (Verdoorn) P.G. Waterman, *Z. capense* is also used in the treatment of snakebite and severe coughs and colds [1]. Previous studies of this genus have reported the isolation of coumarins, lignans, alkaloids, terpenoids, and flavonoids [2]. In our search for biologically active compounds from plant species, the methanol extract of *Z. Capense* roots has been studied. The crude methanol extract was suspended on a methanol-water mixture and sequentially extracted with *n*-hexane and dichloromethane. Preliminary studies of the dichloromethane fraction, using combined chromatographic techniques, have yielded some known benzophenanthridine-type alkaloids, namely decarine, norchelerythrine, and 8-acetonyldihydro-cherlerythrine, and one furoquinoline alkaloid, skimmianine. The lignan, (−)-savinin was also isolated. The structures of these compounds were deduced from their physical and spectroscopic data, including 1D and 2D NMR experiments (^{1}H, ^{13}C, COSY, HMQC, HMBC and NOESY) and comparison with reported data. Further phytochemical study on this plant is going on. **Acknowledgements:** This study was supported by a fellowship from FCT, Portugal (reference number SFRH/BPD/37179/2007). **References:** 1. Tarus, P.K. et al. (2006) S. African. J. Bot. 72:555 – 558. 2. Gray, A.I. et al. (1983) Chemistry and Chemical Taxonomy of Rutales. Academic Press, London, p. 97.

P476 Screening of medicinal plants for antibacterial activity

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Plant-derived compounds have long been playing a crucial role in drug discovery and development, representing a potential source of new anti-infective agents. It was supposed that the antimicrobial compounds from plants may inhibit bacteria through different mechanisms than conventional antibiotics, and could therefore be of clinical value in the treatment of resistant microbial strains [1]. Herein, we report the evaluation of antibacterial activity of fourteen medicinal plants, which were collected in Mozambique. Seventy crude extracts were obtained by both successive extraction methods and by decoction according to traditional use. Four organic solvents with varying polarity (*n*-hexane, dichloromethane, ethylacetate, and 70% ethanol) were used for the extraction. The extracts were tested against two Gram-positive strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 51299, and four Gram-negative strains: *Klebsiella pneumoniae* ATCC 9997, *Klebsiella pneumoniae* ID 2564, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 by broth dilution method. Eight plants extracts showed moderate to significant activity against Gram-positive strains. The *n*-hexane and dichloromethane extracts of *Anacardium occidentale L.* showed highest inhibition against *E. faecalis* ATCC 51299 (MIC = 31 μg/mL), and the 70% EtOH extract of *Adansonia digitata* L. exhibited activity to *S. aureus* ATCC 25923 (MIC = 62 μg/mL). However, no extract showed activity to Gram-negative bacteria with concentrations ranging from 0.9 μg/mL to 500 μg/mL. **Acknowledgements:** This study was supported by a fellowship from FCT, Portugal (reference number SFRH/BPD/37179/2007). **References:** 1. Eloff, J.N. et al. (1998) J. Ethnopharm. 60:1 – 8.

P477 Autophagy cell death process induced in *Trypanosoma cruzi* by piperovatine and piperlonguminine

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Chagas disease is a neglected illness which currently affects 10 – 16 millions of people [1]. As already demonstrated by our group, the amides piperovatine and piperlonguminine obtained from Piper ovatum Vahl have potent effect against epimastigote form of T. cruzi [2]. In the present work, we report the activities of these compounds against proliferation of intracellular amastigote and also determine the mechanism of cell death in epimastigote using flow cytometry and labeling with monodansylcadaverine (MDC) techniques. Piperovatine and piperlonguminine concentration which inhibit 50% of growth (IC50) of amastigotes were 35.0 ± 6.9 μM and 33.9 ± 5.4 μM, respectively. Ultrastructural studies of the parasite showed alterations in cell membrane and presence of multiple vacuoles in the cytoplasm. Additionally, no alterations were observed in cell architecture of nucleus and mitochondria. For membrane integrity assay, treated parasites were incubated with 2 mg/mL propidium iodide (PI) and analyzed in FACSCalibur flow cytometer (Becton-Dickinson, Rutherford, NJ, USA) equipped with the CellQuest software. This study showed an increase in PI signal, with 84% for piperovatine and 86% for piperlonguminine, suggesting alterations in membrane integrity. Besides that, in the study of cell death process, epimastigotes treated with these compounds were incubated with MDC (0.05 mM) and visualized using? uorescence microscope (Media cybernetics, US). Labeling with MDC revealed formation of autophagic vacuoles in 90% of the cells. These results showed in vitro that the mechanism of cell death can be by autophagic process, since the alterations induced had distinct feature from those of typical apoptosis or necrosis [3]. **Acknowledgements:** This study was supported through grants from CNPq, FINEP, Fundação Araucária, and CAPES. **References:** 1. WHO. (2005) Report of the Scienti? c Working Group (SWG) on Chagas Disease, Buenos Aires, Argentina. 2. Veiga-Santos, P. et al. (2008) The biological activity of piperovatine and piperlonguminine isolated from Piper ovatum Vahl in epimastigote form of Trypanosoma cruzi. Planta Medica 75:877 – 1094. 3. Nguewa, P. A., et al. (2004) Programmed cell death in Trypanosomatids: a way to maximize their biological? tness? TRENDS in Parasitology 20:375 – 380.

P478 In vivo study of effect of copaiba oil obtained from *Copaifera martii*, on lesions caused by *Leishmania amazonensis*

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Leishmaniasis is still a severe public health problem, with high rates of morbidity and mortality. Unfortunately, the treatment for this infectious disease is essentially limited to pentavalent antimony (Sb), in use for 50 years [1]. A recent study by our research group showed that copaiba oils obtained from different species of Copaifera show in vitro activity against promastigote forms of Leishmania amazonensis [2]. In the present study, we demonstrated the in vivo activity of copaiba oil obtained from Copaifera martii in BALB/c mice infected with L. amazonensis. For the in vivo tests, Balb/c mice were infected subcutaneously with L. amazonensis (1 x 107 cells/ml) in the right hind footpad. The treatment was started on the 8th week post-infection. Mice were treated topically, orally, orally and topically, or subcutaneously. Treatment with Glucantime® was used as a positive control. The lesion size was measured with a caliper each week during one month of infection. The oral treatment caused a significant ($p < 0.05$) reduction in the average lesion size (1.1 ± 0.4 mm) compared with untreated mice (4.4 ± 1.3 mm). However, topical (4.9 ± 0.3 mm) and subcutaneous (3.0 ± 1.0 mm) treatments showed no significant reduction in the average lesion size ($p < 0.05$). Interestingly, copaiba oil may be a promising oral treatment for cutaneous leishmaniasis. **Acknowledgements:** This study was supported through grants by CNPq, FINEP, PRONEX/Fund. Araucária **References:** 1. Croft, S.L., Seifert, K., Yardley, V. (2006) Indian J Med Res 123:399 – 410. 2. Santos, A.O., Ueda-Nakamura, T., Dias-Filho, B.P., Veiga-Jr, V.F., Pinto, A.C., Nakamura, C.V. (2008) J Ethnopharmacol. 120:204 – 208.

P479 The trypanocidal action of eupomatenoid-5 isolated from *Piper regnellii var. pallescens* may be related to an imbalance between the antioxidant system and ROS

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Due to the severe side effects and variable efficacy, the current treatment for Chagas disease is still unsatisfactory. Natural compounds are good alternative chemotherapeutic agents for treatment of this infection [1]. Our group has reported antiproliferative activity and morphological alterations of eupomatenoid-5 isolated from leaves of Piper regnellii var. pallescens against epimastigotes and intracellular amastigotes of T. cruzi [2]. Here, we assessed the effects of eupomatenoid-5 on trypomastigotes, and investigated the possible mechanism of action of this compound on T. cruzi. Eupomatenoid-5 exhibited activity against trypomastigotes, where 50% of the cells were non-viable with 40.5 μM of the compound. Ultrastructural analyses showed effects on the cytoplasmic membrane and vacuole formation. Treatment with eupomatenoid-5 for 24 h, increased lipoperoxidation 6-fold in trypomastigotes and 2-fold in epimastigotes. Cytometry analysis of rhodamine 123-stained T. cruzi showed depolarization of mitochondrial membrane potential in 29.0 and 62.8% of epimastigotes treated with 34.0 and 51.0 μM of eupomatenoid-5, respectively, after 96 h of incubation. Eupomatenoid-5 also increased G6PD activity by 265, 1,403 and 3,601% after treatment with 34.0, 85.0 and 170.0 μM for 24 h, followed by an increase in H2O2 consumption in epimastigotes. Although T. cruzi possesses different ROS detoxifying mechanisms, they seem to be less efficient than those of mammals. Therefore, the parasite detoxifying systems may be considered a target for drug development. Our results indicate that the trypanocidal action of eupomatenoid-5 may be associated with the impairment of antioxidant systems, causing oxidative stress that can trigger destructive effects on biological molecules such as lipids and proteins, leading to parasite death. **Acknowledgements:** This study was supported through grants from DECIT/SCTIE/MS and MCT by CNPq, FINEP, PRONEX/Fund. Araucária **References:** 1. Ioset, J.R. (2008) Curr Org Chem. 12:643 – 666. 2. Luize, P.S., Ueda-Nakamura, T., Dias Filho, B.P., Cortez, D.A.G., Morgado-Diaz, J.A., Souza, W., Nakamura, C.V. (2006) Parasitology Research 100:31 – 37.

P480 Anti-promastigote activity of dillapiole and isodillapiole against *Leishmania chagasi*

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Leishmaniasis is still a worldwide health problem with 12 million people infected around the world and with 200 million people living at risk areas in 80 countries. Pentavalent antimonals are the main line treatment for leishmaniasis. Disadvantages such as costs, long-term treatment, side effects and low efficacy against and resistance to many strains have been reported. The search for newer substances obtained from natural products that can be used as prototypes potentially superior semi-synthetic analogues is currently an area of great interest. Dillapiole is the major component of the essential oil extracted from *Piper aduncum*. The aim of this work was to evaluate the leishmanicidal activity of dillapiole and the semi-synthetic analogues isodillapiole against promastigotes of *L. chagasi*. The anti-promastigote activity was evaluated as proposed by Delorenzi et al (2001). Promastigotes were incubated in the presence of different concentrations of Dillapiole and Isodillapiole, which were added only once to the cultures. After 3 days at 26 °C, parasite survival was estimated by counting viable or motile forms. Both drugs exhibited a strong anti-promastigote activity, reducing parasite survival in 99 and 96%, respectively, when we used 50 μg/ml of the drugs. Both drugs also showed a concentration dependent activity, however, isodillapiole was less active in lower concentrations. DMSO 1% was used as solvent control. No toxicity to the macrophages was observed after the treatment with the drugs, as measured by their spreading and adherence to glass surface. **Acknowledgements:** Hebron Farmacêutica, MackPesquisa, PIBIC/Mackenzie. **References:** 1. DELORENZI et al. DOI:10.1128/AAC.45.5.1349 – 1354.2001.

P481 Antiviral activity of ethanol extracts of *Ficus benjamina* and its fractions in vitro

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The antiviral activity of *Ficus benjamina* ethanol leaf extract and its fractions against Herpes Simplex Virus -1 and 2 (HSV-1, HSV-2), Varicella-Zoster Virus (VZV), Murine Sarcoma Virus (MuSV) and Moloney Murine Leukemia Virus (MuLV) were investigated in vitro. Ficus binjamina is known to be resistant to various plant viruses (1). Leaf extracts of F. binjamina inhibited all studied viruses. The fraction eluted with 80%-MeOH completely blocked HSV-1, HSV-2. There was an indirect evidence for strong interactions between the plant extracts and the viruses and weak interactions with the cell surface. It is suggested that plant extracts exerted their anti-herpetic effect mainly by blocking the virus access to the host cells. Three flavonoids from this fraction are thought to be responsible for antiviral activities. Identification of their structures is in progress. The fraction eluted with 20%- MeOH (polysaccharide fraction) significantly inhibited VZV, MuSV and MuLV, whereas the fraction eluted with 60%- MeOH (polyphenol fraction) significantly inhibited MuSV and MuLV. **References:** 1. Petrov D.B., Gerberov S.A., Nechaev S.T., Gubarev D.K (1974) Virus infection of decorative plants, Journal of Botany, 4: 23 – 26.

P482 Antimalarial activity of triterpenoids with the cucurbitane skeleton

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Malaria, caused by protozoan parasites of the genus *Plasmodium*, is a devastating infectious disease in tropical and subtropical countries. One of the biggest problems that have hindered the control of malaria is the emergence and spread of drug resistant *Plasmodium* strains, particularly *Plasmodium falciparum*. In order to overcome this problem, new therapeutic agents based on new mechanisms of action or with new structures are urgently needed. Plants have been an important source of medicines against malaria. Quinine and artemisinin, two of the most important antimalarials currently in use, were derived from plants [1,2]. *Momordica balsamina* L. (Cucurbitaceae), a vegetable used as food, has also been widely used in traditional medicine, mainly for the treatment of fever and malaria in Mozambique and South Africa [3]. Continuing our search for biologically active compounds from Momordica balsamina [4,5], the bioassay-guided fractionation of the methanol extract of the aerial parts of this plant led to the isolation of three new cucurbitane-type triterpenoids, balsaminols C-E. Their structures were elucidated on the basis of spectroscopic methods including 2D NMR experiments (COSY, HMQC, HMBC and NOESY). These compounds together with ten cucurbitane-type triterpenoids previously isolated [4,5] were evaluated for their antimalarial activity against two different P. falciparum strains (3D7 and Dd2). Their cytotoxicity was also assayed against human breast cancer cells (MCF-7). Most of the compounds displayed antimalarial activity against both the chloroquine-sensitive strain 3D7 and the chloroquine-resistant clone Dd2 of *P. falciparum*. They were inactive or showed weak toxicity against the MCF-7 cell line. **Acknowledgements:** The authors wish to thank the Science and Technology Foundation, (FCT, grant SFRH/BD/22321/2005). **References:** 1. Turschner, S. et al (2009) Mini-Rev. Med. Chem. 9: 206 – 14. 2. Wells, T.N.S. et al (2009) Nat. Rev. Drug Discov., 8: 879 – 91. 3. Bandeira, S.O. et al (2001) Pharm. Biol. 39: 70 – 3. 4. Ramalhete, C. et al (2009) Bioorg. Med. Chem. 17: 6942 – 51. 5. Ramalhete, C. et al. J. Nat. Prod. 72: 2009 – 13.

P483 Antibacterial activity against *Staphylococcus epidermidis* and inhibition of glucose-stimulated oxygen consumption by *Garcinia mangostana* extract

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Staphylococcus epidermidis, a skin flora, is one of the major opportunistic pathogen which has been recognized as a leading cause of nosocomial infections [1]. Due to multi-drug resistance of *S. epidermidis* [2], the search for medicinal plant-derived antibacterial agents against this pathogen has accelerated in recent years. It has been previously reported that mangosteen, *Garcinia mangostana*, exhibits an antimicrobial action against certain bacteria, fungi and viruses [3]. Our current study is to investigate the antibacterial activity of *G. mangostana* extract against *S. epidermidis*. *G. mangostana* used in this study was collected from Chumporn province located in the southern region of Thailand. The crude extract was prepared by extracting the pericarps with ethanol, and the extract was then used to evaluate the antibacterial activity against *S. epidermidis*. The result showed that the ethanolic extract of *G. mangostana* exhibited the antimicrobial effect against *S. epidermidis* with MIC of 50 μg/ml. In addition, we had observed that the extract inhibited the glucose-induced increase in oxygen consumption of intact *S. epidermidis* cells. This result suggested that the extract interfered with the bacterial glucose metabolism; and this action may play a role in the antibacterial activity of *G. mangostana* extract against *S. epidermidis*. **References:** 1. Ziebuhr, W., Hennig, S., Eckart, M., Kränzler, H., Batzilla, C., Kozitskaya, S. (2006) Int J Antimicrob Agents. 28:14 – 20. 2. Raad, I.; Alrahwan, A.; Rolston, K. (1998) Clin. Infect. Dis. 26:1182 – 1187. 3. Pedraza-Chaverri, J., Cárdenas-Rodríguez, N., Orozco-Ibarra, M., Pérez-Rojas, JM. (2008) Food Chem Toxicol. 10:3227 – 3239.

P484 In vitro anti-bacterial activity of cumin (*Cuminum cyminum* L.) and tarragon (*Artemisia dracunculus* L.) extracts against clinical isolates of *Helicobacter pylori*

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Helicobacter pylori infection is the most common gastrointestinal bacterial disease worldwide. Since antibiotic resistance of *H. pylori* is increasing and sometimes the cure achieved with antibiotics is not successful, so introduction of new therapeutic agents for treatment or prophylaxis is important. Cumin (Apiaceae family) and tarragon (Asteraceae family) are plants that are native to Iran and it has been reported they are traditionally beneficial in gastric problems [1]. In this study their activity against *H. pylori* were examined. Percolated methanol and aqueous extracts of plant leaves were examined against 45 clinical isolated of *H. pylori*. Growth inhibition was determined by the filter paper disc diffusion method on Mueller-Hinton agar containing egg yolk emulsion [2] compared with amoxicillin and metronidazole standard discs [3]. The effect of both methanol extracts was significantly higher than that of the aqueous extracts ($P < 0.001$). Methanol extract of tarragon and aqueous extract of cumin exhibited the most and the least anti- *Helicobacter pylori* activity, respectively. The minimum inhibitory concentrations (MIC_s) of methanol extract of cumin and tarragon were 691 μg/ml. Both of the two methanolic extracts preserved their anti- *Helicobacter pylori* activity after autoclaving for 20 min. Preliminary phytochemical screening of the cumin methanol extract indicated the presence of saponins and of the tarragon methanol extract indicated presence of saponins and tannins. This study demonstrated that tarragon and cumin leaves inhibited the growth of *H. pylori* strains in vitro. **Acknowledgements:** This study was supported by Islamic Azad University, Mashhad Branch, Iran for the Scientific Research Project. **References:** 1. Zargari A, (1997) Medicinal plants. Tehran University Press. Iran. 2. Tabak M, et al. (1999), J. Ethnopharmacol. 67(3): 269 – 77. 3. McNulty C, et al. (2002) J. Antimicrob. Chemother. 49: 601 – 4.

P485 Screening of Turkish plants for antimalarial and cytotoxic effects

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Malaria is the number one parasitic disease worldwide with half of the world's population at risk [1]. Natural products have had an enormous impact in malaria chemotherapy as the majority of current antimalarial agents are natural products or derive from a natural product scaffold isolated from plants traditionally used against malaria. Malaria chemotherapy has become problematic due to resistance development by the parasite against many antimalarial drugs, so new drugs are desperately needed. Previous studies have shown that members of the *Anthemis*, *Salvia* and *Scrophularia* genera display significant antiplasmodial potential [2 – 6]. In this study, we assessed the in vitro activity of the Turkish plants *Anthemis cretica subsp. anatolica*, *A. pestalozzae* (Asteraceae), *Salvia virgata* (Lamiaceae), *Scrophularia lucida* and *S. pinardii* (Scrophulariaceae) against the chloroquine- and pyrimethamine/sulfadoxine-resistant *Plasmodium falciparum* strain K1. The cytotoxicity of the extracts was determined against KB cells to evaluate their selectivity. All plants except *S. virgata* are endemic to Turkish flora and unstudied. The aerial parts and the roots of the plants were extracted separately by maceration with MeOH. All crude extracts showed good to moderate inhibitory potential against *P. falciparum* with no toxicity towards human cells. Hence, they were subjected to a solvent partitioning scheme to yield three subextracts (*n*-hexane, $CHCl_3$ and aq. MeOH). The $CHCl_3$ subextracts exhibited the best antiplasmodial activities with IC_{50} values in the range of 2.2 – 5.6 μg/ml, except for both *Scrophularia* roots

where the *n*-hexane subextracts displayed the best inhibitory effects. **Acknowledgements:** Funding from the School of Pharmacy is gratefully acknowledged. **References:** 1. WHO World Malaria Report (2009) WHO, Switzerland. 2. Tasdemir, D. et al. (2005) Phytochemistry 66:355 – 362. 3. Kamatou, G. et al. (2007) S. Afr. J. Bot. 73:102 – 108. 4. Tasdemir, D. et al. (2008) Phytomedicine 15:209 – 215. 5. Kamatou, G. et al. (2008) S. Afr. J. Bot. 74:238 – 243. 6. Karioti, A. et al. (2008) Phytomedicine 15:1125 – 1129.

P486 Antifungal screening of six *Ficus* species native to Zambia

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The genus *Ficus* (Moraceae) is well documented for antioxidant, anticancer, antidiarrhoeal, antimicrobial, antiplasmodial, antiulcer and gastroprotective activities [1]. In Zambia, the milky latex of some *Ficus* sp. are traditionally used against ringworms, while other plant parts are used to wash wounds, decoctions are also used for chest infections, stomach problems and fevers [2,3]. The folk-medicinal knowledge in Zambia has really not being collected and the potential of the plants not investigated. In this study, we collected the milky latex as well as the leaves and stem barks of six Ficus sp. (*F. sycomorus*, *F. sansibarica, F. ovata, F. wakefieldii, F. lutea and F. natalensis*) that are native to Zambia. The in vitro antifungal activity of the latex (used directly) and the crude MeOH extracts of the leaves and stem barks were assayed against clinical cultures of two fungi causing ringworm infections, *Trichophyton tonsurans, T. interdigitale*, as well as a yeast -*Candida albicans* and a mould -*Aspergillus fumigatus*. The Sabouraud agar plate disc and well diffusion techniques were used with miconazole as the positive control and MeOH the negative control. Except for the milky latex of *F. sansibarica*, all other latexes and the crude extracts were inactive at 100 mg/ml. Relevant studies by other reseachers have however, reported significant activity of the crude MeOH extract of *F. ovata* against a clinical isolate of *C. albicans* [1]. This activity is attributed to the presence of terpenoids, isoflavonoids and phenolic acids. This is the first antifungal screening study evaluating the antifungal activity of native Zambian *Ficus* sp. against fungi. **Acknowledgements:** The Commonwealth scholarship commission and the Rick-Cannell Travel Fund of the School of Pharmacy are acknowledged for funding. **References:** 1. Kuete, V. *et al.* (2009). J Ethopharmacol. 124:556 – 561. 2. Fowler, D.G. 2007. Zambian Plants: Their vernacular names and uses. 3. John Burrows and Sandra Burrows. 2003. Fig of Southern and South-central Africa. Umdaus, Hatfield 0028. South Africa. Check abstract instructions

P487 New polyketides from the endophytic fungus *Corynespora cassiicola* isolated from the Chinese mangrove plant *Laguncularia racemosa*

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The endophytic fungus *Corynespora cassiicola* was isolated from the leaf tissues of the Chinese mangrove medicinal plant *Laguncularia racemosa*. The EtOAc extract of the fungus, which was grown on solid rice medium, exhibited considerable cytotoxic activity against Hela cell line, as well as antimicrobial activity (when tested in vitro). Chemical investigation of the extract yielded twelve new secondary metabolites, including corynecassiicol A (**1**) and its isomer corynecassicol B (**2**), corynesidone D (**3**), seven octalactones, coryneoctalactone A-G (**4-10**) and two decalactones, xestodecalactone D and E (**11** and **12**), together with four known compounds. The structures of the isolated compounds were determined on the basis of one- and two-dimensional NMR spectroscopy as well as mass spectrometry.

P488 Chemical constituents of *Glycosmis parva* and their anti-herpes simplex virus activities

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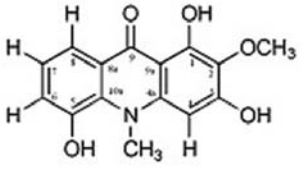
The investigation of EtOAC of branches and leaves of *Glycosmis parva* Craib [1, 2] (Rutaceae) collected in Thailand led to the isolation of a new acridone alkaloid, glycosparvarine (**1**), three new sulfur-containing propanamide derivatives, (+)-*S*-deoxydihydroglyparvin (**2**), (+)-*S*-deoxytetrahydroglyparvin (**3**) [3] and (+)-tetrahydroglyparvin (**4**), together with nine known compounds (*N*-methylatalaphylline, *N*-methylcyclo-atalaphylline-A, glycofolinine, citramine, arborinine, limonin, a mixture of limonexic acid and isolimonexic acid, glyparvin-A and dihydroglyparvin). Antiviral activity evaluation of isolated compounds against herpes simplex virus (HSV) type 1 and 2 disclosed that glycosparvarine (**1**), (+)-tetrahydroglyparvin (**4**) and glycofolinine, showed moderate inhibitory activities with EC_{50} of 348, 229 and 151 μM, respectively. In addition, (+)-*S*-deoxydihydroglyparvin (**2**) exhibited activities with EC_{50} of 29.8 and 44.6 μM against HSV-1 and HSV-2, respectively.



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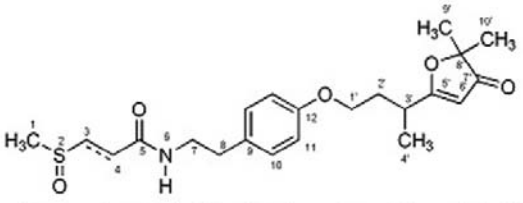

2; unsaturated (double bond on C-3 and C-4))
3; saturated

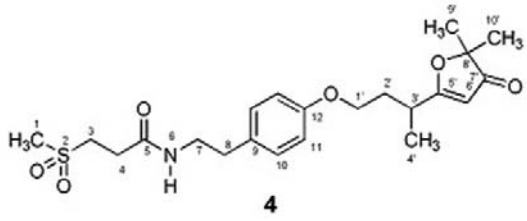

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Fig. 1

Acknowledgements: Royal Golden Jubilee (RGJ) Ph.D. program 2004 (PHD/0212/2547), Thailand Research Fund **References:** 1. Stone, B. C. (1985) Proc. Acad. Nat. Sci. Phila. 137:1 – 27. 2. Hofer, O. *et al.* (1998) Monatsh. Chem. 129: 213 – 219. 3. Chansriniyom, C. *et al.* (2009) Chem. Pharm. Bull. 57:1246 – 1250.

P489 Effects of three functional plant products on growth performance and diarrhea incidence in weaning piglets

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The aim of this investigation was to examine the effect of three functional plant products in the prophylaxis of post-weaning diarrhea. The feeding trial included 184 piglets. On day 3 before weaning the piglets were divided into three experimental groups and one control group by

compensating randomization. The experimental diets were blended with different amounts of either lignocellulose ("Agrocell"), oligogalacturonides ("Enteronid") or herbs ("Herbenterosan", consisting of Tormentillae rhizoma, Matricariae flos, Taraxaci, Rhapontici carthamoides herba, Carvi fructus, Allii sativi bulbus). Lignocellulose and oligogalacturonides are known to affect diarrhoea in weaning piglets favourably [1, 2]. Once a week the piglets and feed residues were weighed. During five days from day 4 following weaning faeces were appraised with a faecal score. Blood samples were collected for the analysis of haptoglobin, sodium, potassium and chloride. The Herbenterosan-group had the lowest group sum in the faecal score from day 4 to 8 (fig. 1). Even though the Agrocell-group had the worst faeces the piglets showed the highest weight gain from day 4 to 11. Compared to the control-group the piglets of all experimental groups gained significantly more weight from day 4 to 11 ($p < 0,05$). During the whole experimental period the piglets of the Herbenterosan-group showed the highest weight (table 1). The control-group showed the lowest weight gain. There was no significant difference in haptoglobin between the groups ($p > 0,05$). The trend of the electrolytes coincided with the trend of the faecal score: groups with a better faecal score had better values of electrolytes and conversely. By using functional plant products post-weaning diarrhea was not prevented. Nevertheless weight gain was affected favorably.

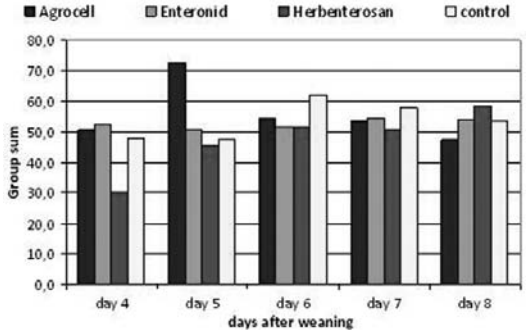

Fig. 1: Faecal score between day 4 and 8 liveweight and gain

Table 1

		C (Control group)	A (Agrocell)	E (Enteronid)	H (Herbenterosan)
starting live-weight (kg, day 0)	M	11,89	11,91	12,02	11,92
	SD	2,25	2,27	2,41	2,35
	N	46	46	46	46
final liveweight (kg, day 25)	M	20,99	21,22	21,43	21,55
	SD	4,55	3,62	4,13	3,92
	N	42	44	43	44
average daily gain (g/piglet/day from day 0 – 25)	M	357	374	373	390
	SD	135	89	108	94
	N	42	44	43	44

M = mean, SD = standard deviation, N = numbers of piglets

Keywords: pig – diarrhoea – average daily gain – feed conversion – haptoglobin – herbs – lignocellulose – oligogalacturonides **References:**
1. Kastner, U., Glasl, S., Follrich, B., Guggenbichler, J.P., Jurenitsch, J. (2002): Saure Oligosaccharide als Wirkprinzip von wäßrigen Zubereitungen aus der Karotte in der Prophylaxe und Therapie von gastrointestinalen Infektionen. Wiener Medizinische Wochenschrift 152: 379 – 381.
2. Kroismayr, A. (2008): Lignocellulose – fresh wood as dietary fibre. PIG PROGRESS 24: 33 – 35.

P490 Antimicrobial activities of extracts and decussatin from *Ficus congensis* (Engl)

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Decoctions prepared from the stembark and leaves of Ficus congensis, Fc (Engl) are used by the Southwestern Egun people of Nigeria in the management of"fevers"(translated in local language as iba) arising from infections. This has served as the driving impetus for the present investigational work on this plant. Hexane (stembark) and Methanol (leaf) extracts of Fc were evaluated for antifungal and antibacterial activities using Disk Agar diffusion (DAD) and Brothmicrodilution (MHB) respectively. Hexane stembark extracts subjected to chromatographic separation, followed by spectral analysis, afforded the xanthone:1-hydroxy3,7,8-trimethoxyxanthone (Decussatin)1as main constituent. The extracts and Decussatin, were screened for antifungal and antibacterial activities. Methanol leaf and Hexane stembark extracts were active against Candida albicans alone (zone of inhibition-20.00 mm &18.9 mm) at MIC of 2mgml-1 and 1mgml-1 respectively, out of the strains of fungi tested-Aspergillus fumigatus, Trichophyton mentagrophytes, Trichophyton rubrum. Methanol leaf extract showed Antibacterial activity against E. coli and B. substilis at MIC(3mgml-1,2mgml-1) respectively. Hexane stembark extract showed Antibacterial activity against E.coli only at MIC of 5mgml-1. No activity was observed in K. pneumonia and S. aureus. However, Decussatin showed little activity at the maximum concentration used;10 and 8 mgml-1for antifungal and antibacterial activities respectively. Spectral analysis were carried with the aid of IR&UV (Perkin Elmer), GC – MS (Agilent 5973 Network Plus),1D&2D NMR (600 MHz, TopsinBruker). At this point, is possible to infer a link between the observed biological activities and folkloric claim of decoctions of Fc. We are reporting the existence of a known xanthone, Decussatin, in Fc which could be of great importance to the chemotaxonomy of this family. This is the first time it is reported in this plant species habiting in a swampy area in Lagos, Nigeria. **Acknowledgements:** The authors are grateful to School of Chemistry and School of Biological Sciences, University of Kwazulu-Natal, Westville Durban, South Africa for their laboratory services. **References:**
1. Dalal, S.R., Sethna, and Shan, R.C.(1953) J.Indian Chem.Soc. 30, 457 – 463.

P491 Triple mode of action of the fresh plant tincture Echinaforce®

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More than 200 viruses cause influenza-like infections (ILI) presenting with nasal complaints, sore throat, cough and sometimes with fever [1]. Most ILI are treated with over-the-counter drugs to reduce the viral spread (antiviral) and the infection-based inflammation (anti-inflammatory), which finally elicits the cold symptoms [2]. Echinaforce® (ECF) is used for the prevention and the acute treatment of URI's and we wanted to elucidate how the efficacy could be explained. Using in-vitro test systems we identified a threefold action for the extract. Already at lowest concentrations ECF fully inhibited the replication of different cold viruses (influenza, respiratory syncytial (RSV) and herpes simlex virus) and inhibited – once the infection had established – the production of various inflammatory mediators (Interleukin IL-6, IL-8 or TNF-a). Moreover we could identify specific anti-bacterial effects against a variety of bacteria like *Haemophilus influenzae*, *Streptococcus pyogenes* and *Legionella pneumophilia*. These pathogens often are associated with secondary infections like pneumonia or bronchitis. Finally, ECF blocked the inflammatory reaction, caused by bacteria, as demonstrated by reduced levels of IL-6 or IL-8. In our experiments we showed that ECF exhibits multiple bioactivities, which could explain the effects as seen in clinical studies. The acute treatment with ECF further might deliver a positive effect to prevent secondary infections, often occurring at a later stage during viral infection. In conclusion, ECF represents an interesting option for the prevention and the treatment of URI, displaying multileveled activities in the management of upper respiratory tract infections. [**References:**
1. Monto AS. Epidemiology of viral respiratory infections. Americal Journal of Medicine. 2002;112(6A):4 – 12. 2. Johnston SL. Problems and prospects of developing effective therapy for common cold viruses. Trends in Microbiology. 1997;5(2);58 – 63.

P492 Anti-viral activities of herbal preparations

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Upper respiratory tract infections (URI) represent the most frequent infectious disease in the western civilisation and 90 – 95% are of viral origin [1]. Many herbal cold remedies own anti-viral activity, which in some cases is directed to viruses not involved in respiratory infections and the term "antiviral" in the context of their use maybe misleading. In

our experiments we compared different herbal preparations, which are traditionally used for the treatment of URI. Thirty (30) preparations from twenty (20) plants were tested for their antiviral activity against the most frequent cold viruses. No significant effect was observed against RV or adenovirus for any of the extracts tested. Membrane-coated viruses like influenza, RSV or HSV were more sensitive to the samples. *Mentha piperita*, *Pelargonium sidoides* and *Cistus incanus* extracts showed moderate to strong activity against H3N2 virus and *Tilia* ssp and *Sambucus nigra* were effective against RSV. Only weak activity was observed against HSV by *Tilia* ssp and *Sambucus nigra*. A combination product of *E. purpurea* herba and root showed extraordinary antiviral effects against influenza, RSV and HSV simultaneously. The effects exceeded the otherwise measured by a factor 4 to 40. Our experiments indicate that many traditionally used plant extract exhibit moderate effects on virulence or replication of the most prominent cold viruses. Some extracts exhibit activity against a particular virus but not against others. A special *E. purpurea* extract however did show remarkable antiviral activity against different respiratory viruses at physiologically relevant concentrations. **Acknowledgements:** Funding from Bioforce AG Switzerland. **References:** 1. Gwaltney JM. Clinical significance and pathogenesis of viral respiratory infections. The American Journal of Medicine. 2002;112 (6A):13 – 18.

P493 Flavonoids from the leaves of *Cryptocarya chinensis* with antituberculosis activity

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Cryptocarya chinensis (Hance) Hemsl. (Lauraceae) is a medium-sized evergreen tree, distributed throughout southern China, Japan, and Taiwan. Flavonoids, pyrones, pavines, aporphines, benzylisoquinolines, lignans, and their derivatives are widely distributed in plants of the genus Cryptocarya, and many of these compounds exhibit cytotoxic and antioxidant activities. In our continuing studies on the antitubercular constituents of Formosan plants, over 400 species have been screened for in vitro antituberculosis activity to date, and *C. chinensis* has been found to be one of the active species. Studies on the neutral $CHCl_3$ fraction of leaves from this species has led to the isolation of two new tetrahydroflavanones, cryptochinone G (**1**) and cryptochinone H (**2**), and two new flavanones, crytoflavanone A (**3**) and crytoflavanone B (**4**), together with 8 known compounds (**5-12**). The structures of these new compounds were determined through spectroscopic analyses, including extensive 2D-NMR, ORD, MS and, IR. Among the isolates, cryptocaryone (**5**), cryptocaryanone B (**6**), and pinocembrin (**7**) showed antituberculosis activities with MICs of 25.0, 25.0 and 3.5 ug/mL against *Mycobacterium tuberculosis*, respectively. The structural elucidation of **1-4** and the antituberculosis activities of the isolates will be discussed in this symposium.

P494 Anti-dermatophyte constituents of the essential oil from the root of *Ferula hermonis*

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In a previous work, the essential oil from the dried root of *Ferula hermonis* Boiss. (Umbelliferae), whose major constituents were alpha-pinene (43.3%), alpha-bisabolol (11.1%) and the unusual acetylene 3,5-nonadiyne (4.4%), showed moderate antifungal activity against *Trichophyton mentagrophytes* [1]. With the aim of characterizing the active constituents, a bioguided fractionation of the essential oil was performed. Twenty seven fractions were obtained and submitted to GC-FID, GC-MS and 13C-NMR analyses, as well as to an agar overlay bioautographic assay for the detection of the activity against *T. mentagrophytes* and *Microsporum gypseum*. Growth inhibition zones of different magnitudes were observed in the case of fraction 2, fraction 10 and from fraction 17 to 27. In fraction 2 the activity was directly related to alpha-pinene, whereas in fraction 10 the major constituent was 3,5-nonadiyne. Successive fractionation of the essential oil allowed the isolation of the main constituents of the active fractions 17 to 27: alpha-bisabolol, alpha-bisabolol oxide B, trans-verbenol, jaeschkeanadiol angelate, and two purified fractions, one of them with 73% of jaeschkenadiol benzoate and the other with 50% of spathulenol. Activity against both dermatophytes was evaluated from the MIC and MFC values, which ranged from 0.25 to 128 μg/ml. The most potent activity was demonstrated by the fraction with 73% jaeschkeanadiol benzoate against *T. mentagrophytes*, with the same MIC and MFC values of 0.25 μg/ml, equivalent to that one of ketoconazole (0.25 μg/ml) and superior to amphotericin B (0.5 μg/ml) and nystatin (2 μg/ml). Both dermatophytes were highly sensitive to the unusual compound 3,5-nonadiyne whose MIC and MFC's for *T. mentagrophytes* were 8 μg/ml, whereas in the case of *M. gypseum* MFC value was about two-fold the MIC value (8 and 16 μg/ml, respectively). **Acknowledgements:** AA Bdwan, JPM (Naor, Jordan) and TS El-Thaher, ARAGEN Biotechnology (Naor, Jordan) for providing plant material. **References:** 1. Al-Ja'fari AH et al. (2008) 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, Athens, Greece.

P495 Antibacterial activity of pimarane-type diterpenes against endodontic pathogens

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In the present work, the in vitro antimicrobial activity of six natural pimarane-type diterpenes and two semi-synthetic derivatives were investigated against a panel of representative microorganisms responsible for dental root canal infections [1] (*Porphyromonas gingivalis*, *Prevotella nigrescens*, *Prevotella intermedia*, *Prevotella buccae*, *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Actinomyces naeslundii*, *Actinomyces viscosus*, *Peptostreptococcus micros*, *Enterococcus faecalis* and *Aggregatibacter actinomycetemcomitans*). The broth microdilution method [2] was used for the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The diterpenes ent-pimara-8(14),15-dien-19-oic acid (1), its sodium salt (2) and ent-8(14),15-pimaradien-3β-ol (3) (Figure 1) were the most active compounds, displaying MIC values very promising [3]. Ours results also allow us to conclude that minor structural differences among these diterpenes significantly influence their antimicrobial activity, bringing new perspectives to the discovery of new chemicals for use as complement of the instrumental endodontic procedures.

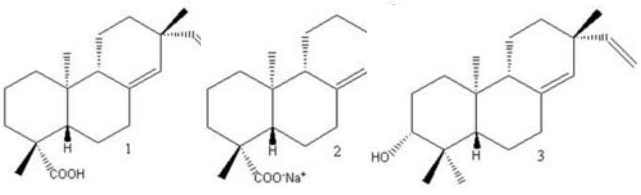

Fig. 1: Structures of the most activity diterpenes

Acknowledgements: FAPESP (Proc. 2009/18278 – 0) and CAPES **References:** 1. Gomes et al. (1996) Endod J. 29: 235 – 241. 2. Porto et al (2009) Molecules 14: 191 – 199. Gibbons (2008) Planta Med. 74: 594 – 602.

P496 In vitro antibacterial activity of saffron (*Crocus sativus* L.) extract and its two major constituents against *Helicobacter pylori*

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Helicobacter pylori (HP) is the principal cause of chronic gastritis, peptic ulcer and gastric carcinoma [1]. Although there are several drug treatment regimes for these infections, sometimes eradication failure and side effects of drugs are observed [2]. Saffron is a spice that is native cultivated in both Iran and Spain and has been reported in the folk medicine as a stomach tonic [3]. Percolated methanol and aqueous extracts of saffron were tested against 45 clinical isolated of HP by filter paper disc diffusion method on modified egg yolk emulsion agar in comparison with amoxicillin (25 μg/disc) and metronidazole (5 μg/disc). There was a significant difference between methanol (17.1 ± 3.1 mm) and aqueous (14.4 ± 2.6 mm) extracts (2 mg/disc) of saffron ($p < 0.001$). The minimum inhibitory concentration (MIC) of the methanol extract was 677 μg/ml determined by agar dilution method. There was a significant difference between safranal and crocin (principal constituents of saffron) activity against 9 clinical strains of HP ($p < 0.001$). The average of MIC and MBC of crocin for these 9 strains were 263 and 300 μg/ml respectively. MIC and MBC of safranal were 16.6 μg/ml. After autoclaving both constituents preserved their antibacterial activity. The methanol extract and crocin preserved their activity at pH = 5. **Acknowledgements:** This study was supported by Islamic Azad University, Mashhad Branch, Iran for the Scientific Research Project. **References:** 1. Nachamkin I. and Skirrow M.B. (1998), Topely and Willson's Mcirobiology and Microbial Infections, Oxford University Press, London. 2. kuipers E, et al. (2003), Curr Opin Pharmacol, 3(5): 480 – 85. 3. Zargari A, (1997) Medicinal plants. Tehran University Press. Iran.

P497 Preparation and characterization of PLGA nanoparticles containing demethylfruticulin A from *Salvia corrugata* Vahl. (Lamiaceae)

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Demethylfruticulin A (SCO1) is the major diterpenoid component of the exudate produced by the trichomes of *Salvia corrugata* leaves. The activity of this new compound against Gram-positive pathogens [1] and the apoptosis effect on mammalian cell lines [2] were investigated in previous works. The aim of this study was to evaluate the possibility of incorporating this natural product into PLGA nanoparticles in order to improve its solubility in the aqueous medium and to favour the interaction with bacterial membrane. Nanoparticles were prepared by an emulsion-diffusion-evaporation method, using Poloxamer 407 as a stabilizer. A Doehlert design for two variables was used to study the influence of the stirring time and the final volume of water on the size (D) and the zeta potential (Z) of the nanoparticles. The obtained nanoparticles showed interesting features: small size (D = 207 ± 8 nm), a negative zeta potential (Z = −14.0 ± 0.4 mV), an increasable drug loading (3% w/w) and high encapsulation efficiency (95% w/w). They also showed a good stability after redispersion in water for 30 days. These preliminary results indicate that PLGA nanoparticles can be designed as a carrier for delivery of SCO1, possibly allowing this new compound to exceed the intrinsic resistance of some bacteria to several antibiotics and to carry out its potential antitumour activity. **References:** 1. Bisio A., Romussi G., Russo E., Cafaggi S., Schito A.M., Repetto B., De Tommasi N. (2008) J. Agric. Food Chem. 56: 10468 – 10472. 2. Giannoni P., Narcisi R., De Torero D., Romussi G., Quarto R., Bisio A. (2010) Phytomedicine 17: 449 – 456.

P498 Antibacterial activity of acyl and epoxide derivatives of β-sitosterol and cycloartanes

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Nowadays, infectious diseases still remain the second leading cause of death worldwide [1]. The ability that microorganisms have to develop resistance to drugs along with population mobility and globalization have turned the possibility of epidemics/pandemics a really serious health problem. Currently, infectious diseases can not only spread faster, but they also appear to emerge more quickly than before [2]. There is, therefore, an increased need for new and more effective antibacterial chemotherapeutic agents. Plants have long been a source of medicines, and an impressive number of modern drugs have been isolated from plants. According to Cragg [3], from the 109 new antibacterial drugs approved by FDA during 1981 – 2006, 69% were natural products or natural product derivatives. The aim of this work was to find out new antimicrobial compounds from plant sources through derivatization of?-sitosterol and tetracyclic triterpenes with the cycloartane scaffold, previously isolated from Euphorbia species [4]. Triterpenes and steroids are a good raw material because they are easily obtained from Euphorbia crude extracts and in large amounts. Sixteen monoacylated derivatives at C-3 were prepared by using different carboxylic anhydrides or acyl chlorides. Epoxide derivatives of both original compounds and esters were also prepared. All compounds were tested against reference and multiresistant bacterial strains: Gram-positive (*Enteroccocus faecalis*, *Staphylococcus aureus* and *Mycobacterium smegmatis*); Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*) and yeast *Candida albicans*. The minimum inhibitory concentrations (MIC) were determined by using the serial broth microdilution method. Several derivatives have showed lower MIC values when compared to parent compounds (MIC: 30 – 73 μM) Remarkable enhancement on the antibacterial activity was observed for all benzoyl and para-substituted benzoyl chlorides (MIC: 28 – 60 μM) against *Staphylococcus aureus* and *Mycobacterium smegmatis*. **References:** 1. Courvalin P (2005) Emerg. Infect. Dis. 11:1503 – 06. 2. The world health report 2007 (2007) http://www.who.int/whr/2007/en/index.html. 3. Cragg, GM, Newman, DJ. (2001) Pharm. Biol. 39:8 – 1. 4. Madureira, AM et al. (2003) Nat. Prod. Res. 17:375 – 80.

P499 New semi-automated method for the creation of a microbial fraction collection developed at Fundación MEDINA

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Recently, several international scientific initiatives have promoted the production of highly diverse new libraries to support current research of pharmaceutical products. However, because just few of the initiatives are successful, there is always a constant need for more chemical diversity. The newly endowed Fundación MEDINA (*Fundación Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía*, Spain) is focusing much of its efforts on new drug discovery from unexplored Natural Product sources. The Fundación Medina's Natural Products Collection covers an uncommonly broad chemical space resulting from a variety of fermentation products prepared through new processes optimized for each type of microbial source. In addition, a collection of known compounds is on hand with which to quickly characterize new bioassays as well as to determine any assay interferences or other shortcomings in rapid and cost-effective fashion. assay interferences or other shortcomings in rapid and cost-effective fashion. We will report here several new semi-automated methodologies of innovative automated extraction and purification techniques, for the generation of new Natural Product collections by using: Different Filamentous fungi, Actinomycetes and other bacteria as source of secondary metabolites Different extraction protocols based on nature of starting materials (resin pre-adsorption of very dilute, water y broths; two-phase solvent extractions for lipid fungal strains and aqueous-based extracts for many actinomycetes) Automated Fractionation protocols tailor-made for the type of starting material (chromatography on highly retentive resins,

gel filtration, regular reverse-phase separations) – Bi-dimensional Fractionation Management making use of different physiochemical properties.

P500 Antimicrobial herbs against *Staphylococcus aureusa* and *Propionibacterium acnes*

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Acne is a common skin condition in adolescence and usually resulted in scarring. Its main effects are psychological, like self-esteem reduction and depression. Pathogenesis of acne are four major factors, including increased sebum production by overactive oil glands, retention hyperkeratosis to block the skin pores, activity of normal skin bacteria (*Staphylococcus aureus* and *Propionibacterium acnes*) and inflammation. In this study, 30 herbal plants were extracted by 50% methanol, and the anti-*S. aureusa* activity of herb extracts were evaluated. The results showed 50% methanol extracts of 7 herbal plants had more significant activity than the others, while the clear zone of *Hydrocotyle verticillata*, *Acanthopanax trifoliatus*, *Wikstroemia indica*, *Berchemia lineata* were all 11.0 mm; *Origanum majorana* was 11.5 mm; *Salvia plebeia* was 13.0 mm and *Myristica fragrans* was 13.5 mm. Among the 30 herb extracts, *M. fragrans* displayed the strongest anti-*S. aureus* activity and the minimum inhibitory concentration (MIC) was 0.64 mg. *P. acnes* was also pathogenesis of acne and was used as target bacteria in this study. The MIC of *M. fragrans* 50% methanol extract was 0.64 mg against *P. acnes*. *M. fragrans*, a high essential oil content spice, showed potential antimicrobial activity and anti-inflammation effects *in vitro* and *in vivo*. The essential oils of *M. fragrans* were extracted by hydrodistillation and analyzed by GC-MS. In agar well-diffusion method, the MIC of the essential oil from *M. fragrans* against *S. aureusa* and *P. acnes* were 80% and 20%, respectively. Therefore, we suggested that *M. fragrans* were a potential material to develop anti-acne cosmetics. **Keywords:** anti-acne, *Staphylococcus aureus*, *Propionibacterium acnes*, *Myristica fragrans* Houtt.

P501 Screening for wound healing effects in terrestrial fungi

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Despite improvements in conventional wound management adequate care of chronic wounds is still a challenge, especially in the light of demographic issues. As treatments are expensive and in part ineffective, new concepts and strategies improving chronic wound care are desirable. Alternative investigation focuses on atmospheric pressure plasmas, different honey types, and natural product research. To screen for stimulatory effects on wound relevant human cells an *in vitro* wound model (scratch assay) using HaCaT human keratinocytes (Boukamp et al., DKFZ Heidelberg) was established. Briefly, HaCaT monolayers were scratched using a 4-rowed cell scraper. Scratch areas were calculated after 24 and 48 hours. To minimize unspecific stimulation of cell migration/proliferation, cells were cultivated in low glucose medium containing only 1% FCS. *Calendula officinalis* crude extract, allantoin, ascorbic acid and dexamethasone served as controls. Watery and ethanolic resp. methanolic crude extracts from fruiting bodies of *Ganoderma lucidum, Ganoderma pfeifferi, Lentinula edodes, Calvatia gigantea* and *Piptoporus betulinus* were tested in different concentrations. A statistically significant reduced time to "wound closure" ($p \leq 0.05$; Tukey HSD) was found for watery and ethanolic *L. edodes* and methanolic *C. gigantea* extracts, allantoin and ascorbic acid. A reduced wound closure speed was found for the ethanolic extracts of both *Ganoderma* species and dexamethasone. In future, this assay shall be used to further screen crude extracts of terrestrial and marine origin for wound related effects. *L. edodes* extract will be fractionated bioassay guided to reveal the active compounds.

P502 Chemical and biological investigations of Manuka honey

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Manuka honey is mainly obtained from New Zealands endemic Myrtaceae *Leptospermum scoparium* J.R. et G.Forst. Its increasing clinical use in wound management originates from its antimicrobial effects. Recent work identified 1,2-dicarbonyl methylglyoxal (MGO) as a major antibacterial compound [1] which appears in Manuka honey in high levels and is formed from dihydroxyacetone during storage [2]. In this study several Manuka honeys were investigated for antibacterial activity, MGO content and phenolic compounds. Antibacterial testing was done by agar diffusion assay as well as in the epidermis model 'cow udder teat' [3]. Aqueous dilutions of Manuka honeys were able to inhibit growth of multi-resistant strains of *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. In these honeys, MGO amounts ranging from 400 to over 1000 mg/kg were found. However, no hydrogen peroxide was detected in Manuka honeys. In comparison: a rape honey contained only 3 mg/kg MGO but high amounts of hydrogen peroxide and showed inhibiting effects on both *Staphylococcus* strains. If MGO was added to a non Manuka honey the resulting bacterial inhibition was the same as for a Manuka honey with comparable MGO amount. Detected and quantified phenolic compounds such as phenyllactic acid or methyl syringate did not exert antimicrobial activity on the tested strains. Osmotic effects did not contribute to inhibiting effect. Therefore, it appears likely that observed clinical benefits of Manuka honeys are proportional to its 1,2-dicarbonyl methylglyoxal content. **References:** 1. Mavric, E et al. (2008) Mol. Nutr. Food Res. 52:483 – 489. 2. Adams, CJ et al. (2009) Carbohydrate Research 343:1050 – 1053. 3. Lukowski, G. et al. (2008) Skin Pharmacol Physiol 21: 98 – 105.

P503 Antimicrobial activity of *Thymus longicaulis* C. Presl essential oil against respiratory tract pathogens

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Thymus longicaulis C. Presl is a small aromatic perennial herb of Lamiaceae family and a typical representative of the Illyric-Mediterranean flora. This species is a traditional remedy for cold, flu, cough, nephritis and abdominal pain [1]. Present study aimed to evaluate composition of essential oil and its *in vitro* antimicrobial activity against major respiratory tract pathogens which show increasing resistance to commonly prescribed antimicrobials [2]. The yield of essential oil obtained by hydrodistillation from aerial plant parts was 12 ml/kg. According to the GC-MS analysis, a total of 41 compounds (99%) were identified. Thymol (46.3%), γ-terpinene (16.2%), thymyl methyl ether (11.4%), and p-cymene (9.4%) were the main components. Antimicrobial activity of the essential oil was determined using microdilution broth assay (tested concentrations were 0.2 – 25 mg/ml). Amoxicillin-clavulanate and fluconazole were used in order to control sensitivity of tested clinically isolated bacterial and yeast strains: *Haemophilus influenzae, Neisseria meningitidis, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes* and *Candida albicans*. The essential oil exhibited antimicrobial activity against all tested pathogens. The most sensitive strains were *H. influenzae* and *S. pneumoniae* (MIC 0.78 mg/ml), while S. aureus was the most resistant (MIC > 25 mg/ml). Our results revealed that *T. longicaullis* from Croatia is rich in essential oil characterized by a high content of thymol. A strong antimicrobial effect of the essential oil proved in this study indicates on its huge potential in the treatment of respiratory tract infections. **References:** 1. Kütür, S. (2007) J. Ethnopharmacol. 111:341 – 364. 2. Klugman KP. (2007) Int. J. Antimicrob. Agents 29:6 – 10.

P504

Antibacterial actvity and synergistic effect investigation of terpenoids from *Copaifera langsdorffii* Desf. against cariogenic bacteria

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In the present work, the anticariogenic activities of nine labdane type-diterpenes and four sesquiterpenes were investigated (Figure). Among these metabolites, (−)- copalic acid (2) was the most active compound displaying very promising MIC values (ranging from 2.0 to 6.0 μg mL-1) against the main microorganisms responsible for dental caries: *Streptococcus salivarius, S. sobrinus, S. mutans, S. mitis, S. sanguinis* and *Lactobacillus casei* [1]. Due to its highest anticariogenic activity, the synergistic effect of 2 combined with the anticariogenic gold standard (chlorhexidine, CHD) was investigated in the checkerboard assays [2] against the most important cariogenic microorganism (S. mutans). No synergistic effect was observed but the MIC values obtained allow us to conclude that 2 is an important metabolite in the search for new effective anticariogenic agents.

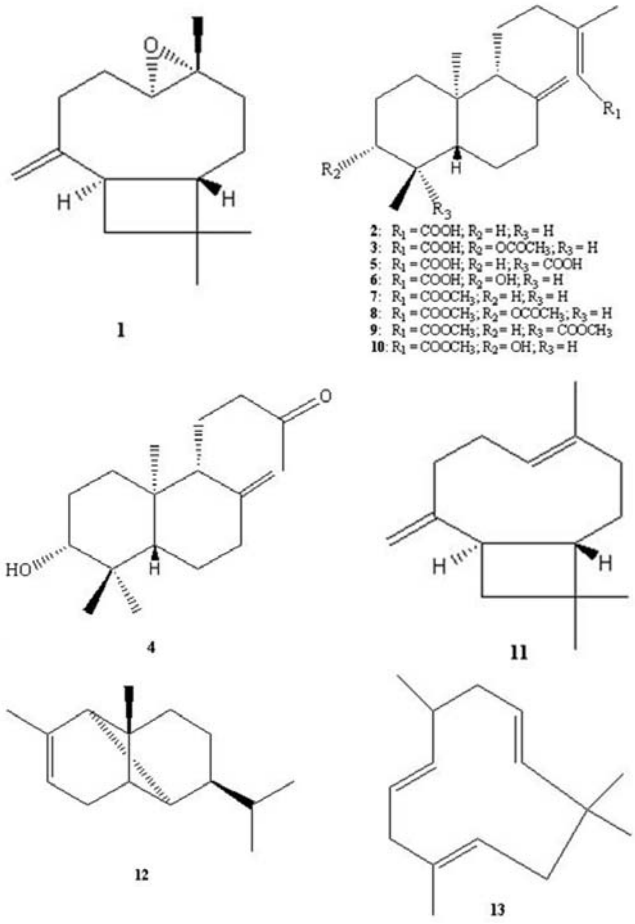

Fig. 1: Chemical structures of *C. langsdorfii* terpenoids

Acknowledgements: FAPESP (Proc. 2009/09438 – 3) **References:** 1. Chung et al. (2006) Phytomedicine 13: 261 – 266. 2. White et al (1996) Antimicrob Agents Chemother 40: 1914 – 1918.

P505

Anti-adenoviral substances isolated from medicinal plants: current status and future prospects

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Development of safe and commonly approved antiviral therapy and/or immunotherapy for infections caused by human double-stranded DNA adenoviruses (HAdVs) in a variety of immunocompromised hosts as AIDS patients, hereditary immunodeficient subjects, pediatric solid organ or haematopoietic steam cell transplant recipients, is still a challenging issue. Thus, the recently reported advances in the development of extraction, identification, cytotoxicity evaluation, proteomic profiling and antiviral activity assays protocols used for search of substances with medicinal plant origin which indicated selective and potent activity against human adenoviruses causing a number of self-limiting mild respiratory, gastrointestinal, genitourinary, ocular and obesity-related [1,2] infectious disease has been reviewed. The performed meta-analysis of these research showed that a still limited set of the plant derived active compounds as some catechins, hydroxyflavones, metoxyflavones, polyphenol acids, depsides, pentacyclic triterpenes, and terpene alcohols has been identified to cause *in vitro* and *in vivo* effects on specific human adenovirus inactivation and growth. A significant anti-HAdV activity at non-toxic concentration for some fractions of ethanolic- or water-type extracts obtained from a restrained number of medicinal plants with ethno-medical background and belonging to different plant families have been also described. However, further and more intensified research is needed to elucidate the active constituents of these plants and to establish detailed mechanism of their anti-adenoviral and virucidal effects to enable development of effective and marketable anti-HAdVs agents. **Acknowledgements:** Copernicus University, Torun, Poland (Internal Grant No. 407/2010). **References:** 1. Jaworowska, A., Bazylak, G. (2006) Postepy Hig. Med. Dosw. 60(1): 227 – 251. 2. Jaworowska, A., Bazylak, G. (2007) Adv. Clin. Exp. Med. 16(6): 743 – 749.

P506

Induction of hemeoxygenase-1 (Ho-1) by phagocytosis of apoptotic cells is a critical modulator of *Trypanosoma cruzi* infection

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Apoptotic cells are rapidly recognized and engulfed by professional phagocytes such as macrophages to avoid secondary necrosis and thus inflammation. Recognition of apoptotic cells polarizes macrophages toward an anti-inflammatory phenotype. However, mechanistic details provoking these phenotype alterations are incompletely understood. Previously our group has shown that there is intense lymphocyte apoptosis in an experimental model of Chagas' disease, a debilitating cardiac illness caused by the protozoan *Trypanosoma cruzi*. Here, we demonstrated a biphasic up-regulation of heme oxygenase-1 (HO-1), a protein that bears an anti-inflammatory potential, in infected murine macrophages, which were exposed to the apoptotic cells. The induction of HO-1 by apoptotic cell uptake or with single treatment with of the HO-1 inducer cobalt protoporphyrin (CoPPIX) correlated with increased number of infected macrophages and number of viable trypomastigote released. Also, induction of HO-1 correlated with increased production of anti-inflammatory factors (TGF-β and PGE-2), and decreased production of TNF-α and nitric oxide (NO). Infected macrophages cocultured with apoptotic cells in the presence of the HO-1 inhibitor tin-protoporphyrin IX (SnPPIX) drastically reduced the numbers of infected cells and trypomastigotes released. These results suggested that induction of HO-1 by uptake of apoptotic cells is a critical modulator of *T. cruzi* infection. **Acknowledgements:** CNPq, FAPERJ, HEBRON Farmacêutica.

P507 The bioactivity of herbal essential oils and ethanol extracts against *Escherichia coli* of animal origin

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Elevated levels of antimicrobial resistance for bacteria prevalent in food-producing animals represent a major concern for both human and veterinary medicine [1] and as an alternative for the control and treatment of the bacteria associated pathologies, herbal products are studied [2, 3]. The research investigated activities of different commercial essential oils and/or ethanol extracts of *Thymus vulgaris* L. (aerial parts), *Salvia officinalis* L. (leaves), *Lavandula officinalis* Mill. (flowers), *Mentha piperita* L. (leaves), *Rosmarinus officinalis* L. (aerial parts), *Ocimum basilicum* L. (leaves), *Mellisa officinalis* L. (leaves), *Origanum vulgare* L. (aerial parts), *Calendula officinalis* L. (flowers), *Arnica montana* L. (flowers), *Echinacea purpurea* L. (aerial parts), *Hypericum perforatum* L. (flowers), *Hippophae rhamnoides* L. (fruits and buds), *Coriandrum sativum* L. (fruits), *Foeniculum vulgare* Mill. (fruits), *Pelargonium graveolens* L. Her (leaves and flowers) against multidrug-resistant E. coli clinical isolates (O_{101}:K_{28}:F_5, O_8:$_{K25}$, O_9:K_{35} -bovine mastitis, n = 7 and calf diarrhoea, n = 3; O_{141}:H_4, O_{138}:H_1, O_{139}:K_{82} -swine oedema disease, n = 10; O_1:K_1, O_2:K_1, O_{78}:K_{80}:F_1 -avian colibacillosis, n = 20). The antimicrobial potency was established by disc diffusion test. Minimal inhibitory (MIC) and bactericidal (MBC) concentrations were determined based on a broth microdilution method. Some essential oils with inhibitory effects on *E. coli* growth enhanced the activity of ampicillin against the tested strains (Etest method). The sensitivity of E. coli to essential oils (20 – 31 mm diameter inhibition zones and MIC and MBC values ranging from 0,125% to 2% (v/v)) suggested that some of the screened herbal products should be considered for further *in vitro* and *in vivo* assays regarding the therapeutic use in animals. **References:** 1. Hammerum, A.M., Heuer, O.E (2009) Clin Infect Dis 48(7):916 – 21. 2. Delamare, A. et al. (2007) Food Chemistry 100:603 – 608. 3. Moreira, MR. et al. (2005) LWT 38:565 – 570.

P508 Antiprotozoal and cytotoxic activities of *Spiranthera odoratissima* A. St. Hil. (Rutaceae)

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Leishmaniasis and Chagas disease affects 30 million people worldwide [1]. The treatments are generally toxic or considered ineffective due to chemoresistance [2]. Plant species have been used by people in the Brazilian Cerrado for the treatment of infectious diseases [3]. In our study, eleven plant extracts of traditional medicine were tested against promastigotes of Leishmania (Leishmania) chagasi, trypomastigotes of Trypanosoma cruzi and NIH-3T3 cells of mammalian fibroblasts. Among these, the ethyl acetate extract of leaves of Spiranthera odoratissima A.St. Hil. (Rutaceae), species used traditionally in the form of decoction in wine to treat acne, boils, kidney infection and inflammation in general [4] exhibited IC 50 de 56.3 g/mL for T. cruzi. The chemical fractionation allowed the isolation of six compounds, among them β-sitosterol and sesamin. β-Sitosterol, a steroid-type triterpene, showed an IC 50 of 92 g/mL in L. (L.) chagasi, 103 g/mL in T. cruzi and 312.5 g/mL in NIH-3T3 cells. Sesamin, a lignin, showed an IC 50 > 100 g/mL against both parasites. Recent studies consider safe extracts with IC 50 values of mammalian cells above 250 g/mL [5]. It is the first time that sesamin is isolated from this plant species.

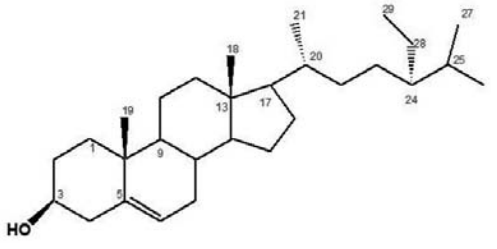

Fig. 1: β-Sitosterol

Acknowledgements: This report was supported by UnB, FAPDF, CAPES and CNPq. **References:** 1. http://www.who.int/neglected_diseases/en. 2. Kumar et al. (2009) Antimicrob. Agents Chemother. 53: 835 – 838 3. de Mesquita et al. (2005) Bioorg. Med. Chem. 13: 4499 – 4506. 4. de la Cruz (1997) Cuiabá: Dissertação de Mestrado. 5. Singh et al. (2008) Parasitol. Res. 103: 351 – 354.

P509 Indigenous knowledge of traditionally used plants from Iran for fever/malaria treatment

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Malaria is an infectious disease caused by several protozoans belonging to the genus *Plasmodium* but *P. falciparum* is the parasite that causes most severe diseases and most fatal cases. Unfortunately, in our days malaria still continues to be an extremely important threat to the health and economic prosperity of the human race, constituting a major cause of morbidity and mortality in tropical countries of Asia, Africa and South America. History shows that plants have been an important source of medicines against malaria with two of the major drugs used in malaria treatment, quinine and more recently artemisinin both having derived from plants. In this study five plants including *Ficus carica, Otostegia persica, Otostegia michauxii, Glycyrrhiza glabra, Matricaria chamomilla* [1 – 4] were selected on the basis of ethnobotanical investigation and searching in Iranian ancient traditional physician's books. The crude methanol extracts were prepared and tested for their in vitro activity against *P. falciparum* and for cytotoxicity against MDBK cells. Extracts from *O. persica, O. michauxii* and *G. glabra* showed antiplasmodial activity. The IC_{50} values of the most active extracts were determined as well as their selectivity towards *P. falciparum* in comparison to their cytotoxic effects against the MDBK cells. *G. glabra* showed better antiplasmodial activity than the other (IC_{50} value 17.5 µg/ml against K1). No cytotoxic activity was shown by methanolic extract of *G. glabra*. **Acknowledgements:** Research Council of Shahid Beheshti University of Medical Sciences, Institute for Medical Research, Kuala Lumpur, Malaysia. **References:** 1. Ibn – sina (2004) Qanoon. Lithography, 1296 A.H. reprinted by Institute of medical history study, Islamic and complementary medicine. Tehran. 2. Jorjani S (1976) Zakhireh Kharazmshahi. Bonyade farhang Iran. Tehran. 3. Aqili Khorasani (1992) Makhzan ol-Advieh. Enghelab e Eslami publishing and Educational Organization. Tehran. 4. Djavanshir K (1999) Vegetation of Bashagerd. Tehran university publication. Tehran.

P510 Antimicrobial activity of the essential oil from the leaves of *Croton cajucara* Benth.

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Croton cajucara Benth. (family Euphorbiaceae), locally known as "sacaca", is a very important plant resource in the Amazon area, being used in folk medicine against gastrointestinal and liver disorders, diabetis and for cholesterol reduction. Several biological effects have been associated to cleordanes present in leaves and bark infusions [1]. A germplasm bank was established for agronomic studies of this species with individuals collected from different areas of the Amazon. Two morphotypes were identified, namely white sacaca and red sacaca. From chemical studies, the essential oils of these plants could be classified in two groups: one rich (up to 45%) in linalool [2], and other containing (up to 44%) of an aromatic sesquiterpene, isolated and identified by NMR as 7-hydroxycalamenene [3]. It was shown that the linalool rich oil is ac-

tive against *Leishmania amazonensis* [4] and oral planktonic microorganisms [5]. Herein we present some results on the antimicrobial activity of the essential oil rich in 7-hydroxycalamenene. Minimum inhibitory concentration (MIC) was evaluated in triplicate according standard methods from the National Committee for Clinical Laboratory Standards (CLSi/NCCLS). Growing inhibition was observed for *Mycobacterium smegmatis*, *M. tuberculosis* (H37Rv), methicillin resistant *Staphylococcus aureus* (MRSA, BMB9393) and *Rhizopus oryzae*. The calculated MIC's were 156 μg/mL for *M. smegmatis*, 4.9 μg/mL for *M. tuberculosis*, 0.0012 μg/mL for the MRSA and 0.15 μg/mL for *R. oryzae*. From the bioautography test, the activity was associated to the presence of 7-hydroxycalamenene in the oil. The results observed were related to an essential oil containing 33% of 7-hydroxycalamenene. **Acknowledgements:** *CAPES, FAPERJ.* **References:** 1. Maciel MAM et al. (2000) J. Ethnopharmacol. 70: 41 – 55. 2. Lopes D et al. (2000) J. Essent. Oil Res. 12: 705 – 708. 3. Quadros AP et al., unpublished results. 4. Rosa MSM (2003) Antimicrob. Agents Chemother. 47:1895 – 1901. 5. Alviano WS et al. (2005) Oral Microbiol. Immunol. 20:101 – 105.

P511 Evaluation of the effects of *Pistacia atlantica* subsp. *mutica* essence on subgingival microorganisms

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Background: Dental plaque and adhesive biofilm to dental surface were a well known major etiologic factor of periodontal diseases. Then control of dental plaque has been respected as an issue of interest. In order to beat the shortcoming of mechanical plague control the chemical ways have been taken into account. The aim of this study was to determine effects of *Pistacia mutica* extract on sub gingival microorganisms. **Methods**: In a single centre, observer blind, cross-over, randomized Latin-square-controlled clinical trial 28 subjects were recruited. In comparison to chlorhexidin (CHX) and placebo (PLC) the effects of *Pistacia mutica* (PM) mouthwash on gram positive and gram negative microorganisms isolated from sub gingival counts were evaluated. Minimum inhibitory concentrations of PM against aerobic and anaerobic bacteria were detected too. **Results**: Mean aerobic bacteria count at baseline was $2.17*10^6$, in PM extract was $7.25*10^4$, in placebo $6.26*10^5$ and in CHX was $9.91*10^3$. Mean anaerobic bacteria count at base line was $6.56*10^6$, in PM extract was $1.97 * 10^6$, in placebo was $6.85 * 10^6$ and in Chlorhexidine was $1.13 * 10^6$. Mean MIC of PM extract was 618 ± 332 μg/ml in anaerobic bacteria in comparison with Chloramphenicol 60 ± 50 μg/ml. Mean MIC of aerobic bacteria in PM extract was 1845 ± 1145 μg/ml in comparison with Chloramphenicol was 45 ± 25 μg/ml. **Conclusion**: Mean aerobic bacteria count in PM extract significantly lower than baseline and placebo but significantly difference was not found in anaerobic bacteria count between PM extract with baseline, placebo and Chlorhexidine. Mean MIC of aerobic and anaerobic bacteria in PM extract was significantly higher than Chloramphenicol. **Key words:** Pistacia Mutica Extract, Aerobic Bacteria, Anaerobic Bacteria, Minimum Inhibitory Concentration

New analytical methods

P512 HPLC-DAD/ESI-MS identification and quantification of flavonoids and hydroxycinnamic derivatives in kale

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Kale (*Brassica oleracea L. var. sabellica L.*) contains high amounts of ascorbic acid and other constitutional compounds like polyphenols. Especially flavonoids, first of all quercetin, are known for antioxidative activity and other beneficial effects to human health [1]. The main concern of this project is to find flavonoids as potential inhibitors of sodium glucose co-transporter (SGLT1) to reduce the intestinal uptake of glucose thereby avoiding elevated postprandial blood glucose concentrations. Flavonoids and hydroxycinnamic acids of various sources of kale were characterized and identified by HPLC-DAD/ESI-MS analysis. More than twenty phenolic compounds including glycosides and acylated glycosides of quercetin and kaempferol as well as derivatives of sinapic acid could be identified. Depending on the starting plant material three flavonoid aglycones could be quantified after acidic hydrolysis: kaempferol as the main aglycone, followed by quercetin and isorhamnetin. The total flavonoid content in kale ranged from 1550 – 5000 ppm of fresh weight. In order to produce a final product with high flavonoid content an aqueous kale juice was prepared and processed using an adsorber resin (AMBERLITE™ FPX66). The identification of apigenin, rhamnetin and dihydrokaempferol (in traces) in flavonoid enriched kale extracts with LC ESI (+) MS is reported for the first time. The influence of different extraction methods on the total yield of flavonoids was investigated. Some polyphenol containing extracts were tested for their inhibition of SGLT1 in special electrophysiological assays. **Acknowledgements:** We thank the Federal Ministry of Education and Research for financial support, Project-No. 315371E. **References:** 1. Olsen, H. et al. (2009) J. Agric. Food Chem. 57: 2816 – 2825.

P513 Quality control analysis of geraniin in dried leaves of *Phyllanthus muellerianus* (KUNTZE) EXELL

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P. muellerianus is a woody climber and the leaves are used for treatment of wounds, skin eruptions, urethral discharge and dysentery [1 – 3]. We investigated the wound healing properties of *P. muellerianus* through bioguided fractionation; activity was found to be attributed to geraniin, major compound in the leaves [4]. **Aims:** (1) To develop simple method of extraction for *P. muellerianus* with maximum yield of geraniin (2) to develop and validate suitable analytical methods [5, 6] for quality control of geraniin. **Methods:** 1 g of dried powdered leaves in 10 mL MeOH/H_2O (7/3) was extracted by ultrasonic bath for 15 min to yield the test solution for HPLC-analysis. **Results:** Different extraction procedures were compared and ultrasonic extraction was found to be the most efficient method for the extraction of geraniin. Among different solvents, MeOH/H_2O (7/3) was found to bring highest yields of geraniin (> 4%). Peak identification was done by spiking and co-injection with reference compound. The content of geraniin was determined to be 4.3%. *Linearity and range* - 6 conc. (100 – 1000 μg/mL) showed linearity: y = 384.01x – 1191.1, R^2 = 0.9979. *Accuracy and recovery:* Spiking experiments were performed as follows: 6 conc., addition of geraniin from 0%-186%. The x-intercept of calibration curve (y = 379.21x + 148077, R^2 = 0.9914) gave the amount of geraniin in extract. Comparison with results obtained from standard calibration procedures gave recovery of > 99%. *Limit of detection*: 0.5 μg/mL; *Limit of quantitation*: 2.5 μg/mL *Precision*: Intra-assay and intermediate precisions were 4.8 and 9.6% (% RSD) respectively. **References:** 1. Burkill (1994), *Useful plants of west tropical Africa*, 121 – 122. 2. Irvine, *Woody plants of Ghana* (1961). 246 – 247. 3. Agyare et al., (2009). *J. Ethnopharmacol.* 125:393 – 403. 4. Agyare et al., (2010) *Phytomedicine* (submitted). 5. Text on Validation of Analytical proceedings – ICH Harmonized Tripartite Guideline, ICH; ICH-Q2A. 6. Validation of Analytical Procedures: Methodology – ICH Harmonized Tripartite Guideline, ICH; ICH-Q2B.

P514 Localization of arabinogalactan-proteins (AGP) in aerial parts of *Echinacea purpurea* and of AGP binding sites in rabbit Peyer's patches by immunofluorescence

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Echinacea purpurea preparations are used as unspecific immunostimulants. Besides alkamides [1], high molecular weight arabinogalactan-proteins (AGPs) are supposed to belong to the immunomodulatory active compounds of E. purpurea. In plants these AGPs are thought to influence plant growth, development and programmed cell death [2]. The localization of AGPs in plant tissues of *E. purpurea* helps to understand the physiological role of AGPs and optimize the production of pharmaceutical preparations. For immunolocalization of AGPs, the β-glucose-Yariv-reagent (βGlcY), binding selectively to AGPs, was used to label AGPs in sections of stem and leaf stalks from *E. purpurea*. Compar-

able to roots of *E. purpurea* [3], AGPs are mainly located in cell walls of xylem tracheary elements, especially in pit channels. In contrast to roots, the aerial parts of the plant show additionally very strong immunolabeling of sclerenchyma cells and of companion cells of the phloem. The pharmacological effects of AGPs after oral application of *E. purpurea* preparations are thought to be mediated by Peyer's patches of the ileum [4]. Cryo sections of rabbit Peyer's patch tissue were incubated with AGPs from Echinacea and AGP binding sites were detected using immunohistochemistry and confocal microscopy. AGPs from the aerial parts of *E. purpurea* strongly bind to intraepithelial lymphocytes in pockets of membranous (M) cells. As these sites are believed to be critically involved in the initiation of specific immune responses as well as the generation of immune tolerance [5], AGPs from *E. purpurea* might directly influence the immune protection of the human organism. **Acknowledgements:** We thank Rottapharm/Madaus GmbH, Köln, for financial support of this work. **References:** 1. Woelkart K et al. (2007) Planta Med 73: 615 – 623. 2. Nothnagel E A (1997) Int Rev Cytol 174: 195 – 291. 3. Classen et al. (2009) Planta Med 75: 1526 – 1533. 4. Bodinet C et al. (2004) Drug Res 54: 114 – 118. 5. Brandtzaeg P et al. (1999) Immunol Today 20: 141 – 151.

P515 Localization of mistletoe lectins I-III in *Viscum album* L. by immunofluorescent labeling

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Because of immunomodulatory effects mistletoe extracts play an important role as phytotherapeutic adjuvants in cancer therapy. Mistletoe lectins I-III contribute to the in vivo activity of mistletoe preparations [1]. They consist of two chains: the A chain is a homologous protein for all mistletoe lectins and responsible for the inhibition of translation. The B chain is necessary for binding to specific carbohydrate epitopes; the protein sequence of this chain varies from lectin to lectin. The localization of bioactive lectins in *Viscum album* L. is of significance to understand the physiological role of lectins in the plant and to optimize the production of pharmaceutical preparations. We investigated the cellular distribution of lectins in different parts of the plant by immunofluorescent labeling. Leaves and internodes of shoots of *Viscum album* L. were treated with a monoclonal antibody against the A-chain of mistletoe lectins I-III. A secondary fluorescent-labeled antibody was added and the sections were analyzed by confocal laser scanning microscopy. The mistletoe lectins I-III were located mainly in cells of the parenchyma of the cortex, in sclerenchyma tissue next to the vascular bundles and in the companion cells of the phloem. Especially in parenchyma cells of the third and fourth internode a high content of lectins could be detected, whereas parenchyma cells of the leaves showed weak labeling only. **Acknowledgements:** We thank Rottapharm/Madaus GmbH, Köln, for financial support of this work. **References:** 1. Seifert G et al. (2008) Cancer Lett 264: 218 – 228.

P516 Rapid analysis of N-phenylpropenoyl-L-amino acids (NPA) from cocoa by UPLC and CE

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The occurrence of N-phenylpropenoyl-L-amino acids (NPA) in *Theobroma cacao* L. was firstly described in 1998 [1]. Since that NPA were shown to be key contributors to the astringent taste of cocoa beans and nibs [2], and to be stimulators of mitochondrial activity and proliferation rate of human liver cells and keratinocytes [3]. Furthermore the aspartic acid amide of caffeic acid showed strong antiadhesive properties against the adhesion of *Helicobacter pylori* to human stomach tissue [3]. Preliminary data on absorption and bioavailability of NPA in humans are available [4]. Recently a stable isotope dilution analysis with LC-MS/MS (MRM) detection was developed and 14 NPA were quantified in cocoa samples [5]. As SIDA-methods often suffer from the availability of reference compounds, there is need for alternative methods. The aim of our work was to establish a fast method for analysis of NPA in cocoa and cocoa products. We developed two methods on the basis of alternative separation principles: chromatography (→ UPLC) and electrophoresis (→ CE). Both methods were validated according to ICH guidelines and enabled us to quantify the main NPA in different cocoa products. After defatting with petroleum benzine, extraction was done using acetone-water (7:3). To avoid interference caused by accompanying polyphenols subsequent SPE on quaternary amine sorbent yielded NPA-enriched fractions. UPLC separation of compounds 1 – 5 (Image 1) was done in less than 4 minutes. CE offers a suitable alternative with the disadvantage of a lower sensitivity. Highest amounts of NPA were found in cocoa beans, followed by nips and chocolate.

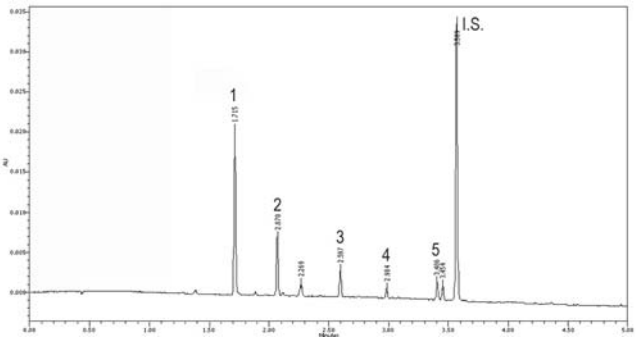

Fig. 1 UPLC/PDA (320 nm): **1** K-Asp, **2** pC-Asp, **3** K-Dopa, **4** K-Tyr, **5** pC-Tyr, **I.S.** Rosmarinic acid

References: 1. Sanbongi C et al. (1998) J. Agric. Food Chem. 46:454 – 7. 2. Stark T and Hofmann T (2005) J. Agric. Food Chem. 53:5419 – 28. 3. Hensel A et al. (2007) Planta Med 73:142 – 50. 4. Stark T et al. (2008) Mol. Nutr. Food Res. 52:1201 – 14. 5. Stark T et al. (2006) J. Agric. Food Chem. 54:2859 – 67.

P517 Efficient silkworm expression of a single-chain variable fragment antibody against ginsenoside Re and its application in ELISA for quality control of total ginsenosides in various ginseng

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Panax ginseng and its related species are well known as important traditional medicines which have been widely used in Asia, especially China, Japan and Korea, for thousands of years. Ginseng has been shown to have various pharmacologic activities such as tonic, immunomodulatory, anti-mutagenic, and anti-aging activities [1 – 2]. A single-chain variable antibody fragment (scFv) against its major compound, ginsenoside Re (G-Re) has been successfully expressed in the silkworm larvae using the *Bombyx mori* nucleopolyhedrovirus (BmNPV) bacmid DNA system. The baculovirus donor vector for expression of scFv against G-Re (GRe-scFv) was constructed to contain a honeybee melittin sequence signal (HMSS) to accelerate secretion of recombinant GRe-scFv into the hemolymph of silkworm larvae. Functional GRe-scFv was purified by cation exchange chromatography followed by immobilized metal ion affinity chromatography. The yield of purified GRe-scFv was 6.5 mg per 13 silkworm larvae, exhibiting higher yield than that expressed in *E. coli* (1.7 mg per liter of culture medium) [3]. GRe-scFv retained similar characteristics of the parental monoclonal antibody (MAb) against G-Re (MAb-4G10), making it possible to develop an indirect competitive enzyme-linked immunosorbent assay (icELISA) for the quality control of total ginsenosides in various ginsengs. The detectable range for calibration of G-Re by developed icELISA was 0.05 – 10 μg/ml. These results clearly suggested that the silkworm expression system is quite useful for the expression of functional scFv that frequently require time- and cost-consuming refolding when expressed in *E. coli*. **References:** 1. Lee, TK. et al. (2005) Mutagenesis 20: 237 – 243. 2. Kiefer, D. et al. (2003) Am. Fam. Physician 68: 1539 – 1542. 3. Pongkitwitoon, B. et al. (2010) J. Nat. Med. in press.

P518 Flash chromatography on cartridges for the separation of plant extracts – Rules for the selection of chromatographic conditions, and comparison with MPLC

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Flash chromatography on cartridges has become increasingly popular for the rapid purification of compounds, mainly of synthetic origin. In contrast, its application for natural product isolation is poorly documented, and easy-to-use procedures for optimization of the separation conditions are lacking. Using Sepacore® cartridges (Büchi Labortechnik), we have established empirical rules for the selection of chromatographic conditions with an emphasis on gradient mode. Reversed phase HPLC separations can be transposed by increasing the gradient time by a factor 2 – 4. For normal phase separations, solvent compositions resulting in *Rf* values of 0.15 – 0.2 on TLC for the most lipophilic and the most hydrophilic constituents, respectively, should be selected as gradient endpoints. We applied these rules to the separation of complex plant extracts, with *Curcuma xanthorrhiza*, *Piper nigrum* and *Salvia milthiorrhiza* as examples of medicinal and commercial importance. The performance of the cartridges was compared to that of classical MPLC (medium pressure liquid chromatography) glass columns. Sepacore cartridges enabled a good separation of compounds with a broad range of polarity, as typically found in plant extracts. The chromatographic resolution remained, however, lower than that achieved by MPLC on columns packed with material of smaller particle size. For poorly soluble extracts, solid introduction gave better results than liquid injection. Despite lower resolution as compared to MPLC, pre-packed cartridges are an attractive alternative for the purification of extracts and crude fractions due to their ease of use and speed of separation.

P519 Content of antidepressant hyperforin correlates with translucent glands in *Hypericum perforatum* L.

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Hypericum perforatum L. synthesizes two therapeutically important compounds, hypericin and hyperforin, which have recently been shown to accumulate in secretory structures: the dark glands and the translucent glands respectively [1]. Photographic images of *H. perforatum* leaves were computer-analyzed to produce measures of total gland area and total leaf area. A single leaf was placed between two microscope slides and positioned in a microscope fitted with light externally and from below. Since the translucent glands span the depth of the leaf, they clearly show when lit from behind, and could be captured in high-definition images (CCD camera "Evolution LC", exposure at 700 ms). The images were captured as TIFF files, and processed in computer program Image-Pro Plus 5.1 (conversion to grey tones; masking of anything outside the leaf outline; and finally count and size-measure of any bright objects (the translucent glands) of a defined roundness (0 – 2 arbitrary units) and within a defined size (0.001 – 1 mm2). The leaves were then subjected to extraction and HPLC analysis for hyperforin content. The content of hyperforin in a *H. perforatum* leaf correlated with the total area of translucent glands (correlation 0.71), whereas there was no correlation with the leaf area (correlation 0.09). This means that it is not the biomass of a plant, but the number and size of translucent glands that influence the yield of hyperforin in a crop. This applied method indicates that hyperforin content in *H. perforatum* leaves could be estimated without use of analytical chemistry, simply by observation and calculation of translucent gland area. **References:** 1. Soelberg J. et al. (2007) Ann Bot 99: 1097 – 1100.

P520 Simultaneous determination of eight marker components in the traditional herbal medicine, Sipjundaebo tang by HPLC-DAD

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Sipjundaebo tang, a traditional herbal medicine, has been used in the treatment of anemia, inflammation and tumor. For simultaneous determination of eight components, namely hydroxymethylfurfural (5-HMF), paeoniflorin, ferulic acid, cinnamaldehyde, decursinol, 6-gingerol, decursin and glycyrrhizin in Sipjundaebo tang, a high performance liquid chromatography-diode array detector method was established. To develop and validate this HPLC-DAD method, C18 column (S-5 µm, 4.6 x 250 mm) was used with gradient mobile phase, water and methanol containing 0.1% TFA at the column temperature of 35 °C. UV wavelength was set at 230, 254, 280 and 300 nm. Validation of the chromatography method was evaluated by linearity, precision and accuracy test. Calibration curve for eight marker components showed good linearity with R2 > 0.9998. Limits of detection (LOD) and Limits of quantification (LOQ) ranged from 0.01 µg/ml to 0.13 µg/ml and 0.03 µg/ml to 0.41 µg/ml, respectively. The relative standard deviations (RSDs) value of precision test, intra-day and inter-day test, was less than 0.61% and 0.42%, respectively. The results of accuracy test varied from 95.1% to 104.8% with RSD < 2%. In conclusion, this developed simultaneous determination method was efficient for the quality evaluation of Sipjundaebo tang.

P521 Quantitative detection of sinigrin in crude extracts of horseradish (*Armoracia rusticana*) roots by Enzyme-linked Immunosorbent Assay (ELISA)

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The glucosinolate sinigrin is an important ingredient in roots of horseradish (Armoracia rusticana Gaertn., Mey., & Scherb.). Glucosinolates are characterized as sulfur- and nitrogen-containing plant secondary metabolites, possessing several health benefits [1] and are predominantly found in members of Brassicaceae. Following disintegration of root cells, during processing of the roots, sinigrin is degraded by myrosinase thereby releasing isothiocyanate which causes the spicy taste of horseradish-containing food. HPLC has been used as the standard method for detection of sinigrin or other glucosinolates. We developed an enzyme-linked immunosorbent assay (ELISA), rarely employed for quantitative detection of glucosinolates [2, 3], in order to estimate the sinigrin content in horseradish root extracts. We immunized Balb/c mice with BSA-linked sinigrin to obtain sinigrin-specific polyclonal antibodies. Quantification of sinigrin was achieved using a competitive ELISA against a sinigrin-ovalbumin conjugate used as the antigen. Values determined by this ELISA were compared with HPLC measurements suggesting that this method can be readily used as a rapid and high-throughput technique for measuring sinigrin-containing horseradish extracts. **References:** 1. Traka M. et al. (2009): Glucosinolates, isothiocyanat and human health. 2. Hassan F. et al. (1988) J. Agric. Food Chem. 36: 398 – 403. 3. van Doorn H. E. et al. (1998) J. Agric. Food Chem. 46: 793 – 800.

P522 Absorption study of Chios mastic gum triterpenic acids after oral administration in mice by LC-MS/MS

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Chios mastic gum is the resin that is obtained from the trunk and branches of *Pistacia lentiscus L* var. *chia*. Although mastic gum is used extensively as a constituent of functional foods or as an herbal drug, the oral absorption of its major constituents remains unclear. In this context, high performance liquid chromatography (HPLC) coupled to a hybrid quadrupole linear ion trap mass spectrometer (QqLIT) was employed for the measurement of mastic triterpenic acids in mouse plasma. 24Z – Isomasticadienonic acid (IMNA) and 24Z – isomasticadienolic acid (IMLA) were selected as model compounds, based on their activity against *Helicobacter pylori* [1]. Their concentrations were determined simultaneously in mouse plasma after oral administration of mastic gum (40 mg/Kg). In addition, the absorption kinetic properties of IMNA and IMLA were recorded after oral administration of a polymer free mastic extract (40 mg/Kg) in order to evaluate the role of the natural poly-β-myrcene (that constitutes ~25% of raw mastic) in the absorption procedure. The results of the absorption study for the triterpenic acids indicated that they were quickly absorbed showing a peak concentration at 1 h after administration of the polymer free extract. Circulating IMNA was found approximately 3000 ng/mL and circulating IMLA approximately 150 ng/mL. Interestingly, in the case of the administration of raw mastic, the absorption curve was significantly modified. Both triterpenic acids were quickly absorbed but no peak time was observed and additionally the maximum measured concentrations were 10-fold lower than in the case of the polymer free extract.

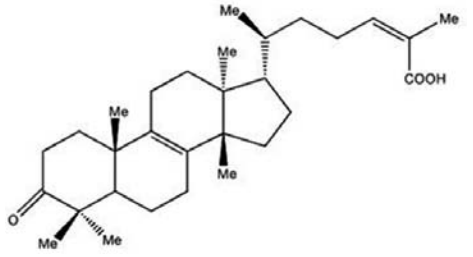

Fig. 1: IMNA

References: 1. Paraschos S. et al. (2007) Antimicrobial Agents and Chemotherapy. 51: 551 – 559.

P523 Micellar electrokinetic capillary chromatography (MECC) for rapid analysis of *Dipsacus sylvestris* HUDS. (Dipsacaceae) and differentiation from other *Dipsacus* species

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The biennial *Dipsacus sylvestris* HUDS. (Dipsacaceae), introduced to Europe in antiquity, grows to a basal rosette with a strong tap root in its first year of cultivation, followed by the flowering period in the second year. Significant constituents are phenolic acids, flavonoids [1], iridoids and bis-iridoids [2]. In opposite to the well-established Traditional Chinese Medicine (TCM) plant Dipsacus asperoides CHENG., *D. sylvestris* has mainly historical usage. To characterize possible antiinfectious compounds [3] different extracts of Dipsaci sylvestris radix were analysed by TLC, capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) allowing the differentiation from the TCM drug. Especially MECC (λ= 234 nm; borate buffer 45 mM, SDS 20 mM, pH 9.4) yields typical fingerprint electropherograms mainly consisting of iridoids and phenolic acids (Figure). MECC results were comparable to HPLC measurements and showed reproducibility of peak areas and relative migration times.

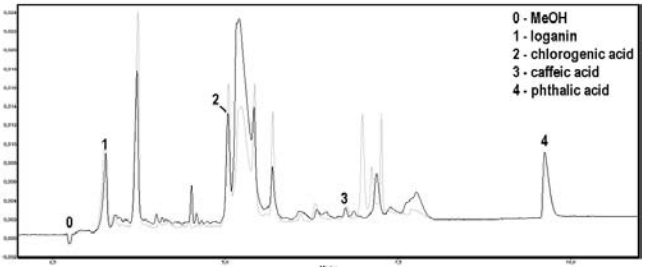

Fig. 1: MECC of MeOH extracts of Dipsacus sylvestris (black)/D. asperoides (grey)

Acknowledgements: Leipzig University, Sabine Liebold, Lothar Hennig
References: 1. Plouvier, V. (1966) Compt. Rend. Serie D 262: 1368 – 1371. 2. Jensen, S.R. et al. (1979) Phytochemistry 18: 273 – 277. 3. Liebold, T. et al. (2008) Planta Med 74: 995.

P524 Analysis of the constituents of aqueous preparations of *Stachys recta* by HPLC-DAD and HPLC-ESI-MS

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Herbal teas are one of the most common forms of oral aqueous preparations, used also externally in traditional medicine. They are obtained from one or more herbal substances by means of decoction, infusion or maceration [1]. In continuing our studies on traditional preparations of herbal drugs [2,3] we report the investigation of infusions and decoctions of Stachys recta L. (yellow woundwort). A method based on HPLC/DAD coupled to an ESI interface was developed for the determination of phenolic constituents in three aqueous preparations of S. recta. The assay was simple, effective and permitted the quality control of S. recta decoctions and infusion. The method was validated for the linearity, repeatability of the standards and samples, time precision, limits of detection and quantification. Overall, 30 constituents were detected and identified, belonging mainly to three classes of compounds: caffeoylquinic acids, phenylethanol glycosides and flavonoids. 15 of them were quantified having a lower limit not less than 0.02% of the lyophilised extracts. Only 7 of them were previously reported in this species, while 23 were identified for the first time as constituents of S. recta. HPLC-DAD-ESI-MS analysis provided evidence for the certain identification of the main constituents and in some cases of their isomers. Eight constituents were isolated and their structure elucidated by HPLC-ESI-MS and 1D- and 2D-NMR spectroscopy. Among the investigated preparations, the infusion is the best method to extract the native costituents of the plant, while decoction is a more aggressive treatment and causes partial degradation of some acylated flavonoids. **References:** 1. European Pharmacopoeia 6th edn, Vol. 1, Council of Europe, Strasbourg, 2008. p. 685. 2. Bilia, AR et al. (2007) J. Pharm. Biom. Anal. 44: 70 – 78. 3. Bilia, AR et al. (2002) Drug Dev. Ind. Pharm. 25: 611 – 22.

P525 Development of a validated LC-PDA method for the quantification of anti-inflammatory secondary metabolites from Ratanhiae radix

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Roots of *Krameria lappaceae* (Dombey) Burdet et Simpson, listed in many pharmacopoeias (ÖAB90, *Ph. Eur. 6.0*) are traditionally used against oropharyngeal inflammation due to their high tannin content. We could show in previous studies that lignan derivatives contribute strongly to the anti-inflammatory properties of Ratanhiae radix. All eleven isolated constituents [1], including ratanhiaphenol I, II and conocarpan, exerted a pronounced topical anti-inflammatory potential *in vivo* (ID_{50} values 0.3 – 0.6 μM/cm^2) and could also inhibit the NF-κB activation in a dose-dependent manner [2]. Besides gravimetrical analysis of the ratanhiaphenols I, II and III the content of these compounds in the

drug has never been determined. Therefore, we developed a validated LC-PDA method (according to the ICH-guidelines) for the quantification of the eleven active lignan derivatives in the roots (ASE extraction). The method is also suitable to determine the lignan derivatives in the tincture (pre-purification over polyamide). Separation was achieved on a phenyl-hexyl column using a solvent gradient consisting of 0.02% aqueous TFA and a mixture of acetonitrile/methanol (v/v; 75/25). The two major compounds were identified as conocarpan (**1**) and ratanhiaphenol II (**2**) with contents of 0.61%± 0.02% and 0.56%± 0.02% in the roots and 0.16 mg/ml (RSD 1.1%) and 0.17 mg/ml (RSD 0.4%) in the tincture, respectively. The recovery rates, determined in two different concentrations for both sample types, ranged from 95.5 to 104.8% for **1** and **2**. The outcome of this study confirms that the isolated lignan derivatives are present in concentrations relevant for the previously determined anti-inflammatory activities. **Acknowledgements:** This work was granted by the Austrian Science Foundation (NFN: DNTI, S 10703-B03) **References:** 1. Baumgartner L et al. (2010) Book of Abstracts; International Symposium Drugs from Nature Targeting Inflammation, SL6. 2. Baumgartner L et al. (2009) Planta Med 75:PA20.

P526 New and fast HPLC method for analysis of flavonoids in honey and propolis samples

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Flavonoids present in honey and propolis exhibit a wide range of biological effects, including antibacterial, anti-inflammatory and anti-allergic activities. The presence of specific flavonoids and their content can be used to distinguish botanical and geographical origin [1]. Usual methods for flavonoid analysis are time consuming HPLC assays using combinations of aqueous acid solutions and methanol or acetonitrile. Such long methods are not practical in routine analysis [2,3,4]. Thus, a relatively fast HPLC method for determination of flavonoid content in propolis and honey was developed, with gradient elution over 30 minutes and using ammonium formiate buffer (pH 4,5) and methanol as mobile phases. A column with sub-2-micron particles was used at a presure not exceding 360 bar. The method was validated and it is suitable for identification and quantification of six flavonoids in propolis and honey (quercetin, chrysin, naringenin, pinocembrin, pinostrobin, galangin). Flavonoid content of several propolis and honey samples originating from Bosnia and Herzegovina was assayed using the developed method. Propolis samples generally contained all said flavonoids, with the exception of the propolis samples from Mostar (pinostrobin and naringenin are not present) and Brcko (naringenin not present). Flavonoid content was 2,38 mg/g to 117,05 mg/g. Out of the three honey samples bought in a general store, one was proven to be a forgery. Flavonoids (naringenin, pinocembrin, chrysin and glangin) were found in two remaining honey samples and the total content was 1,16 μg/g to 1,65 μg/g. Based on these results, the presented HPLC method is a reliable and accurate tool for analyzing flavonoids in honey and propolis samples. **References:** 1. Pyrzynska K., Biesaga M. (2009) Trends in Analytical Chemistry 28: 893 – 902. 2. Sabatier S. et all. (1992) J. Food Sci. 57: 773 – 777. 3. Merken H.M., Beecher g.R. (2000) J. Agric. Food Chem. 48: 577 – 599. 4. Hadjmohammadi M.R. et all (2009) Chromatographia 69: 1 – 7.

P527 A new method for the production of enriched polyphenolic extracts from propolis using adsorption resins

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In previous works we have presented several applications for the production of enriched polyphenolic extracts from raw materials (olive mill waste water [1], winery byproducts, sesame [2]) using adsorption resins. Herein we describe the development of a new method for the production of enriched extracts from propolis. More specifically, two raw propolis samples (from geographically distinct regions of Greece: North Aegen and Crete) were first submitted to hydrodistillation in order to obtain the contained essential oil (0.14% and 0.12%) and the aqueous residue was filtered to remove the insoluble material (mainly non volatile terpenes and hydrocarbons: 61.2% and 60.5%). The filtrate was allowed to cool and the solidified wax (34.2% and 34.0%) was removed. The obtained clear solution was passed through a column containing adsorption resin XAD-4. The adsorbed polyphenols were desorbed using methanol and the obtained extract (1.8% and 1.5%) was analyzed by HPLC-UV. The contained phenolic acids (e.g. caffeic, p-coumaric, ferulic) and flavonoids (e.g. pinobanksin) showed 50 – 100 fold increased concentration in comparison with the raw propolis. In parallel, the essential oil obtained during the first step was analysed by GCMS showing α-pinene as the major component (40.3%, 46.6%) followed by at least 25 common constituents. Chiral GCMS analysis showed significant differences in the (+)/(−) α-pinene ratio between the two samples suggesting that this factor could be very useful in the discrimination of propolis samples **Acknowledgements:** We would like to thank APIVITA S.A. for the supply of the propolis samples **References:** 1. Agalias, A. et al. (2007) J. Agric. Food Chem. 55:2671 – 2676. 2. Grougnet, R. et al (2006) J. Agric. Food Chem. 54:7570 – 7574.

P528 HPTLC detection: an appropriate preparation of spraying and dipping solutions

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In thin-layer chromatography, for the purpose of post-chromatographic derivatization, the specific derivatization reagent solution can be transferred onto the plate by spraying or dipping. This TLC step of detection, or derivatization, was studied with a view to elaborating a standardized approach. The focus was on the solvent used to prepare the derivatization solutions, and also on the concentrations of these reagent solutions. For this purpose, qualitative results from both spraying and dipping experiments were compared. As herbal raw material, four flavonoid-containing drugs were used: ginkgo leaf, birch leaf, passion flower, and hawthorn leaf and flower. The specific derivatization reagent was natural product/macrogol 400; on the one hand dissolved in methanol, and on the other hand dissolved in ethyl acetate/dichlormethane [1]. The spraying as well as dipping procedures were carried out with appropriate automatic devices. Qualitative results showed that, in each case, derivatization reagents dissolved in ethyl acetate/dichlormethane showed concise zones, and weakly pronounced zones were also clearly visible. By contrast, dipping the plate into a derivatization solution containing methanol led partly to indistinct zones. Thus, post-chromatographic derivatization by dipping required an adaptation of the original spraying reagent composition, i.e. specific solvent and concentration, to obtain comparable results between spraying and dipping. Such standardization of TLC steps may be helpful in the goal of harmonizing Ph Eur monographs. **Acknowledgements:** We thank SWISSMEDIC, Swiss Agency for Therapeutic Products, Pharmacopoeia division, for the financial support. **References:** 1. Reich, E., Schibli, A. (2007): High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Thieme, Stuttgart.

P529 Quantitative analysis of cycloartane glycosides in black cohosh rhizomes and dietary supplements by RRLC-ELSD and RRLC-qTOF-MS

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Actaea racemosa L. (syn.: Cimicifuga racemosa (L.) Nutt.), commonly known as black cohosh, is a perennial herb growing in temperate regions of the Northern hemisphere [1]. The centre of its distribution is in the Southeastern United States, where Native North Americans used the rhizome of black cohosh for the treatment of rheumatism and menstrual disorders [1,2]. In the present study, a fast and reproducible RRLC-ELSD method for the quantitative analysis of 17 cycloartane glycosides and the aglycone cimigenol in black cohosh rhizomes and dietary supplements has been developed. Separation of the 18 triterpenes was achieved within 16 min using reversed phase material and a gradient elution system consisting of water, acetonitrile and methanol as mobile phase. The method was validated for accuracy (recovery rates from 96.79 to 102.86%), repeatability (R.S.D.? 6.94%), precision (intra-day variation? 5.98%, inter-day variation? 3.74%) and sensitivity. Detection limits of 2.50 – 3.75 μg/mL and quantification limits of 7.50 – 10.00 μg/mL were determined. Calibration curves were set in a range from 5 –

1000 µg/mL, with $R^2 > 0.998$ for all constituents investigated. Peak purity analysis and peak assignment were accomplished by means of RRLC-qTOF-MS and in comparison with reference compounds. Thereby three different sources (ESI, APCI and APPI) were applied and studied for their ionisation potential in regard to the respective cycloartane derivatives. One of the isolated black cohosh constituents, 24-O-acetylhydroshengmanol-3-O-α-L-arabinopyranoside, could be identified as new natural compound. **References:** 1. Britton, N., Brown, A. (1913) An illustrated flora of the Northern United States, Canada and the British Possessions -Vol. II. Charles Scribner's Sons. New York. 2. Hardy, M. (2000) J. Am. Pharmaceut. Assoc. 40:234 – 242.

P530 Identification of triterpenoids from the fruiting body and mycelium of *Antrodia camphorata* with HPLC-ESI/MS

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Antrodia camphorata Wu, Ryvarden & Chang (Polyporaceae) is an endemic parastic fungus in Taiwan. It grows slowly on the heartwood of *Cinnamomum kanehirai* and produced orange-red color fruiting body on the surface of wood. The fruiting body of *A. camphorata* has been used as antidote for food or drug intoxication and treatment for hepatoma. The extract or compounds isolated from fruiting body or mycelium of *A. campharata* showed immuno-enhance, neuroprotective, hepatoprotective, cytotoxicity on tumor cells, anti-inflammatory, and antioxidative, activities. The bioactive components of *A. camphorata* were identified as polysaccharides, triterpenoids, steroids, bezenoids, and maleic acd/succinic acid derivatives. High-performance liquid chromatography-photodiode array detector-electrospray ionization mass spectrometry (HPLC-PAD-ESI/MS) was developed to characterize the triterpenoids in *A. camphorata* mycelium and fruiting body. Zhankuic acid D, dehydroeburicoic acid (or antcin B), dehydroesulfurenic acid, zhankuic acid, antcin C, and antcin A were identified with selected ion monitoring (SIM) of 481, 467, 483, 485, 469, and 453 amu ions from ethanol extract of *A. camphorata* fruiting body. Antcin C, antcin A, zhankuic acid C, dehydresulfurinic acid, and dehydroeburicoic acid (or antcin B) were identified with SIM of 469, 453, 485, 483, and 467 amu ions from ethanol extract of *A. camphorata* mycelium. This HPLC-PAD-ESI/MS is suitable to identify the triterpenoids in the commercial products of *A. camphorata* mycelium or fruiting body.

P531 Quantitative NMR: A useful tool for the content assignment of reference standards for quality control of herbal medicinal products

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The quality control, in-process control and stability testing of herbal medicinal products is performed by the use of characteristic markers as reference standards. The content of these reference standards is usually assigned indirectly by the combination of two chromatographic methods, water content by Karl Fischer and determination of residue solvents and of inorganic impurities. However, this procedure is not very accurate if the purity of the material is below 99.5%. As these marker substances are usually isolated from plants it is very difficult to achieve the needed high purity like 99.5% with a reasonable effort. Employment of quantitative NMR (qNMR) spectroscopy as a relative primary method increases the reliability of the content assignment of reference standards and their qualification [1]. The basis of qNMR is the direct proportionality of the signal intensity with the number of protons contributing to the resonance line [2, 3]. The marker compound is evaluated against an internal standard which is weighed together with the marker compound into an NMR tube. The application of qNMR for the content assignment of herbal markers was comprehensively validated within a public-funded project [4]. This technique was applied for the content assignment of a series of substances with relevance for the use as reference standards for herbal medicinal products. Results will be presented for different markers and the chances and limitations of this way of content assignment will be discussed.

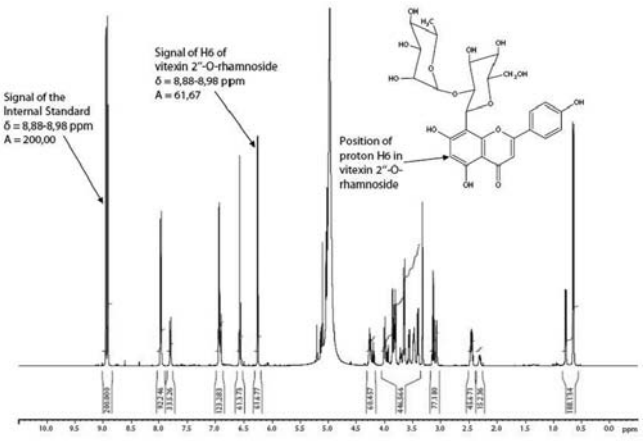

Fig. 1

References: 1. Veit, M.; Wissel, S. pharmind 2008, 1, 135 – 138. 2. Malz, F.; Jancke, H.: Validation of quantitative NMR. Journal of Pharmaceutical and Biomedical Analysis 2005, 38, 813 – 823. 3. Jancke, H. NMR als primäre Methode. Nachrichten aus Chemie, Technik und Laboratorium, 1998, 46 722. 4. Malz, F.; Jancke, H. Validation of quantitative NMR. Journal of Pharmaceutical and Biomedical Analysis 2005, 38, 813 – 823.

P532 Analysis of artemisinin in plant extracts of *Artemisia annua* L. cultivated in Brandenburg

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Artemisia annua L. (Asteraceae) has been used by Chinese folk medicine for many centuries in the treatment of illnesses like fever and malaria. In the 1970 s artemisinin, the active agent of the plant, was discovered and isolated. Combination therapies that include artemisinin are the preferred treatments for malaria and are both effective and well tolerated in patients. Today the most used method of artemisinin production is the extraction of plant material [1]. The demand of artemisinin for drug production is very high and can only be satisfied by farming of Artemisia annua at big areas. For a study of optimal farming the cultivation of A. annua was carried out in different variants like open ground and greenhouse in Brandenburg. For the selective determination of the content of artemisinin in raw impure extracts a lot of chromatographic and spectroscopic methods are described [2, 3]. A recent method for the characterization of plant material is metabolic profiling of extracts. By usage of spectroscopic methods like NMR a comprehensive classification of plant material is possible and provides valuable information about secondary metabolite constitutions at selected harvesting time points or planting regions. We used the direct determination of artemisinin by 1 H-NMR spectroscopy without purification of the raw extracts. Quantification was carried out analyzing the NMR chemical shifts of artemisinin. Using a reference standard of pure artemisinin we determined artemisinin contents between 0.02 and 0.5%. **Acknowledgements:** The authors like to thank the Ministerium für Wirtschaft of Brandenburg and the EU for their support. **References:** 1. Lapkin, A. A. et al. (2006) J. Nat. Prod. 69: 653 – 1664. 2. Castilho, P. C. et al., (2008) Phytochem. Anal. 19: 329 – 334. 3. Marchand, E. et al. (2008) Biomed. Chromatogr. 22: 454 – 459.

P533 Development of HPTLC method for quantification of flavonoids in extracts of the selected *Potentilla* species

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Potentilla species (Rosaceae) and their extracts have been highly valued in many different ethnic cultures for hundreds of years throughout the world. Extracts from P. species were and are still applied for the treatment of inflammations, wounds, infections due to bacteria, virus or fungi, diarrhoea, diabetes mellitus and several more ailments. Most of the biological activities of P. extracts can be explained with the high content of polyphenolics e.g. tannins (proanthocyanidins and hydrolysable tannins), numerous flavonoids, coumarins, polyprenols, phenolic carboxylic acids as well as triterpenoids [1]. Our previous study for the simultaneous determination of polyphenolics from P. species has been developed and validated using HPTLC precoated silica gel 60F254 plates with toluen\ethyl formate\formic acid (6:4:1, v/v/v) [2]. The aim of the present study was to determinate of three flavonoids: isoquercitrin (IQ), quercetin 3-glucuronide (QG) and rutin (RT) in the ethyl acetate extracts obtained from aerial parts of the selected Potentilla species: P. nepalensis and Drymocallis rupestris (P. rupestris) by using HPTLC-densitometry method. Chromatography was performed in CAMAG ADC2 (Automatic Development Chamber). HPTLC-DIOL silica gel F254 plates were applied. As a mobile phase ethyl acetate/methyl ethyl ketone/diisopropylether/formic acid (3:10:4:1, v/v/v/v) was used (distance of 7.5 cm). Densitometry was carried out by using of Shimadzu CS-9301PC densitometer. The absorption spectra were recorded at 350 nm. The proposed HPTLC method was found to be simple, precise and accurate for the quantification of these compounds in plant materials. **Acknowledgements:** This study is financially supported by the Polish Ministry of Science and Higher Education (Grant No. N N405 621638) **References:** 1. Tomczyk, M., Latté, KP. (2009) J Ethnopharmacol 122:184 – 204. 2. Tomczyk, M. et al. (2010) Phytochem Anal 21:174 – 179.

P534 Characterization of salvianolic acid B and verbascoside cyclodextrin complexes by innovative NMR methods. Evaluation of their stability

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Salvianolic acid B is a potent antioxidant with anti-thrombotic, antihypertensive and hepatoprotective activities obtained from *Salvia miltiorrhiza* L. root [1]. Verbascoside is a phenylpropanoid glycoside widely spread in nature with remarkable antioxidant, neuroprotective and hepatorpotective properties [2]. Both constituents are very water-soluble but unstable in aqueous solutions at pH > 5 [3]. In the present study both substances were enclosed in β-cyclodextrin in order to increases their water stability and the complexes were characterised by ^{1}H-NMR complexation shifts analysis, ^{1}H-NMR diffusion measurements, ROESY, NMR titration study and Job's plot. Diffusion ordered spectroscopy (DOSY) has been used extensively to study supramolecular aggregates and solubilisation processes in order to determine diffusion coefficients for the individual signals in a spectrum [4]. The DOSY spectra demonstrated that in the presence of β-CyD the translational diffusion of verbascoside and salvianolic acid B are sizably slowed down with respect to the free drugs. In the case of salvianolic acid B induced shifts and intermolecular ROE signals demonstrated that two caffeoyl moieties are deeply inserted in the cyclodextrin cavity, while one caffeoyl moiety hangs out from the wider rim. The Job's plots confirmed the 3:2 inclusion complex. In the case of verbascoside induced proton shifts and intermolecular ROE signals demonstrated that the caffeoyl moiety is deeply inserted in the cyclodextrin cavity. The Job's plots confirmed the 1:1 inclusion complex. Stability studies made by qNMR showed that both complexes increased the stability of the molecules. **References:** 1. Lin, YL et al. (2006) J. Ethnopharm. 105: 215 – 222. 2. Guangmiao, F. et al. (2008) Cur. Med. Chem.15: 2592 – 2613. 3. Meng, W. et al. (2009) Nat. Prod. Comm. in press. 4. Bilia, AR. et al. (2002) J. Pharm. Sci 91: 2265 – 2270.

P535 Quantification of xanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin in hop extracts and derived capsules using secondary standards

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Hop (*Humulus lupulus*) is a frequently used estrogenic herbal medicinal product, containing the prenylflavonoids xanthohumol (XN), isoxanthohumol (IXN), 8- and 6-prenylnaringenin (8-PN and 6-PN). Although many analytical methods have been developed for the quantification of these compounds, there still is a lack of validated methods for routine control. Therefore we have developed an accessible HPLC-DAD method using quercetin and naringenin as secondary standards for the analysis of hop extract and capsules. After optimization of sample preparation and HPLC conditions, the analysis was validated according to the ICH guidelines. The response function of XN, 8-PN, quercetin and naringenin showed a linear relationship. For the determination of XN, a calibration line of at least three concentrations of quercetin was constructed. The correction factors for XN (quercetin) and 8-PN (naringenin) were validated and determined to be 0.583 for XN, and 1.296 for IXN, 8-PN and 6-PN. The intermediate precision was investigated and it could be concluded that the standard deviation of the method was equal considering time and concentration (RSD of 2.5 – 5%). By means of a recovery experiment, it was proven that the method is accurate (recoveries of 96.1 – 100.1%). Several hop-containing preparations available on the Belgian market were analysed in order to check their compliance to the guideline concerning the maximal daily intake of selected medicinal plants, estabilised by the Advisory Committee for Plant Preparations. For hop the maximal daily dose is limited to 400 μg of 8-PN. These analyses showed that the reported method was applicable for this conformity testing.

P536 Fluorometric determination of ascorbic acid in the absence of the oxidant in juices of common citrus

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A simple and sensitive fluorimetric method for the determination of ascorbic acid (AA) in juices of common citrus is described. The method is based on the condensation reaction between AA and o-phenylenediamine (OPDA) in the abscence of the oxidant. The keto-form of AA reacts with OPDA in alkaline medium and forms an N-heterocyclic compound with large π-bond system, which can emit strong fluorescence. The fluorescence intensity is measured at exitation and emision wavelengths of 330 nm and 430 nm, respectively. Under optimum conditions, a linear relationship is obtained between the fluorescence intensity and the concentraction of AA in the range 0.05 – 50 μg mL-1. The detection limit (3σ) was found to be 0.54 μg mL-1 of AA (σ from 5 determination of 10 μg mL-1). A relative standard deviation of 1.4% was recorded for 8 measurements of 1 μg mL-1 standard AA solution. The efect of the reaction time is also studied. The fluorescence intensity of the system reached a maximum immediatelly after all the reagens were added and remained stable at least for 45 minutes. This method is simple, sensitive and easy for the determination of AA in juices of common citrus. This is first report that analyses of AA in biological samples were done without oxidant.

P537 Method development and optimization for isoflavone quantification in dry soy extract containing products

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Isoflavones are secondary metabolites belonging to flavonoids found in a variety of plants, especially soy (*Glycine max* L.). Due to the estrogen-like activity of these compounds several soy products are on the market and

applied to relieve menopausal symptoms. The aim of our study was to develop a simple method for the determination of isoflavones in soy extract containing products. The main isoflavones of soybean are genistin, glycitin, daidzin and their respective acetyl, malonyl and aglycone forms. Quantitative measurement of each individual isoflavone is difficult because some of the reference compounds are not widely commercially available [1]. Keeping in mind that the biological effects of soy isoflavones depend upon aglycone form, hydrolysis of glycosides is a practical method for the quantitative determination of total isoflavones and assessment of biological value of extracts [2]. The objective of our investigation was to establish an acid hydrolysis method for the quantitative HPLC determination of total isoflavones including daidzein, genistein and glycitein. To optimize the extraction and hydrolysis of isoflavones, the effect of HCl concentration (1.5 – 6 N), hydrolysis time (25 – 210 min) and temperature (30 – 100 °C) on total isoflavone aglycone content was studied using an RP-HPLC-DAD method. By mathematical fitting and optimization methods optimum hydrolysis conditions for maximizing the quantification of isoflavones were determined (t = 94 min, c_{HCl} = 5.09 N, T = 80 °C). The experimentally verified model has a good coefficient of R^2 (0,9928) and the recovery of isoflavones was 97.81 – 102.76%. The developed method allows a reliable and maximum determination of isoflavones in dry soy extract containing products. **References:** 1. Rostagno, M.A. et al. (2009) J Chrom A 1216: 2 – 29. 2. Chiang, W.D. et al. (2001) Food Chemistry 72: 499 – 503.

P538 Phytochemical analysis of the stems from cultivar varieties of *Rubus idaeus*

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The stems of red raspberry (*Rubus idaeus* L., Rosaceae) are used in folk medicine of several countries of Eastern Europe as anti-inflammatory and antipyretic remedies in the treatment of flulike infections. There is no literature data on a chemical composition of this plant material, in contrary to fruits of *R. idaeus* rich in anthocyanins and ellagic acid derivatives [1]. The aim of the study was to recognize active biologically compounds present in the stems of twelve cultivars of red raspberry. Different groups of simple phenols and polyphenols were analysed in the obtained methanol extracts by chromatographic methods – TLC, HPLC/DAD/MS and two dimensional techniques, including "comprehensive" LCxLC [2]. Sanguiin H-6 and ellagic acid were shown to be the major compounds in all analysed cultivars. Other phenolic compounds, cinnamic and benzoic acid derivatives and flavonols, flavan-3-ols and procyanidins were detected in minor concentrations as free forms (aglycones) or glycosides. Among flavonols, hyperoside and isoquercetin were dominant compounds. The stems of *Rubus idaeus* seems to be a rich source of phenols possesing antioxidant [4] and antiviral activity [5], what can explain its traditional use as curative in a cold. **Acknowledgements:** Polish State Committee for Scientific Research (KBN) Grant **References:** 1. Rao A.V., Snyder D. M. (2010) J. Agric. Food Chem. 58: 3871 – 3883. 2. Dugo P. et al. (2009) J. Chromatogr. A.1216: 7483 – 7487.

P539 Improved separation of green tea catechins and xanthines with calixarene bonded silica packings in narrow-bore HPLC systems

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Epigallocatechin gallate (EGCg), the predominant green tea catechin, is recommended as the health-promoting and complementary agent in the current management of variety infectious diseases with approved antivirals and antibiotics [1]. However, in commonly applied isocratic HPLC assays with octadecylsilica-C 18 packed columns the baseline separation of critical pairs as catechine/epigallocatechine and caffeine/EGCg was often failed. In presented isocratic narrow-bore HPLC procedure, the three different calixarene bonded stationary phases were successfully applied with AcN-2.65 mM H_3PO_4 (10:90, v/v), pH 3.0, as mobile phase to enable a complete separation of six catechins and six xanthines present in aqueous-AcN extracts of green tea. Compared to the retention with C 18 columns, the *trans* isomer (catechine) was eluted before the *cis* isomer (epicatechine) on each calixarene-based packing studied. However, when the dihydroxyphenyl substituents in catechine moiety were replaced by trihydroxyphenyl fragment, as was also in case of its gallate esters, the *cis i*somers were eluted first. These phenomena showed that selectivity of catechins separation with calixarene stationary phases was closely related with stability of their inclusion (1:1) host-guest complexes formed between analyte molecules and calixarene cavities [2]. In case of xanthines the increased size of substituents attached to the N1 nitrogen atom of the pyrimidine ring, caused enhanced retention of these compounds in proposed HPLC systems. The distribution profiles of catechins and xanthines in series of commercially available green teas and catechins-based nutraceuticals were determined with the developed HPLC procedure to offer more detailed data for standardization of such health-promoting products. **Acknowledgements:** Nicolaus University, Torun, Poland (Internal Grant No. 407/2010). **References:** 1. Song, J.M., Seong, B.L. (2007) Expert Rev. Anti Infect. Ther. 5(3): 497 – 506. 2. Bazylak, G. et. al. (2008) Curr. Drug Discov. Technol. 5(2): 177 – 189.

P540 Quantitative determination of stachydrine via HPTLC in cardioactive *Leonurus* and *Leonotis* drugs

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Tea preparations from aerial parts of the Central and Eastern European Lamiaceae *Leonurus cardiaca* L. are traditionally used as a remedy against tachyarrhythmia, heart failure, and other cardiac disorders [1 – 3]. Nevertheless, a scientific basis for these therapeutic effects had not been established [4] until just recently an antiarrhythmic refined extract was prepared from this drug via bioassay guided fractionation [1], making the development of improved methods of extract characterisation necessary. In Chin.Ph. [5] the "alkaloids" of the tea preparation of the closely related and similarly used East Asian *Leonurus japonicus* Houtt. [6] are regarded as the active constituents and quantified photometrically after Reinecke's complexation, calculated as stachydrine. Unfortunately, both the quantification method by complexation and the accompanied TLC method of the Chin.Ph. are deemed to be "not reproducible" by current experimental literature, such as Heuberger et al. 2008 [7]. Therefore, a state of the art HPTLC method was developed, using a CAMAG automatic HPTLC system with scanner and winCATS analysis software for reproducible stachydrine quantification. Silica gel 60 F254 HPTLC plates were scanned at 517 nm after development with MeOH:CH_2Cl_2:$NH_3$25% (8:2:3) and derivatisation with Vágújfalvi solution. The related cardioactive [8 – 10] South African *Leonotis leonurus* (L.)R.Br. was co-investigated. These measurements revealed stachydrine contents (w/w) of 0.2 to 1.1% for the *L. japonicus* drug, 0.3% for the material from *L. leonurus*, 0.5 to 1.5% for the *L. cardiaca* samples, and 6.6% for the antiarrhythmic refined extract of *L. cardiaca*. **References:** 1. Ritter M. et al. (2010) Planta Med 76: 572 – 82. 2. Barnes J. et al. (2002) Monograph Motherwort in: Herbal Medicines, Pharmaceutical Press, London. 3. Fuchs L. (1543) New Kreutterbuch, Basel edition. 4. *Leonuri cardiacae* herba, in: Kommentar zum Europäischen Arzneibuch (2005). Wissenschaftliche Verlagsgesellschaft, Stuttgart. 5. Pharm. P. Rep. China, Eng. Edition Vol 1. Beijing: Chem. Ind. Press, 1997/2000. 6. Luo X: Compendium of Materia Medica, book III, Beijing: Foreign Languages Press, 2003. A translation of the "Bencao Gangmu", first published by Li Shizhen, 1593. 7. Heuberger H. et al. (2008) Z Arzn Gew Pfl 13 (4): 173 – 181. 8. Burger A, Kabatembe J (2008) Planta Med 74: 991. 9. Watt JM, Breyer-Brandwijk MG (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, London. 10. Hutchings A (1996) Zulu Medicinal Plants. Natal University Press, Pietermaritzburg.

P541 Leonurine in *Leonurus* and *Leonotis* drugs? Detection and quantitative determination by a newly developed HPLC method

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Both aerial parts and seeds of *Leonurus japonicus* Houtt. are regarded as two of the most efficient remedies for the treatment of abnormal men-

struation, postpartum abdominal pain, and blood circulation disorders in TCM and Japanese Kampo medicine [1 – 3]. These pharmacological effects are generally attributed to the "alkaloid" leonurine (4-[amino(imino)methyl]aminobutyl4-hydroxy-3,5-dimethoxybenzoate) [4]. Interestingly, in European herbalism *Leonurus cardiaca* L. (Ph.Eur.) is used with similar indications such as gynaecological disorders [5,6] and especially heart disease [7], in which case its efficacy was recently proven by the development of a cardioactive refined extract via bioassay guided fractionation [8]. Although the presence of leonurine in *L. cardiaca* was reported in one single publication [9], these results were never reproduced. Nevertheless, it has repeatedly been quoted as an active constituent of this drug, even in current textbooks on pharmacognosy [10,11]. In the present study, a comprehensive, highly reproducible HPLC method for the detection and quantitative analysis of leonurine in plant drug material is reported for the first time. The South African herb Leonotis leonurus (L.)R.Br., used by native healers with similar indications [12], was co-investigated. Leonurine contends (w/w) between 0.001 and 0.104% were detected in the nine different *L. japonici* herba samples, of which only one failed to contain measurable amounts of leonurine. Instead of their similar application, no leonurine was detected in *L. japonicus* seeds or in the *L. cardiaca* and *L. leonurus* samples, indicating that apart from leonurine additional ingredients might contribute to the well proven clinical efficacy of the examined traditional medical plants. **References:** 1. Luo X: Compendium of Materia Medica, book III, Beijing: Foreign Languages Press, 2003. A translation of the "Bencao Gangmu", first published by Li Shizhen, 1593. 2. Pharm. P. Rep. China, Eng. Edition Vol 1. Beijing: Chem. Ind. Press, 1997/2000. 3. Kataoka N, Nakamura A (1992) Yamato Manyo Flowers. Tohoshuppan, Oosaka. 4. Kubota S, Nakashima S (1930) Folia Pharmacologica Japonica 11: 153 – 167. 5. Culpeper N: Culpeper's Complete Herbal & English Physician, London: Parkgate Books, 1997. Identical text to the original publication of 1652. 6. Welch JM (1883) Transactions of the National Eclectic Medical Association 10: 79. 7. Fuchs L. (1543) New Kreutterbuch, Basel edition. 8. Ritter M. et al. (2010) Planta Med 76: 572 – 82. 9. Gulabov AZ, Tchervenkova-Veleva VB (1970) Travaux scientifiques – Chimie 8 (1): 129 – 132. 10. Hiller K, Löw D: *Leonuri cardiacae* herba/Herzgespannkraut. In: Wichtl M (editor): Teedrogen und Phytopharmaka, ein Handbuch für die Praxis auf wissenschaftlicher Grundlage. Stuttgart: Wissenschaftliche Verlagsgesellschaft, 2009. 11. Teuscher E, Melzig MF, Lindequist U (2004) Biogene Arzneimittel. Wissenschaftliche Verlagsgesellschaft, Stuttgart. 12. Burger A, Kabatembe J (2008) Planta Med 74: 991.

P542 Quantitative HPTLC determination of rosmarinic acid and antioxidant activity of *Origanum onites* L. water and 70% methanol extracts

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Turkey is regarded as an important gene-center for the family Lamiaceae (Labiatae). The family is represented by 45 genera, 546 species and 730 taxa in Turkey. The rate of endemism in the family is 44.2% [1 – 2]. The flora of Turkey has 23 species of Origanum (15 endemic). They are known and used as thyme or "kekik" which is the name given those species with a thymol/carvacrol type odor in Turkey. The members of the Lamiaceae are common mainly in the mountainous areas of the Mediterranean parts of Turkey and the composition of their essential oils is detailed by Baser (1 – 3). In this study rosmarinic acid levels of origanum onites water and 70%methanol extracts were determined with HPTLC using Camag TLC scanner at 330 nm. ethyl acetate:toluene:-formic acid (5:4:1) used as mobil phase. Extracts were also investigated for total phenolics content and antioxidant activity using Dpph bioauthography, and abts radical scavenger activity. Rosmarinic acid level of water extract and 70%Methanol extract were found 2.36% and 2.29% respectively. Results can comparable with HPLC results (4) The extracts showed remarkable antioxidant activity. **References:** 1. Baydar, H., et al., Food Control, 2004. 15(3): p. 169 – 172. 2. Baser, K.H.C., E Acta Horticulture, 1993. 333: p. 217 – 238. 3. Baser, K.H.C., Lamiales Newsletter, 1994. 3 p. 6 – 11. 4. Exarchou, V., et al., J. Agric. Food Chem, 2001. 49(1): p. 2 – 8.

P543 CE and SDS-PAGE fingerprint analysis and identification of several fermentation products obtained by the *Candida* and *Penicillium* species used for homeopathic starting materials

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Fermentation cultures of Candida albicans (strain DSMZ 4884), C. parapsilosis (strain DSMZ 5197), Penicillium brevicompactum (strain DSMZ 3961), P. chrysogenum (strain DSMZ 5753), P. glabrum (strain DSMZ 5752) and P. roquefortii (strain DSMZ 5504) are prepared using various purification steps and cell mill treatment as well as sterilization and lyophilisation procedures to produce water-soluble substances [1 – 6]. Homeopathic (isopathic) dilutions of these starting materials are used as drug products in various European countries under the trade names of Albicansan, Pefrakehl, Stolonikehl, Notakehl, Quentakehl, Fortakehl and Exmykehl. The identification of the drug substances are performed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and capillary electrophoresis (CE) producing characteristic fingerprints. The SDS-PAGE analysis shows substance-specific bands with the following relative molecule masses (Mr). The signals obtained by CE and detection at 260 nm show fingerprint electropherograms that are specific for every substance preparation, making it possible to identify each individual cultured strain. Molecule Masses from extracts of Candida and Peniciliium species

Table 1

Candida sp.	Mr [kDa]									
Penicillium sp.	**> 100**	**100 – 90**	**90 – 80**	**80 – 70**	**70 – 60**	**60 – 50**	**50 – 40**	**40 – 30**	**30 – 20**	**< 20**
C. albicans			85	77				37/34	26	16
C. parapsilosis	118			78			41	37	28	
P. brevicompactum						59		34	27/25	
P. chrysogenum				76					24	16
P. glabrum		96				57	41		24	
P. roquefortii					67		50	38	24	

References: 1. German Homeopathic Pharmacopoeia (HAB) draft monograph (2009) "Candida albicans e volumine cellulae (lyophil., steril.)". 2. German Homeopathic Pharmacopoeia (HAB) draft monograph (2009) "Candida parapsilosis e volumine cellulae (lyophil., steril.)". 3. German Homeopathic Pharmacopoeia (HAB) draft monograph (2010) "Penicillium brevicompactum e volumine cellulae (lyophil., steril.)". 4. German Homeopathic Pharmacopoeia (HAB) draft monograph (2009) "Penicillium chrysogenum e volumine cellulae (lyophil., steril.)". 5. German Homeopathic Pharmacopoeia (HAB) draft monograph (2009) "Penicillium glabrum e volumine cellulae (lyophil., steril.)". 6. German Homeopathic Pharmacopoeia (HAB) draft monograph (2009) "Penicillium roquefortii e volumine cellulae (lyophil., steril.)".

P544 Zebrafish bioassay-guided microfractionation combined with CapNMR a comprehensive approach for the identification of anti-inflammatory and anti-angiogenic constituents of *Rhynchosia viscosa*

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Zebrafish have recently emerged as an attractive *in* vivo system for functional genomics and drug discovery [1]. Because of their small size, rapid development, optical transparency, and high genetic, physiologic, and pharmacologic similarity with humans, zebrafish embryos and larvae are also an ideal model for natural product discovery. As zebrafish bioassays require only microgram amounts of crude extracts, chromatographic fractions, and pure compounds, we are developing a zebrafish-based natural product discovery platform that takes advantage of modern techniques for the isolation and structural elucidation of natural products, such as UHPLC-TOF-MS profiling, LC-MS microfractionation and subsequent capillary NMR characterization [2]. An in vivo, zebrafish-based anti-inflammatory screen of methanolic extracts of East African medicinal plants resulted in the identification of *R. viscosa*, which revealed potent inhibition of leukocyte migration after larval tail trans-

ection in the presence of bacterial lipopolysaccharides. This extract also displayed anti-angiogenic activity in transgenic zebrafish with vasculature-specific expression of GFP. Intriguingly, *R. viscosa* is used by traditional healers in Tanzania for the treatment of inflammatory skin disorders and insect bites, corroborating our findings in zebrafish. Microfractionation of the crude methanolic extract was performed, and individual fractions tested for bioactivity in zebrafish. One highly active fraction was subjected to HR-ESI-MS and CapNMR analysis, resulting in the identification of genistein – a known inhibitor of inflammation and angiogenesis. These results indicate the potential of zebrafish bioassay-guided microfractionation, in combination with sub-milligram NMR techniques, to rapidly identify bioactive natural products. **References:** 1. Crawford, A. et al. (2008) Planta Med., 6, 624 – 632. 2. Glauser, G. et al. (2009) J. Agric. Food Chem., 57, 1127 – 34.

P545 Mass spectrometry in microbial metabolomic analysis as an analytical tool for dereplication strategy

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Metabolomics is the quantitative analysis of wide arrays of metabolites, including seoncdary metabolites, in biological samples. Metabolic profiling focuses on a group of metabolites while metabolic fingerprinting deal with global screening approach to classify samples based on metabolite patterns. The application of MS for analysis of secondary metabolites has grown over the last two decades, and today MS is the most important detector method in biotechnology [1]. Dereplication accelerates the discovery of novel natural products by eliminating repetitive work on known compounds. MS has been used for the dereplication of natural products because it is sensitive and provides information about the molecular mass, molecular formula and substructure of molecules [2], allowing dereplication of natural products at their early stages of purification and characterization. The aim of this study was to analyze metabolite profiles of crude extracts obtained from actinomycetes in differents cultivation medium by direct infusion MS and LC/MS. There are differences in the production of secondary metabolites in response to the growth conditions. The dereplication strategy for the analysis of the secondary metabolites has been developed. First, full scan total ion chromatogram (TIC), scanning m/z 100 – 1200, was acquired in both positive and negative modes. Ionization of compounds was better in positive mode. The dereplication was carried out in the mass range of m/z 750 – 900. The data suggest that the secondary metabolites located in this mass range belong to class of macrotetrolides and macrolides antibiotics (Figure 1). Compounds were isolated by a bioassay-guided fractionation using the microalga *Chlorella vulgaris* as test organism.

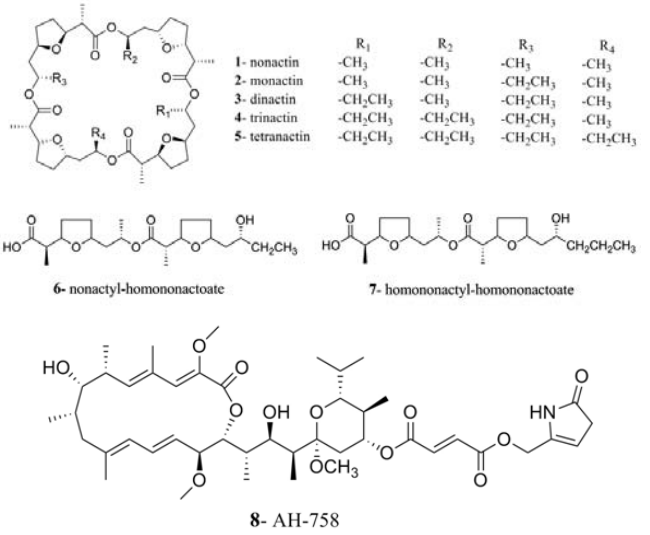

Fig. 1: Chemical structures of macrotetrolides and 16-membered macrolide antibiotics

Acknowledgements: FAPESP, CAPES and CNPq **References:** 1. Dettmer, K.; Aronov, P.A.; Hammock, B.D. Mass. Spectrom. Rev. 2007, 26, 51 – 78. 2. Lang, G.; Mayhudin, N.A.; Mitova, M.I.; Sun, L.; Van der Sar, S.; Blunt, J.W.; Cole, A.L.J.; Ellis, G.; Laatsch, H.; Munro, M.H.G. J. Nat. Prod. 2008, 71, 1595 – 1599.

P546 Specific HPLC determination of toxic alkaloids in aconite roots compared with amount ot total alkaloids determined by titration

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Aconite roots are known to be very toxic due to diterpene ester alkaloids [1]. Therefore, in Chinese medicine they are only used after processing. Hydrolysis of the ester groups decreases toxicity [2]. Nevertheless, several cases of poisoning by unprocessed or improperly processed aconite roots have been reported [3]. Recently we suggested a specific sample preparation method and HPLC assay for the analysis of toxic aconite alkaloids [4]. Now, an improved method with an even better resolution is presented. Using this method, we have determined the content of mesaconitine, aconitine and hypaconitine in 30 commercial samples of processed aconite roots. In most of the samples, toxic aconite alkaloids were not detectable, or only traces were found. However, in four samples we could detect more than 0.04% of hypaconitine and mesaconitine, the highest with a content of 0.16%. Therefore, this method is suggested as a purity test for the European Pharmacopoeia. Acute toxicity of batches high in hypaconitine and mesaconitine was also confirmed in CFLP mice. In the aconite monograph of the German Homeopathic Pharmacopoeia, alkaloids are determined by a titration method. We compared the results of HPLC analysis of toxic alkaloids (mesaconitine, aconitine and hypaconitine) with the results obtained by the titration method, and found no correlation. Samples which were lacking mesaconitine, aconitine and hypaconitine, still contained up to 0.2% alkaloids determined by titration. Therefore, titration of alkaloids is not appropriate as an assay for toxic alkaloids in aconite roots.

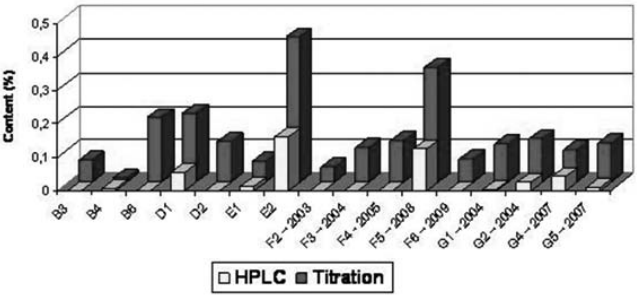

Fig. 1

References: 1. Xiao, P.-G. et al. (2006) Acta Phytotaxon. Sin. 44, 1 – 46. 2. Bisset, N.G. (1981) J. Ethnopharmacol. 4: 247 – 336. 3. Singhuber, J. et al. (2009) J. Ethnopharmacol. 126: 18 – 30. 4. Csupor, D. et al. (2009) J. Chromatrogr. A 1216: 2079 – 2086.

P547 Fluorometric determination of ascorbic acid in the abscence of the oxidant in juices of common citrus

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A simple and sensitive fluorimetric method for the determination of ascorbic acid (AA) in juices of common citrus is described. The method is based on the condensation reaction between AA and o-phenylenediamine (OPDA) in the absence of the oxidant [1]. The fluorescence intensity is measured at excitation and emission wavelengths of 330 nm and 430 nm, respectively. Under optimum conditions, a linear relationship is obtained between the fluorescence intensity and the concentration of AA in the range 0.05 – 50 μg mL^{-1}. The content of AA (mg/100 mL) in fresh juice of white grape was 30.5, red grape 28.0, orange 15.8 and lemon 16.8 mg. The results obtained with our method are comparable with other methods [2]. The detection limit (3σ) was found to be 0.54 μg mL^{-1} of AA (σ from 5 determination of 10 μg mL^{-1}). A relative standard deviation of 1.4% was recorded for 8 measurements of 1 μg mL^{-1} standard AA solution. The recovery for 50 μg mL^{-1} of AA added to samples of fresh citrus juices was between 101.5 and 104.6%. The effect of the reaction time is also studied. The fluorescence intensity of the system reached a maximum immediately after all the reagents were added and remained stable at least for 45 minutes. Presented method is simple, sensitive and easy for the determination of AA in the biological samples. This is first report that analyses of AA in biological samples were done without oxidant. **References:** 1. X, W. et al. (2003) Talanta 59, 95 – 99. 2. Eitenmiller R.R., Ye, L., Landen, W.O. Jr.(2008) Vitamin analysis for the health and food sciences, CRC Press.

P548 Comparative quantification of phosphatidylcholine in sea urchins eggs by instrumental TLC with various detection techniques

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The aim of work was comparison of two detection methods of TLC plates for quantification of phosphatidylcholine (PC) in sea urchins eggs. Lyophilized sea urchins eggs from Barents Sea were used. Lipids fraction was extracted with chloroform/methanol (2/1) by sonification in 30 min. A samples were spotted on Silica gel 60 F 254 s glass plates (Merck, Germany) using a Linomat V system (Camag, Switzerland). The plates were developed in mixture of chloroform/methanol/acetic acid/water (7/2/0.8/0.5) [1]. PC was quantified by direct densitometric scanning of the developed plate at 202 nm and after derivatization with 2% phosphomolybdic acid solution at 700 nm using a Camag TLC Scanner 3. The best separation of PC, sterols, fatty acids and triglycerides was obtained. Characteristics of various techniques of PC quantification are resulted in Table 1. The quantitative results of both detection methods did not show any statistically significant differences between each other.

Table 1: Characteristics of various techniques of PC quantification

Parameter	Detection at 202 nm	Detection at 700 nm after derivatization
Regression equation*	Y = 522.4+571.7X	Y = 382.1+1763.8X
r	0.9992	0.9997
sdv,%	2.14	1.01
Linearity, μg/spot	2.0 – 10.0	2.0 – 5.5
LOD/LOQ, μg/spot	0.45/1.36	0.13/0.40

*Y – spot area in AU; X – amount of analyte in spot in μg

Thus, for PC quantification in sea urchins eggs can be used both the standard approach with plate derivatization and direct detection at 202 nm. **References:** 1. Essig, S., Kovar, K.A. (2001)J AOAC Int. 84(4): 1283 – 1286.

P549 High-speed counter-current chromatography: an effective method for the isolation of flavonoids and profisetinidin from fustic (*Cotinus coggygria* Scop.)

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While chromatographic methods such as column chromatography and semi-preparative HPLC are widely employed during purifications of plant extracts, high-speed counter-current chromatography (HSCCC) is only occasionally utilized. This method based on liquid-liquid partition is advantageous as it avoids the loss of analytes due to irreversible adsorption. The present research focuses on the analysis of the diethyl ether-soluble fraction of a crude extract from *Cotinus coggygria* (Anacardiaceae) wood. After preliminary separation through vacuum liquid chromatography over RP-18 material (solvent: acetonitrile/water gradient), two of the resulted fractions (A-3, CH_3CN/H_2O 10/90, v/v and A-8, CH_3CN/H_2O 36/64, v/v) were purified on Sephadex LH-20 (solvent: methanol), yielding enriched fractions; four thereof were selected for HSCCC separations. An optimized solvent system was developed, comprising a mixture of hexane, ethyl acetate, methanol and water (1/2.5/1/1; all v/v). For separations, a P.C. Inc. (Potomac, USA; HSCCC multilayer coil, series 690) instrument was used. The system was operated in the "tail to head" mode, with the upper solvent layer as mobile phase. HSCCC using the above solvent system afforded the isolation of seven compounds: fustin (**1**), dihydroquercetagetin (**2**), epifisetinidol-(4β→8)-(+)-catechin (**3**), fisetin (**4**), quercetin (**5**), butein (**6**), and the new natural compound C-3/C-3" dimer of 3′,4′,7-trihydroxyflavanone (**7**). Their structure was elucidated by 1D (^{1}H NMR, ^{13}C NMR) and 2D (HSQC, HMBC) NMR experiments. The here described HSCCC solvent system is suitable for the separation of medium-polarity phenolic compounds from *C. coggygria* wood.

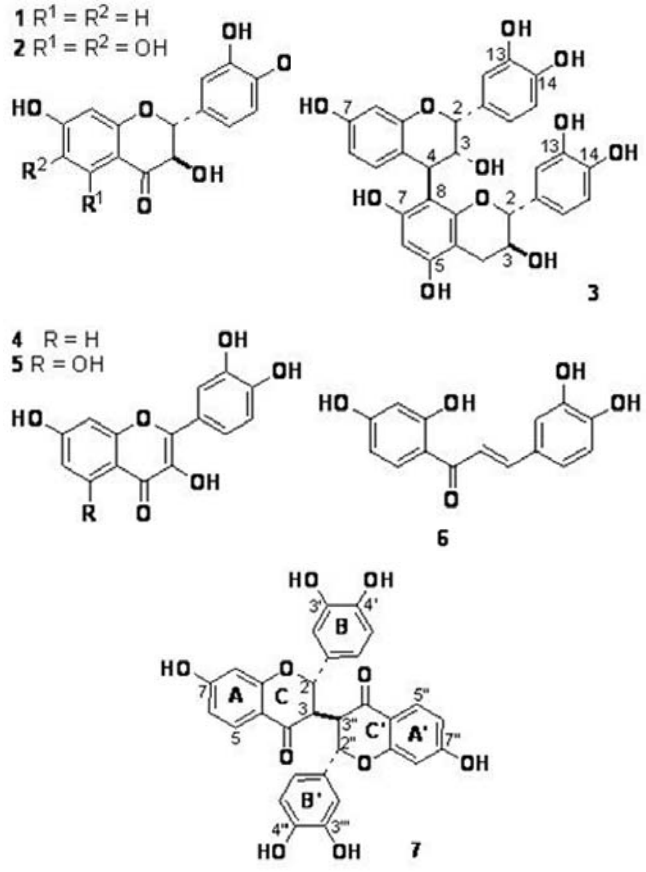

Fig. 1

Acknowledgements: The authors wish to thank the Austrian Science Fund (FWF) for financial support (P18379, Inhibitors of acetylcholinesterase from plants). Prof. Judith Rollinger (Institut für Pharmazie, Uni-

versität Innsbruck) is acknowledged for measuring IR-spectra, and Prof. Dr. Ernst Ellmerer (Institute of Organic Chemistry, University of Innsbruck) for NMR measurements.

P550 Two new cycloartane glycosides from *Astragalus cicer* L. (Fabaceae)

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The genus *Astragalus* contains more than 2000 species and represents one of the largest genus in the family of Fabaceae. It is widely distributed in the world of which about 70 species have been found in the Mediterranean region [1]. Some of them are well-known for their pharmacological properties, particularly hepatoprotective, immunostimulant and antiviral activities [2]. Many phytochemical studies on *Astragalus* genus were realized bringing out the presence of saponins with cycloartane-type genins [1,3]. Meanwhile no phytochemical investigations have been made on Astragalus cicer L. In this study we present the isolation and structural elucidation of two new cycloartane-type saponins named cicerosides A and B (**1**,**2**) together with the known coumpound eremophiloside B [4] from the aerial parts of *A. cicer* L. The n-butanol fraction from the methanol extract of the aerial parts was submitted to several solid/liquid preparative chromatographic methods such as CC on Sephadex LH-20, flash chromatography and MPLC over normal and reversed phase RP18 silica gel. The structures were established mainly by 600 MHz 1D and 2D NMR techniques (COSY, TOCSY, NOESY, HSQC, HMBC) and mass spectrometry. The structure of compound **1** was elucidated as 3β,6α,16β,24(R),25-pentahydroxycycloartan-3-yl-3-O-(α-Lrhamnopyranosyl)-6-O-(α-L-rhamnopyranosyl)-16-O-acetoxy-24-O-(β-D-glucopyranosyl)-25-O-β-D xylopyranoside, named ciceroside A and compound **2** as 3β,16β,24(R),25-tetrahydroxycycloartan-6-on-3-yl-3-O-(α-L-rhamnopyranosyl)-24-O-(β-D-glucopyranosyl)-25-O-β-D-xylopyranoside, named ciceroside B. **References:** 1. Verotta, L. et al. (2001) Studies in Natural Products Chemistry. Elsevier. Amsterdam. 25:179 – 234. 2. Rios, JL. et al. (1997) Phytotherapy Res. 11:411 – 418. 3. Linnek, J. et al. (2009) Nat. Prod. Comm. 4:477 – 478. 4. Peronne, A. et al. (2008) Tetrahedron 64:5061 – 5071.

P551 A rapid simultaneous quantification of five biologically active polyisoprenylated benzophenones using liquid chromatography-tandem mass spectrometry (MRM) method in two *Garcinia* species from Cameroon

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Garcinia is a plant genus of the family Clusiaceae native to Asia, Tropical and Southern Africa and Polynesia. *Garcinia preussii* Engl (synonym *Garcinia epunctata* Stapf) is traditionally used in Western Africa to treat stomachache, and it is popular as chewstick [1]. These species are known to be a rich source of polyisoprenylated benzophenones derivatives with a large spectrum of biological activities such as antioxidant, antiviral and anticancer properties [2]. A sensitive, rapid and simple reversed-phase high-performance liquid chromatography-electrospray ionization mass spectrometry method has been developed for the identification and quantification of five polyisoprenylated benzophenones, garcinol, 7epi-garcinol, isogarcinol, clusianone and 7epi-clusianone, in the extracts of the bark, fruit and leaves of *Garcinia preussii* and of the bark and roots of *Garcinia brevipedicellata*. The separation of garcinol and 7epi-garcinol was achieved on a RP-18 column using a solvent system consisting of a mixture of acetonitrile-water-formic acid as a mobile phase in a gradient elution mode. The identification of the five compounds was determined on a triple quadrupole mass spectrometer with ESI interface operating in the negative mode. A multiple reaction monitoring (MRM) method was developed for the quantification of these five polyisoprenylated benzophenones in the extracts of the two *Garcinia* species. **References:** 1. Bouquet A., (1969) Féticheurs et médecines traditionnelles du Congo (Brazzaville). ORSTOM. Paris. 2. Ciochina R. et al. (2006) Chem. Rev. 106 (9): 3963 – 3986.

P552 Chemotaxonomic study of the Polygalaceae family: saponins from *Securidaca welwitschii*

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From a chemotaxonomic point of view, in a continuation of our study on saponin constituents of the Polygalaceae family [1 – 3], we have examined the saponin fraction of stem barks of *S. welwitschii*. Five new presenegenin glycosides **1-5** were isolated by successive MPLC over silica gel, together with one known oligosaccharide multi-ester, **1-4** as two inseparable (E)/(Z)-isomers of a 3,4-dimethoxycinnamoyl derivative (1/2 and 3/4). Their structures were elucidated mainly by 2D-NMR techniques and mass spectrometry as 3-O-(β-D-glucopyranosyl)presenegenin 28-O-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-4-O-[(E)-3,4-dimethoxycinnamoyl]- β-D-fucopyranosyl ester (**1**) and its (Z)-isomer (**2**), 3-O-(β-D-glucopyranosyl)presenegenin 28-O-β-D-galactopyranosyl-(1→4)- β-D-xylopyranosyl-(1→4)-3-O-acetyl-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-4-O-[(E)-3,4-dimethoxycinnamoyl]- β-D-fucopyranosyl ester (**3**) and its (Z)-isomer (**4**), and 3-O-(β-D-glucopyranosyl)presenegenin 28-O-β-D-galactopyranosyl-(1→3)- β-D-xylopyranosyl-(1→4)- α -L-rhamnopyranosyl-(1→2)-O-β-D-fucopyranosyl ester (**5**). **References:** 1. Mitaine-Offer, A. C. et al. (2009) Phytochemistry. 71: 90 – 94. 2. Mitaine-Offer, A. C. et al. (2002) J. Nat. Prod. 65: 553 – 557. 3. Haddad, M. et al. (2003) J. Nat. Prod. 66: 372 – 377.

P553 Application of HPTLC-MS for the identification of flavonoids in herbal extracts

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Only recently, a very convenient and universal HPTLC-MS Interface became available, which semi-automatically can extract zones of interest and direct them into a LC-MS system [1]. Substances are directly extracted from a TLC/HPTLC plate and mass spectra are obtained within a minute. So far, the method has hardly been applied for the analysis of plant extracts [2]. We now have tested the HPTLC-MS Interface for investigation of flavonoid containing herbal drugs. Extracts and pure compounds have been applied as bands onto HPTLC plates using an automatic TLC sampler. Chromatography was performed in an automatic developing chamber with humidity control. Separated zones were eluted from the plate with the TLC-MS Interface using acetonitrile as solvent delivered by an HPLC pump at 100 µl/min. The interface was hyphenated to a Finnigan LCQ Deca XP Plus ion trap mass spectrometer equipped with an electro spray ionization (ESI) source. Rutoside, hyperoside, vitexin, quercetin and rosmarinic acid as pure substances were used to optimize extraction, detection and identification by HPTLC-MS. It was possible to identify hyperoside, vitexin, quercetin and rosmarinic acid also in an extract of Thymus vulgaris. Therefore, the HPTLC-MS

interface proved to be a quick and powerful tool for the on-line identification of flavonoids in TLC separations. It can complement the classical TLC detection tools. **References:** 1. Luftmann, H. et al. (2007) Rapid Commun Mass Spectrom 21: 3772 – 3776. 2. Reich E, Widmer V. (2009) Planta Med. 75(7):711 – 718.

P554 Quantitative analysis of isoflavones in 11 *Trifolium* spec. from Turkish flora and in some commercially available *Trifolium* products

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The genus *Trifolium* is represented by 96 species in Turkish Flora [1]. Among these species *Trifolium pratense* L. is marketed for use in alleviating menopausal symptoms. *T. pratense* contains the isoflavones, daidzein, formononetin, biochanin A, and genistein. It has been shown that the isoflavonoids lower the cholesterol level, play a role in prevention and medication of some cancer types and help to reduce menopausal symptoms [2 – 5]. The purpose of this study was to quantify four isoflavones in the 11 *Trifolium* spec. collected from nature and in some herbal products to determine which sample contains the highest isoflavone amount. A rapid HPLC-DAD method used for determination of isoflavonoids [6]. The method was validated according to the guidelines. *T. canescens* and *T. triocephalum* were found as the samples contain higher isoflavonoid amount than *T. pratense* [Fig. 1].

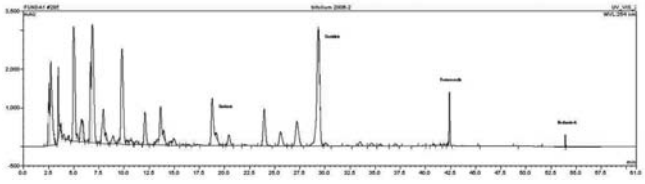

Fig. 1: HPLC Chromatogram of T. canescens

References: 1. Zohary, M. 1970. Trifolium L. In P.H. Davis (Ed) Flora of Turkey and the East Aegan Islands, Edinburg University Press. Vol.3, p. 412 – 413. 2. van der Weijer, P., Barentsen, R. (2002) Maturitas 42 187 – 193. 3. Cassidy, A., Bingham, S., Setchell, K. (1995) Br. J. Nutr. 74 587 – 601. 4. Jared, RA., Keikha, M., Dowling, J. McPherson, SJ., Clare, AM., Husband, AJ., Pedersen, JS., Frydenberg, M., Risbridger, GB.(2002) Cancer Epidemiol. Biomarkers Prev. 11 1689 – 1696. 5. Nestel, P., Cehun, M., Chronopoulos, A., DaSilva, L., Teede, H., McGrath B. (2004) Eur. J. Clin. Nutr. 48 403 – 408. 6. Krenn, L., Unterrieder, I., Ruprechter, R. (2002). Journal of Chromatography B 25, 123 – 128.

P555 Low molecular weight volatiles in Portuguese *Ficus carica* varieties

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Ficus carica L. is one of the earliest cultivated fruit trees. As a seasonal food, fig represents an important component of the Mediterranean diet [1]. In this work, the volatile composition of two characteristic Portuguese white varieties ("Pingo de Mel" and "Branca Tradicional") was determined by using an HSSPME coupled to GC/FID methodology. Samples were separated into leaves, pulps and peels and were submitted to different treatments (freezing and lyophilization). The developed procedure is rapid, sensitive, reproducible, and accurate. The detection limit values for volatiles ranged between 0.007 and 166.2 μg/L, and the method was precise. Recovery values were generally high. Considering its rapidity and low cost, this technique may be very useful for the quality control of fig fruits and leaves. The two analyzed varieties presented a similar profile composed by acetaldehyde, ethyl acetate, methanol, ethanol, hexanal, limonene, (E)2hexenal and octanal. Total volatiles content decreased in the following order: leaves (16593196 mg/kg) > peels (10032086 mg/kg) > pulps (6221087 mg/kg). Methanol was the major volatile in all samples representing 55 to 87% of total identified compounds. **Acknowledgements:** Fundação Calouste Gulbenkian, Branca M. Silva. Fundação para a Ciência e a Tecnologia, Andreia P. Oliveira (SFRH/BD/47620/2008). **References:** 1. Oliveira, A.P. et al (2010) Food Chem. 121: 1289 – 1295.

P556 Determination of the fine structure of polysaccharides by degradation with specific recombinant enzymes

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Structural analysis of polysaccharides is a difficult task. The common method for determination of linkage type is methylation analysis, leading to partial methylated alditol acetates which are analysed by GLC-MS. To gain further information polysaccharides are often partially degraded, e.g. through treatment with mild acid or periodate, result in the disadvantage of relatively weak specificity. We used recombinant enzymes to degrade a complex polysaccharide. In contrast to enzymes isolated from microorganisms, which often include accompanying enzymatic activities, recombinant enzymes demonstrate a high level of purity. As a model we analysed the complex polysaccharide part of an arabinogalactan-protein from oat which consists mainly of galactose and arabinose residues. After degradation with the highly specific recombinant enzymes endo-β-1,6-galactanase [1], exo-β-1,3-galactanase [2] and exo-α-1,5-arabinofuranosidase [3], the remaining polysaccharide was subjected to linkage analysis. The released mono- and oligosaccharides were analysed by high performance anion-exchange chromatography and pulsed amperometric detection (HPAEC-PAD) [2]. The results allow postulation of the arabinogalactan fine structure and give further information concerning the overall structure of the molecule. **References:** 1. Ichinose H et al. (2008) Appl Environ Microbiol 74(8): 2379 – 2383. 2. Ichinose H et al. (2005) J Biol Chem 280(70): 25820 – 25829. 3. Ichinose H et al. (2008) Appl Microbiol Biot 80(3): 399 – 408.

P557 Development and validation of a LC-MS/MS method based on a new 96-well HybridSPE™-precipitation technique for quantification of CYP450 substrates/metabolites in rat plasma

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A rapid and selective high throughput HESI-LC-MS/MS method for determining eight cytochrome P450 (CYP) probe drugs in one step extraction and single run was developed and validated. The four specific probe substrates midazolam, dextromethorphan, tolbutamide, theophylline and their metabolites 1-hydroxymidazolam, dextrorphan, hydroxyl(-methyl)tolbutamide, 1,3-dimethyluric acid, together with the deuterated internal standards, were extracted from rat plasma using a novel 96-well Hybrid-SPE™-precipitation technique. This novel technology com-

bines the simplicity of precipitation with the selectivity of SPE and hence leads to much cleaner extracts than with conventional procedures. The bioanalytical assay was based on reversed phase liquid chromatography coupled with tandem mass spectrometry in the positive ion mode using selected reaction monitoring (SRM) for drug (-metabolite) quantification. All analytes were separated simultaneously in a single run that lasted less than 11 min. The intra- and inter-day precisions for all eight substrates/metabolites were 1.62 – 12.81% and 2.09 – 13.02%, respectively, and the relative errors (accuracy) for the eight compounds ranged from −9.62 – 7.48% and −13.84 – 8.82%. Hence, the present method provides a robust, fast and reproducible analytical tool. The method enables the determination of four major drug metabolising cytochrome P450 (3A4, 2C9, 1A2, and 2D6) enzymes and can be used as a common high throughput analytical assay for *in-vivo* herb-drug interaction studies.

P558 Validation of TLC procedures for the identification of *Cimicifuga racemosa* and *Hypericum per-foratum* during quality control of a Cimicifuga/Hypericum fixed combination

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For quality control of pharmaceuticals, validated analytical procedures are required. Identity verification of herbal substances and phytopharmaceuticals is usually performed by thin layer chromatography (TLC). Cimicifuga/Hypericum film-coated tablets contain dry extract preparations of *Cimicifuga racemosa* and *Hypericum perforatum*. Two TLC procedures on silica gel 60 F_{254} HPTLC plates were validated for this fixed combination: (a) for detection of characteristic constituents of *C. racemosa*, such as triterpene glycosides, (b) for detection of marker compounds typical for *H. perforatum*, such as hypericin. Mobile phases were (a) ethyl acetate: methanol (85:15), (b) toluol: ethyl acetate: formic acid: water (5:15:2:1). The typical validation characteristic for identification tests is their specificity. A characteristic necessary for any analytical procedure is its robustness [1]. For verifying the specificity of both procedures, test solutions of the herbal medicinal product, of herbal substances and of individual placebos were analysed. The procedures were suitable to detect specifically characteristic constituents of both herbal substances as mentioned above. Identification of both herbal substances was possible without any restriction. Specificity was also tested by comparison of two different stationary phases. HPTLC plates were essential (Cimicifuga), respectively prefered (Hypericum) instead of aluminium foil plates. For verifying the robustness of both procedures, three parameters were modified: sample preparation: variation of numbers of extraction cycles during soxhlet extraction; chromatography: variation of the mobile phase; variation of detection (duration of heating, respectively drying conditions). All tested variations led to chromatograms comparable to those obtained under standard conditions. Therefore, robustness was proven, too. **References:** 1. ICH guideline Q2(R1) Validation of analytical procedures: text and methodology; current step 4 version, Nov. 2005 (http://www.ich.org).

P559 HPLC and 2D NMR analyzes as a tool for the quality control of herbal medicinal products: the case of an antihypertensive herbal supplement containing not declared ajmaline and reserpine

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In this study HPLC-DAD-ESI-MS, including HPLC-ESI-MS^n analyses and 2D NMR techniques were applied for the quality control of a herbal supplement. Several reports were received by the pharmacovigilance system by patients who fainted after consuming the product "Olivis", a dietary supplement as an adjunctive treatment for hypertension. Declared components of this product are extracts from *Olea europea*, *Crataegus oxyacantha*, *Fumaria officinalis, Capsella bursa pastoris*. "Olivis" sample was subjected to 2D NMR and HPLC-DAD-ESI-MS analyses in order to identify the marker consituents of the different Herbal Drugs. Comparison of the NMR and chromatographic profiles of the extracts with the original product showed the lack of constituents responsible for antihypertensive activity, such as oleuropein, protopine. Since signals corresponding to polysaccharides submerged those of the other characteristic compounds, fractionation of the preparation was carried out in order to separate the bulk of saccharides. HPLC-DAD and HPLC-ESI-MS analyses of the fractions showed the presence of an alkaloid, ajmaline, as the principal constituent of this product, as well as alkaloids structurally related to ajmaline. HPLC-ESI-MS^n analyses revealed a chromatographic peak identified as reserpine. Phytochemical fractionations led to the isolation of ajmaline, whereas quantitation showed that ajmaline prevailed against reserpine indicating that a *Rauwolfia* species other than *R. serpentina* was used for this product. *Rauwolfia* extracts have been banned but they are available via internet. The present study shows the importance of extensive controls using combined analytical tools of the botanical products on the market to assure their quality and as a consequence their safety profiles.

P560 Compared the separation efficacy of xanthones from mangosteen with three gel-filtration columns

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Mangosteen, *Garcinia mangostana* Linn (Guttiferae), is a popular refreshing juicy fruit in Taiwan. The rinds of mangosteen have been used as a traditional medicine in Thailand for the treatment of trauma, diarrhea, and skin infections. Xanthones are regarded as the major bioactive compounds of the mangosteen hulls which have been confirmed to consist of antimicrobial, anti-inflammatory, antioxidative, and inhibit human immunodeficiency virus infection activities. In present, three gel filtration columns packed with Sephadex LH-20, Toyopearl HW-40, and MCI-gel CHP-20P respectively were used to compare the separative efficacy for the xanthones from mangosteen hulls. The same amount of crude extract of mangosteen hulls was dissolved in methanol and separated by each column with methanol elution. Each eluate fraction was detected by high-performance liquid chromatography coupled with electron-spray ionization mass spectrometry. The result display Sephadex LH-20 has the best separation efficacy. The elution orders for the three major xanthones were β-mangostin, α-mangostin, and γ-magostin. It suggested that the separation mechanisms were complex with gel filtration and adsorption. This method can be used for the xanthones fractionation from crude extract of mangosteen.

P561 Effect of extraction conditions on the content of soluble oxalate in aqueous infusions of green and herbal teas

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Green (GTs) and herbal (HTs) teas are recommended as the alternative hot beverages for subjects who tend to form calcium oxalate stones [1]. The aim of study was to compare the effect of extraction procedures [2] on the content of soluble oxalate (SOX) in drinkable infusions of six GTs and seven HTs sold in Poland. The traditional method of extraction using boiling water at 100 °C was compared with the microwave extraction at 80 °C (EWPM), the ultrasound extraction at 40 °C (EWU-40) and the ultrasound extraction at 60 °C. The highest amount of SOX, as determined by oxidimetric and HPLC methods, was obtained in the infusions made by the EWPM procedure, i.e. 7.73 – 14.89 mg/g (dry mass) for studied GTs, and in range of 3.53 – 18.11 mg/g for studied HTs. The lowest values were obtained after the EWU-40 procedure (5.06 – 10.88 mg/g for GTs, and 1.50 – 11.03 mg/g for HTs). Highest average content of SOX were observed in brews obtained from green whole leaf teas by the EWPM method (14.89 mg/g). The reduced content of SOX was observed in brews obtained by EWPM method from the highly crushed, ready-to-use, express green teas (7.73 mg/g). Similarly, the highest content of SOX after the EWPM extraction was obtained for herbal whole leaf teas as in the case of peppermint (18.11 mg/g), salvia (12.23 mg/g), and nettle (11.76 mg/g). These results could be used for standardization of analyti-

cal methods applied to determination of SOX in imported green teas and domestic herbal teas. **Acknowledgements:** *Nicolaus Copernicus University, Torun, Poland (Internal Grant No. 407/2010).* **References:** 1. Massey, L.K. (2007) J. Am. Diet. Assoc. 107(7): 1191 – 1194. 2. Honow, R., Hesse, A. (2002) Food Chem. 78(4): 511 – 521.

P562 HS-SPME method to monitoring "in vivo" volatile compounds emitted from *Achillea collina* infested by aphids

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Plant leaves normally release small quantities of volatile compounds, but when it is damaged by herbivorous insects, many more volatiles are produced. The emitted volatile fraction plays a key role in plant-environment interaction, being involved in important processes in plant life cycle [1]. Determination of volatile compounds release from living plants are usually performed by enclosing the whole plants or some of its parts in glass o plastic chambers, followed by collection of the emitted compounds in sorbent traps and chromatographic analysis of the adsorbed compounds. This general procedure has some drawbacks for example some material employed as trapping can introduced artifact. This work describes the application of an Headspace Solid Phase Micro-Extraction (HS-SPME) and Gas-Chromatographic Mass-Spectrometric (GC-MS) method to characterize the volatile compounds emitted by leaving leaves of *Achillea collina* Becker ex Rchb, infested by aphids (*Macrosifonella*) using a sampling glass chamber specially designed for this task. In particular, the headspaces volatiles were extracted using a divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS, 70/30 μm) fiber. The extraction conditions including extraction temperature and time were also optimized using the total peak area as index. The best response was obtained at room temperature for 240 min. As a result, many volatile compounds appeared as new compounds after infestation (α-fenchene, aromadendrene and pinocarvone). Other compounds showed an increase trend after infestation by aphid such as camazulene, followed by β-pinene, α-bergamotene and β-phellandrene. In conclusion, the present method is simple and effective and can be used to study the "in vivo" volatile compound emissions from medicinal plants. **References:** 1. Pareja M. et al. (2007). J Chem Ecol 33: 695 – 710.

P563 Development of enzyme-based rapid screening methods for bioactive constituents in plant extracts

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In recent years, there are more and more researches with developments of hyphenated techniques for the rapid identification of antioxidants in complex plant extracts, but the limitations for this on-line analysis may be critical to control the stability and activity of biological reactions [1]. A rapid method for detection and identification of antioxidant compounds in plant extracts was developed by a combination of microplate array analysis using xanthine oxidase, tyrosinase, lipoxygenase as models and HPLC [2,3]. As for the enzymatic reaction with plant extracts, the activity of the enzyme can be kept stable under the experimental conditions. With this simple and rapid method, we can estimate the required minimum screening dose of enzyme for bioactive constituents from plant extracts by HPLC. In addition to the reversible inhibitory effect of enzyme on plant extracts demonstrated by both microplate array analysis and HPLC, we will testify the irreversible inhibitory effect of enzymes with trace amounts of bioactive compounds by LC-TOF/MS. **References:** 1. Tang, D. et al. (2008) J Sep Sci. 31:3519 – 26. 2. S. Zhu; S. et al. (2006) Fitoterapia 77:100 – 108. 3. Chen, C.H. et al.(2009) Molecules 14: 2947 – 2958.

New Targets for herbal medicines

P564 The herbal combination preparation STW 5 is active in DSS-induced colitis

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Herbal extracts often have strong anti-inflammatory properties. To these belongs the phytomedicine STW 5, a combination preparation of nine herbal extracts used in the therapy of functional gastrointestinal diseases, including irritable bowel syndrome (IBS). Earlier pharmacological studies, e.g. in TNBS induced colitis, an experimental model for Crohn's disease, suggest that STW 5 has anti-inflammatory properties. So we decided to test it also in a model of Dextran Sodium Sulphate (DSS) induced colitis, which relates more to ulcerative colitis in man. DSS was administered to rats in the drinking water for 7 days, leading to lesions in the colon, associated with changes in plasma levels of various relevant mediators. STW 5 was administered once daily orally in 3 dose levels (1, 2.5 and 5 ml/Kg) during DSS administration. It reduced the symptom score, colon shortening and colon mass index in a dose dependent manner. The levels of TNFα and myeloperoxidase activity (as inflammation parameters) as well as the levels of glutathione, glutathione peroxidase, and superoxide dismutase (as oxidative stress parameters) were measured in colonic tissue as well as in the blood. The changes in these parameters induced by DSS were favourably influenced by STW 5 in a manner comparable to sulfasalazine (300 mg/Kg) which was used as a reference standard drug. The findings point to the potential therapeutic usefulness of STW 5 not only in IBS, but also in ulcerative colitis.

P565 Rosemary extract enriched in carnosic acid shows anti-obesity and anti-diabetic effects on *in vitro* and *in vivo* models

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Rosemary (*Rosmarinus officinalis* L.) leaf extract standardized to 20% carnosic acid (RE) has shown good antioxidant properties in both ORAC in vitro and LDL oxidation ex vivo assays [1]. Regulating fat absorption by inhibiting the action of the pancreatic lipase activity is an effective way to reduce body weight and obesity [2]. PPARγ represents the major target for the glitazone type of drugs used for the treatment of type 2 diabetes [3]. Therefore, we evaluated the in vitro capacities of RE to inhibit pancreatic lipase activity compared to tetrahydrolipstatin, and to activate PPARγ in a cell based assay compared to rosiglitazone. In addition, we studied the effects of a low-fat diet (LFD), high-fat diet (HFD), and high-fat diet at 500 mpk RE (RED) on C57BL/6J mice during 16 weeks. In vitro results showed that RE was able to inhibit pancreatic lipase activity by 69.8% at 100 μg/ml ($P< 0.001$) and activate PPARγ by 23% at 50 μg/ml ($P< 0.05$) as compared to their respective positive controls. In the in vivo experiment, after a 16 week treatment and compared to both control groups, we observed that RED reduced by 67.7% the body weight gain ($P< 0.001$) and by 79.4% the adipose tissue gain ($P< 0.001$), without affecting the food intake. RED lowered total cholesterol increase by 68.4 ($P< 0.01$). Glycemia was reduced by 72.0% ($P< 0.05$). RED did not show any modification in the safety parameters measured as liver weight gain, AST and ALT levels. These results encourage conducting further studies on obesity and diabetes control using RE. **References:** 1. Ibarra, A. et al. (2010) J Med Food In press. 2. Kim, HY and Kang, MH. (2005). Phytother Res 19:359 – 361. 3. Evans, RM. et al (2004). Nat Med 10:355 – 361.

P566 Effects of some essential oils and saponins on lysozyme activity in human monocytes and epithelial cells

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Lysozyme is one of the most important factors of innate immunity, possessing antibacterial, antiviral, antitumor and immune modulatory activities. Human lysozyme is produced by phagocytic cells and a variety of epithelial cells. In a recent study we have investigated the effect of some essential oils and saponins on the secretion and expression of lysozyme activity in the human monocytic cell line THP-1, and the human colonic epithelial cell line HT-29. The selection of these natural substances was based on their antibacterial, antiviral, anti-inflammatory or immune-stimulating properties. The influence on lysozyme secretion, as an important defense molecule of the human innate immune system, may provide a new mechanism which could explain these properties. Lysozyme activity was determined using highly sensitive fluorescence-based assay [1]. Some saponins from natural origin had stimulating effects on lysozyme activity secretion within one-hour-incubation in both monocytic and epithelial cells. Primulic acid, ginsenosid-Rd, quillaja and gypsophila saponins were proved to induce lysozyme activity secretion in both cell lines. Aescin could stimulate lysozyme activity secretion only in the monocytic cells, while it had no effect when incubated with the epithelial cells. Stimulating effect of essential oils on lysozyme activity in both monocytic and epithelial cell lines could be less frequently observed. Orange blossom oil had stimulating effect on lysozyme activity secretion in both cell lines. Inducing effect on lysozyme activity could also be detected by tea tree oil and majoran oil when incubated with the epithelial cells, whereas no effect or inhibitory effect was observed in the monocytic cells. **References:** 1. Helal, R., Melzig, M.F. (2008) Pharmazie 63(6):415 – 419.

P567 The effect of ginger juice on the gastrointestinal tract is not fully explained by its known constituen

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Ginger, a traditional Chinese herbal medicine, is used to treat digestive disorders in particular to alleviate symptoms of nausea [1]. [6]-Gingerol (6G) is one of the main constituents in ginger and is reported to have anti-oxidant properties [2]. The aims of this study were to use HPLC and ICP-AES to measure the concentration of 6G, and elements in fresh ginger rhizome juice (GJ) and investigate their effects on the upper gastrointestinal tract. In vitro isometric recording was used to investigate GJ (200 µL), 6G [1.59×10^{-5}M], a selected combination of elements (K [4.6×10^{-2}M], Mg [7.4×10^{-3}M], Mn [8.3×10^{-4}M], Na [1.1×10^{-3}M], Ca [5.1×10^{-4}M]), and a "faux" ginger juice on contractile activity of proximal and distal stomach and duodenal segments from *Suncus murinus* (house musk shrew). The concentration of 6G in GJ was 239.4 ± 7.9 mg/L. GJ caused a pro-longed inhibitory effect on duodenal contractions and a biphasic effect on the stomach resulting in an overall increase in tension at 25 minutes. "Faux" GJ did not fully account for the motility effects of GJ, indicating other bioactive constituents were present in GJ (e.g. [6]-shogaol). GJ was most effective on the duodenum (48.3% inhibition of contraction tone), this could be a target for an enteric coated ginger capsule for gastrointestinal disorders. **References:** 1. Lien, HC. et al. (2003) Am. J. Physiol. Gastrointest. Liver Physiol. 284:G481-G489. 8:125 – 132. 2. Kim, JK. et al. (2007) Free Radic. Res. 41:603 – 614.

P568 Determination of dibenzylbutyrolactone-type lignans in *Centraurea* species and analysis of arctigenin's anticancer effect

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Centaurea species (*Asteraceae*) are mainly considered as weeds or ornamental plants but rarely as a herb. Dibenzylbutyrolactone-type lignans, matairesionoside and arctiin, and their aglycones, matairesinol and arctigenin are present in *Centaurea* generea. Arctigenin exhibit antitumor effect against colorectal [1], pancreatic [2] and skin cancer [3]. Isolation of lignans was published mainly for taxonomical classification. We investigated *Centaurea americana*, *C. calcitrapa, C. cyanus, C. dealbata, C. montana* and *C. scabiosa* fruits to quantify their lignan composition by HPLC-UV [4, 5]. Matairesinoside and matairesinol were the major lignan components in *C. scabiosa* with 51.5 mg/g and 11.7 mg/g, while arctiin was in *C. dealbata* with 58.7 mg/g. Arctigenin was present in all *Centaurea* species, with highest of 6.3 mg/g in *C. americana*. The total lignan content ranged from 4.53 mg/g (*C. cyanus*) to 73.8 mg/g (*C. dealbata*) with RSD% between 3.2 – 6.9. Lignans of the selected *Centaurea* species were determined and from pharmaceutical point of view *C. dealbata* seems to be the best source of arctiin and *C. scabiosa* of matairesinoside for further utilization. In the anticancer experiments with arctigenin, inbred C57Bl/6 mice were transplanted with C38 colorectal tumor. Tumor growth rate in mice treated with 50 mg arctigenin/kg body weight was the smallest. The size of the tumors on the 21st day in group was as big as the tumors on the 14th day in the control group. Arctigenin in 50 mg/kg dose was effective against C38 colon cancer. Arctigenin's antitumor activity against C38 colon cancer is promising in *in vivo* experiments. **References:** 1. Hausott, B et al. (2003) J. Cancer Res. Clin. Oncol. 129: 569 – 576. 2. Awale, S. et al.(2006) Cancer Res. 66: 1751 – 1757. 3. Takasaki, M. et al. (2000) Cancer Lett. 158: 53 – 59. 4. Boldizsár, I et al. (2010) J. Chrom A 1217:1674 – 1682. 5. Sedlák, É et al. (2008) Chrom 68: S35-S41.

P569 Suppression of histamine-induced increase of endothelial permeability via nitric oxide production by *Bixa orellana* leaves extract

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Previously reported pharmacological activity of *Bixa orellana* L. (Bixaceae) includes its ability to neutralize edema-forming effects of *Bothrops asper* venom (Nunez V., et al, 2004). Study on the mechanism of its anti-edema activity is thus far lacking. The purpose of this study was to examine the effects of aqueous extract of *B. orellana* (AEBO) leaves on endothelial permeability and the permeability-regulator molecule, nitric oxide (NO), during inflammatory stimulation by histamine. This study demonstrated that AEBO (0.1 mg/ml – 0.4 mg/ml) significantly ($p < 0.05$) suppressed histamine-induced increased endothelial permeability in human umbilical veins endothelial cells (HUVECs), where maximal inhibition was 90.2% at concentration and time point of 0.4 mg/ml and 15 min, respectively. Histamine-mediated NO formation in HUVECs was significantly reduced by all concentration of AEBO in a dose-dependent manner. 0.4 mg/ml showed maximal inhibition where it reduced NO level from 12.51 ± 0.07 µM to 11.3 ± 0.07 µM (65.4% inhibition). On the other hand, 0.1 and 0.2 mg/ml of AEBO suppressed NO production at 21.70% and 34.50%, respectively. To verify that AEBO will produce similar effects to exogenous source of NO as to endogenous NO, NO donor, sodium nitroprusside (SNP) was used. AEBO showed significant effects in scavenging NO radicals released by SNP where maximal inhibition was 51.2% at 0.4 mg/ml. These results indicate that AEBO suppressed increased endothelial permeability by re-establishing normal NO production in HUVECs. This study justifies the use of Bixa orellana in traditional medicine by showing its potential in regulating endothelial cell barrier function. **References:** 1. Nunez V. et al., (2004). Braz. J. Med. Bio. Res. 37: 969 – 977.

P570 Screening of Chinese medicinal plants for inhibition of NF-κB1 gene expression

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Patients suffering from widespread chronic inflammatory diseases – like rheumatoid arthritis and asthma – have to endure serious side-effects due to long-term therapy based on the first-line therapeutics non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. Therefore, research into alternative therapies has been intensified in recent years, especially into the Traditional Chinese Medicine (TCM), that comprises a huge variety of anti-inflammatory plants. A crucial mediator of cellular inflammation is the Nuclear Transcription Factor kappa B (NF-κB) leading to transcription of various pro-inflammatory genes. In this study 68 TCM plants have been examined regarding their potential to decrease the mRNA-level of NF-κB1 in an *in vitro* gene expression assay. THP-1 cells were incubated with herbal extracts (20 µg/ml) and stimulated with LPS, whereas parthenolide served as positive control. The expression level of NF-κB1 mRNA was determined by relative quantification using real-time PCR. The following extracts showed considerable inhibitory effects (> 40%) on NF-κB1 gene expression: *Aristolochia debilis* DCM and MeOH, *Asari species* DCM, *Cassia species* MeOH and *Chrysanthemum indicum* DCM. These extracts were further investigated with a cell viability assay (XTT). The extracts of *Asari species* and *Aristolochia debilis* showed a high toxicity, whereas those of *Cassia species* and *Chrysanthemum indicum* did not affect cell survival. Since the inhibitory effect on NF-κB1 gene expression may contribute to the anti-inflammatory activity, further investigations of the active compounds are in progress. **Acknowledgements:** This work was granted by the Austrian Science Foundation (NFN: Drugs from Nature Targeting Inflammation, S 10705-B03).

P571 Therapeutic and prophylactic effects of betulin emulsions on patients treated with chemotherapy causing hand-foot syndrome

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Betulin-emulsions (BE-emulsion) consist of a triterpene extract (TE) from birch cork. The main TE-component is BE (81%). TE is able to stabilize a W/O emulsion without additives [1]. Experimental studies suggest that the BE emulsion exhibits anti-inflammatory and regenerative effects on irritated skin [2]. Thus, TE supports the natural process of epidermal regeneration and the reconstitution of the epidermal barrier function. Commonly, chemotherapy causes skin alteration accompanied with inflammation and the destruction of the epidermal barrier. The hand-foot-syndrome (= inflammatory skin alterations on the palm and sole of foot) is one of the side effects of capecitabine (Cap) and doxorubicine liposomal (DL). Different stages are defined: 1 no impairment of workaday life; paresthesia, dysesthesia, tingling, painless swelling, 2 erythema, painful swelling, tingling or burning, flaking; 3 flaking, ulceration and functional impairment. Case studies showed positive effects of the therapeutic and prophylactic use of BE-emulsions on skin alteration caused by chemotherapy. 13 patients were treated with skin alterations stage 2 – 3 and 13 patients received prophylactic application. The application of BE-emulsion for 7 and more days, decreased the alterations by reducing inflammation and ulceration of all 13 patients to stage 0 – 1. Prophylactic treatment of 13 patients protected their skin in all cases. In 5 cases only alterations of stage 1 were observed after chemotherapy with Cap or DL under supportive care. The present results suggest that the use of BE-emulsion is a promising treatment option of skin alterations caused by chemotherapy and everyffective in prophylactic application. **References:** 1. Rolf Daniels, Melanie N. Laszczyk: Betulin für tensidfreie Emuldionen. Pharmazeutische Zeitung, 2008; 153: 34 – 35. 2. M. N. Laszczyk, I. Reitenbach-Blindt, W. Gehring: Regenerative und antientzündliche Effekte von Betulin-Emulsionen bei gestörter epidermaler Barrierefunktion. Aktuelle Dermatologie, 2009; 35: 1 – 5.

P572 Anti-adhesive activity of herbal extracts against *Campylobacter jejuni*

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Campylobacter jejuni is one of the most common bacterial causes of diarrhoea in the industrialised world [1], being associated with the occurrence of Guillain-Barré Syndrome (GBS) [2] and induces diseases partially through intestinal adherence [3]. With increasing reports of C. jejuni drug resistance against standard antibiotics [4], investigations into anti-adhesive agents for the prevention of bacterial infection [5] are highly significant. Given the consumer-driven development towards holistic and integrative healthcare [6], research into additional anti-*Campylobacter* effects of phytotherapeutics that are already used for their beneficial effects on bowel and digestive functions is crucial. Dilutions of 21 herbal extracts were screened for anti-adhesive activity against *C. jejuni* using modifications of previously published anti-adhesion assays [7, 8]. Anti-adhesion effects with IC 50 values < 3 mg/mL were obtained for 7 ethanolic plant extracts, with ginger, cayenne and licorice displaying the highest anti-adhesion activity against *C. jejuni* (IC 50: < 0.1 mg/mL, 0.29 mg/mL and 0.65 mg/mL, respectively). Such marked activities could well be clinically relevant. In addition, differences in anti-adhesion activity were found for two different Echinacea species with *E. purpurea* displaying significantly higher and dose dependent anti-adhesion activity than *E. pallida* var. angustifolia. No significant anti-adhesion activity (IC 50 values > 35 mg/mL) was found for agrimony, andrographis, chamomile, fennel, meadowsweet and wormwood extracts. This study provides evidence for additional beneficial effects of marketed phytotherapeutics in gastrointestinal disorders. Further research is required to identify i) synergistic effects of different herbal extracts, ii) anti-adhesive potential of as yet unknown compounds and iii) anti-adhesive activities of known herbal constituents. **References:** 1. Young KT., et al. Nat Rev Microbiol, 2007. 5(9): p. 665 – 79. 2. Mishu, B. et al. Clin Infect Dis, 1993. 17(1): p. 104 – 8. 3. Park, S.F., Int J Food Microbiol, 2002. 74(3): p. 177 – 88. 4. Allos, B.M., Clin Infect Dis, 2001. 32(8): p. 1201 – 6. 5. Wittschier, N., et al., J Pharm Pharmacol, 2007. 59(6): p. 777 – 86. 6. Hirschkorn, K.A., Sociol Health Illn, 2006. 28(5): p. 533 – 57. 7. Beil, W. et al., Phytomedicine, 2007. 14 Suppl 6: p. 5 – 8. 8. O'Mahony, R., et al., World J Gastroenterol, 2005. 11(47): p. 7499 – 507.

P573 The protective effect of luteolin on amyloid β protein (25 – 35)-induced neurotoxicity in primary rat cortical neuron cells and possible mechanisms

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It is well known that neurodegeneration of the amyloid β peptide (Aβ) plays a major part in the memory dysfunction observed in early stages of Alzheimer's disease which shows a significant extent of oxidative damage. The flower bud of *Lonicera japonica* Thunb. has been shown to possess antibacterial, antipyretic and anti-inflammatory effects. Luteolin, belongs to favonoid compounds, is a main active constituent of Lonicerae Flos. In modern pharmacological studies, Luteolin possesses DNA protective effect, anti-inflammatory, anti-oxidant, and is a free radical scavenger.

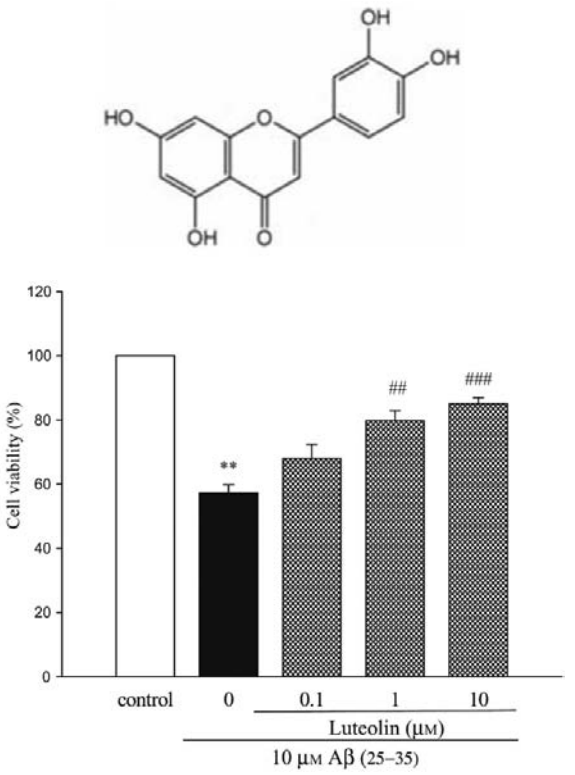

Fig. 1: Chemical structure of Luteolin and protective effect of Luteolin

The present study was carried out to investigate the neuroprotective effect of Luteolin on amyloid β (25 – 35)-induced neuro-toxicity using cultured rat cortical cells. After exposure of primary cultures of rat cortical cells to 10 μM Aβ (25 – 35) for 48 h, it exhibited marked apoptotic death. Pretreatment with Luteolin (1, 10 μM) significantly protected cortical cell cultures against Aβ (25 – 35)-induced toxicity. Luteolin (1, 10 μM) showed a concentration-dependent inhibition on 10 μM Aβ (25 – 35)-induced apoptotic neuronal death, as assessed by MTT assay. Furthermore, Luteolin reduced apoptotic characteristics by DAPI staining. For Western blot analysis, the results showed that protective effect of Luteolin on Aβ (25 – 35)-induced neurotoxicity was mediated by preventing of p-ERK, JNK, p-JNK, p-P38 and caspase 3 activations in rat primary cortical cells. Taken together, the results suggest that Luteolin prevents Aβ (25 – 35)-induced apoptotic neuronal death through inhibiting the protein level of JNK, ERK and p38 MAP kinases and caspase 3 activations. **References:** 1. Hirano T, Higa S, Arimitsu J et al. 2006. Luteolin, a fl avonoid, inhibits AP-1 activation by basophils. Biochem Biophys Res Commun 340: 1 – 7. 2. Brown JE, Rice-Evans CA. 1998. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radic Res 29: 247 – 255.

P574 Influence of Saint John's wort (*Hypericum perforatum*) constituent hyperforin on phagocytosis and inducible nitric oxide synthase (iNOS) in microglia

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Extracts of the flowering upper parts of St. John's wort (*Hypericum perforatum* L.) are used effectively for the treatment of mild to moderate depression, and there is already broad knowledge about the mode of action of extracts and constituents thereof on neurons [1, 2]. However, there is still considerable lack in scientific knowledge about the impact on microglia, the immunocompetent cells of the brain. We investigated the effects of St. John's wort extract and its constituent hyperforin on nitric oxide (NO) production via iNOS in N11 and BV2 mouse microglia. Moreover, the influence on transcription factor activation and phagocytosis was analyzed. We found that extracts of St. John's wort efficiently suppress LPS-induced NO release and identified hyperforin as the responsible compound, being effective at concentrations between 0.25 and 0.75 μM. Reduced NO production was mediated by diminished iNOS expression on the mRNA- and protein-level. In addition, at similar hyperforin concentrations, zymosan phagocytosis was reduced to 20 – 40% and CD206 macrophage mannose receptor expression was down regulated. The observed effects correlated with a suppression of the activated state of Nf-kappaB and phospho-CREB, while c-JUN, STAT1 and HIF-1alpha activity as well as COX-2 expression remained unaffected by hyperforin [3]. **Acknowledgements:** We thank Steigerwald Arzneimittel GmbH (Darmstadt, Germany) for financial support. **References:** 1. Barnes J. et al. (2001) J Pharm Pharmacol 53:583 – 600. 2. Butterweck V. (2003) CNS Drugs 17:539 – 562. 3. Kraus B. et al. (2010) Naunyn Schmiedebergs Arch Pharmacol. 2010 Apr 6. [Epub ahead of print]; doi: 10.1007/s00210 – 010 – 0512-y.

P575 Triterpenes from birch bark extract beneficially affect wound healing

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Triterpenes are the active compounds in birch bark extract which was shown to exert wound healing effects in patients. In this study we investigated the wound healing activity of a triterpene extract (TE) prepared from the outer bark of birch and three of its components, betulin, lupeol and betulinic acid using the scratch assay and 3T3 Swiss albino mouse fibroblasts. The whole extract as well as the three isolated triterpenes enhanced cell numbers in the artificial wound in a concentration-dependent manner. Noteworthy, addition of all three single effects resulted in a similar effect as observed for the whole extract. Cell proliferation as well as cell migration was influenced, with a higher impact on proliferation. Moreover, studies on the underlying molecular mechanism were undertaken. During the inflammatory phase of wound healing, a variety of proinflammatory compounds like cytokines, chemokines and prostaglandins (PG) such as PGE_2 are released [1]. The key enzyme for the PGE_2 production is COX-2. Interestingly, measurement of COX-2 mRNA levels by qRT-PCR in HaCaT cells treated with the birch bark extract revealed a time-dependent increase of COX-2 mRNA, which is mainly caused by betulin. TNFα-prestimulated HaCaT cells, representing a model of inflammatory skin, showed synergistic effects on COX-2 mRNA levels under the extract stimulation. Further studies are in progress to clarify the molecular mechanism of the wound healing effect. **Acknowledgements:** Financial support from the Federal Ministry of Economics and Technology is gratefully acknowledged. **References:** 1. Futagami, A. et al. (2008) Lab Invest, 28(11):1503 – 13.

P576 Induction of apoptosis by γ-humulene in HT29 human colorectal carcinoma cell lines

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The present study was designed to investigate whether γ-humulene exerts cytotoxic activity against colorectal cancer cells by inducing apoptosis and to examine the possible mechanism in the phenomenon. Inhibition of proliferation of γ-humulene on HT29 colorectal cancer cells was determined by the MTT assay. Apoptosis of γ-humulene-treated cells was determined by morphological analysis and quantities by flow cytometry after staining with propidium iodide (PI). Cell cycle and the cell surface expression of the CD95/CD95 ligand were evaluated by flow cytometry. Caspase activities were also analyzed. **Results:** Treatment with γ-humulene of colorectal cancer cells not only inhibited cell proliferation, but also induced apoptosis. Treatment with γ-humulene resulted in an up-regulation of the CD95 receptor and CD95L on the cell surface. γ-humulene-treated cells showed the activation of caspase-8 and caspase-3. Both zVAD-FMK (a broad range caspase inhibitor) and IETD-FMK (a caspase-8 inhibitor) showed apparent inhibition of the apoptosis-inducing effect. Our results suggest that γ-humulene triggers apoptosis in colorectal cancer cell lines *via* the activation of the CD95

receptor/ligand system, and that this agent may be useful for developing new therapeutic regimens for the treatment of colorectal carcinoma.

P577 Influence of isolated compounds of the TCM formulation HLJDT on apoptosis in yeast under stress conditions

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Apoptosis is very important for the development and homeostasis of organisms. Various stimuli e.g. low doses of H_2O_2 can induce apoptosis in *Saccharomyces cerevisiae* [1]. *S. cerevisiae* wildtype BY4741 were used as test organisms. Apoptosis under stress conditions induced by H_2O_2 or acetate was investigated using the TCM formulation Huang-Lian-Jie-Du-Tang (HLJDT; it consists of Gardeniae fructus, zhi zi; 1.5 parts, Scutellariae radix, huáng qín; 1 part, Phellodendri cortex, huáng bai; 1 part, Coptidis rhizoma, huáng lián; 1.5 parts.) and its main compounds (baicalein, baicalin, berberine, geniposide, jatrorrhizine, wogonin) as substrates. In preliminary investigations the influence of the incubation time as well as the influence of different concentrations of the substrates on the model organism was determined. HLJDT did not show any anti apoptotic effect. After H_2O_2 treatment baicalein reduced the cell death significantly. Berberine and geniposide prevented the yeast cells from dying under acetate stress conditions. These pure compounds which reduced apoptosis under stress conditions may play an important role in future in the therapy of neurodegenerative diseases. **Reference:**
1. Madeo F. et al. (1999) J. Cell Biol.145, 757 – 767.

P578 Chronological aging experiments in yeast with the TCM formulation HLJDT

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Similarities of the morphological and physiological changes to mammalian cells make yeast a simple model system for cellular and perhaps organismic aging [1]. During the study of the chronological aging experiments differences between cells of *Saccharomyces cerevisiae* wildtype BY4741 treated with the TCM formulation Huang-Lian-Jie-Du-Tang (HLJDT) and its isolated pure main compounds (baicalein, baicalin, berberine, geniposide, jatrorrhizine, wogonin) in comparison to untreated yeast cells were investigated. HLJDT consists of Gardeniae fructus (zhi zi; 1.5 parts), Scutellariae radix (huáng qín; 1 part), Phellodendri cortex (huáng bai; 1 part) and Coptidis rhizoma (huáng lián; 1.5 parts). In preliminary investigations the most effective concentrations of the lyophilized decoction and its main ingredients were figured out. Different liquid media seem to influence the results. A significant life-span extension detected as colony forming units (CFU) could be observed with the decoction over a period of 15 and 18 days, respectively. The pure compounds showed only weak effect on the prolongation of the life-span of the yeast cells. These results might underline the use of the TCM formulation HLJDT to prevent age-related diseases. **Reference:**
1. Fröhlich, K.-U, Madeo, F.(2001) Exp. Gerontol. 37: 27 – 31.

P579 The neuroprotective effect of schizandrin on glutamate-induced neuronal excitotoxicity

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Glutamate (Glu) receptor-mediated toxicity is an important mechanism of neuronal damage in various pathologic conditions including ischemia, trauma, and neurodegeneration. The excitotoxic neuronal death induced by Glu has been shown to occur through both necrosis and apoptosis depending on Glu exposure. Fructus Schizandrae is widely used as a tonic in traditional Chinese medicine. Fructus Schizandrae contains dibenzocyclooctadiene lignans such as schizandrin. Schizandrin possesses many biological properties, including anti-inflammatory, antitumor, and a potentiating effect on glutathione mediated anti-oxidation. However, there has been less information concerning its protective function against Glu-induced neurotoxicity.

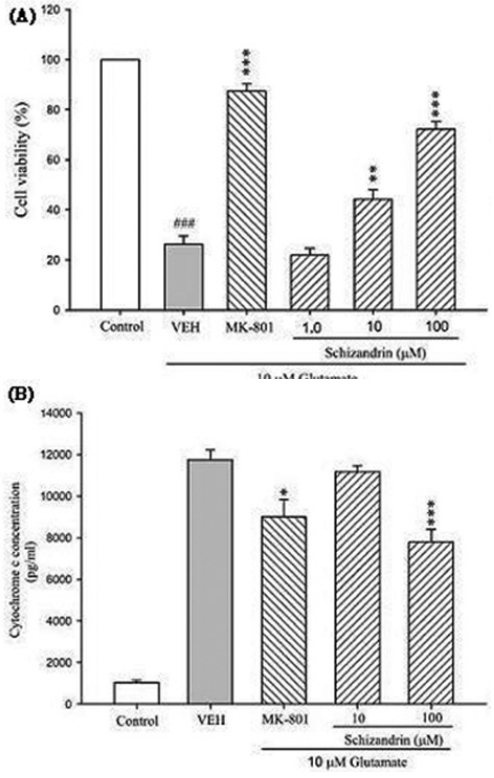

Fig. 1: Neuroprotective activities of Schizandrin against Gluinduced neurotoxicity and repression of cytochrome C release

The neuroprotective effect of schizandrin on the glutamate (Glu)-induced neuronal excitotoxicity and its potential mechanisms were investigated using primary cultures of rat cortical cells. After exposure of cortical cells to Glu for 24 h, cortical cell cultures exhibited apoptotic death. Pretreatment of the cortical cell cultures with schizandrin significantly protected cortical neurons against Glu-induced excitotoxicity. The neuroprotective activity of schizandrin was the most robust at the concentration of 100 μM. Schizandrin reduced apoptotic characteristics by DAPI staining in Glu-injured cortical cell cultures. In addition, schizandrin diminished the intracellular Ca^{2+} influx, inhibited the subsequent overproduction of NO, ROS, and preserved the mitochondrial membrane potential. Furthermore, schizandrin increased the cellular

level of glutathione (GSH) and inhibited the membrane lipid peroxidation malondialdehyde (MDA). Schizandrin attenuated the protein level changes of caspase-9, caspase-3, and cleaved poly(ADP-ribose) polymerase (PARP). Taken together, these results suggest that schizandrin protected primary cultures of rat cortical cells against Glu-induced apoptosis through a mitochondria-mediated pathway and oxidative stress. **References:** 1. Hsieh MT, Tsai ML, Peng WH, Wu CR. Effect of Fructus schizandrin on cycloheximide-induced amnesia in rats. Phytother Res. 1999;13:256 – 257.

P580 Investigations into the bioactive entities of hawthorn extract WS® 1442 responsible for its endothelial barrier protecting activity

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Our previous studies indicate that hawthorn (*Crataegus* spp.) extract WS® 1442 effectively protects against endothelial barrier dysfunction and subsequent edema formation *in vitro* and *in vivo* by influencing key regulation systems of endothelial permeability. Aim of the present study was to gain a first insight into the bioactive principles of this multi-component system. We used 4 different fractions (A-D) of WS® 1442 (Sephadex LH-20 column chromatography, kindly provided by Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany) and examined their impact on key parameters of endothelial barrier function in human endothelial cells. Fractions B (small phenolic compounds, flavonoids), C (oligomeric proanthocyanidins), and D (polymeric proanthocyanidins) inhibited the thrombin-induced endothelial hyperpermeability (Transwell® assay). Interestingly, these fractions differenzially affected the signaling pathways triggered by WS® 1442: Fractions C and D induced a clear augmentation of cAMP concentrations (ELISA), leading to an increase in VE-cadherin stability and an enhancement of cortical F-actin bundles (confocal microscopy). In contrast, the thrombin-induced rise of intracellular calcium was primarily attenuated by fraction B (ratiometric imaging). Fractions B and C were further sub-fractionated (preparative RP-HPLC, Schwabe). Concerning fraction B, two sub-fractions out of ten clearly affected the calcium signaling. Four out of six sub-fractions of fraction C induced cortactin phosphorylation and cortical F-actin redistribution. Currently, these sub-fractions undergo further investigations focusing on their impact on calcium signaling. In summary, we showed for the first time that the different signaling mechanisms triggered by the extract can clearly be assigned to distinct fractions, i. e. phytochemical groups of WS® 1442.

P581 Hepatoprotective effect of *Mahonia oiwakensis* stems against carbon tetrachloride hepatotoxicity

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This study using *in vitro* and *in vivo* models investigates hepatoprotective activities of the ethanol extract of *Mahonia oiwakensis* stems (MOS_{EtOH}). Anti-oxidative activity of MOS_{EtOH} was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. Wistar rats were orally pretreated with MOS_{EtOH} (20, 100 and 500 mg/kg) and silymarin (200 mg/kg) for three consecutive days with administration of carbon tetrachloride (CCl_4) (1 ml/kg, 50% CCl_4 in olive oil). The results showed that MOS_{EtOH} exhibited anti-oxidative activity in the DPPH (IC 50, 0.743 mg/mL) assay. Treatment with MOS_{EtOH} (100 and 500 mg/kg) or silymarin decreased the AST and ALT levels in serum when compared with CCl_4-treated group. Histological analyses also show that MOS_{EtOH} and silymarin reduced the incidence of liver lesions including vacuole formation, neutrophil infiltration and necrosis of hepatocytes induced by CCl_4 in rats. Additionally, MOS_{EtOH} and silymarin attenuated the decreased protein activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd) and increased malondialdehyde (MDA) and nitric oxide (NO) contents in liver as compared with CCl_4-treated group. In conclusion, the MOS_{EtOH} (100 and 500 mg/kg) has a strong hepatoprotective effect on CCl_4-induced hepatic injury in rats and can be used as pharmacological agent for the prevention of liver disorders.

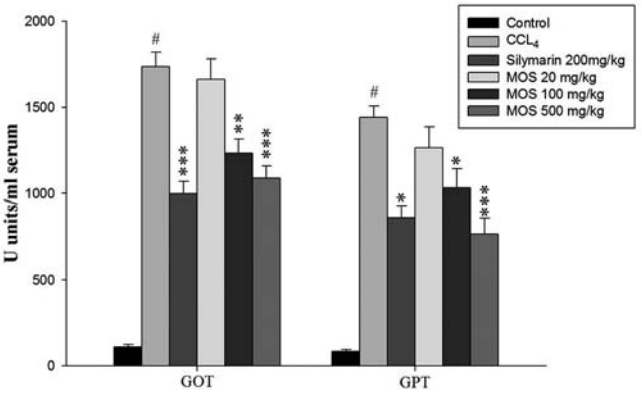

Fig. 1

P582 *Lindera obtusiloba* BL. 70% EtOH extract induced ERK activation inhibits melanin synthesis in mouse B16F10 melanoma cells

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Lindera obtusiloba BL. (LOB) is known to contain various bioactive constituents such as geranyl acetate, L-phellandrene, linderic acid and tsudzuic acid [1], and the plant is used for antioxidative activities and liver protection. In this study, we investigated the effects of a 70% ethyl alcohol leaf extract of LOB on melanogenesis using cultured mouse B16F10 melanoma cells. In mammalian melanocytes, melanin is synthesized within melanosomes that contain at leat three structurally related enzymes: tyrosinase, tyrosinase related protein-1 (TRP-1), and TRP-2 [2]. Microphthalmia-associated transcription factor (MITF) is involved in the pigmentation, proliferation, and survival of melanocytes [3]. Further more, MITF strongly stimulates tyrosinase and TRP-1 promoter activities, indicating that MITF is an important transcriptional regulator of melanogenesis [4]. Our results show that LOB was found to down-regulate microphthalmia-associated transcription factor (MITF) and tyrosinase, and western blotting showed that LOB induces the activation of extracellular signal-regulated kinase (ERK). These results suggest that the ERK pathway is involved in the melanogenic signaling cascade [5], and the ERK activation by LOB reduces melanin synthesis via MITF down-regulation. **References:** 1. Elwood, J. M. and Jopson, J. (1997) Int. J. Cancer. 73: 198 – 203. 2. Kobayashi, T. et al. (1994) EMBO J. 13: 5818 – 5825. 3. Streingrimsson, E. et al (1994) Nat. Genet. 8: 256 – 263. 4. Bertolotto, C. et al (1998) Mol. Cell. Biol. 18: 694 – 702. 5. Englaro, W. et al. (1998) J. Biol. Chem. 273: 9966 – 9970.

P583 Oral administration of *Vaccinium uliginosum* L. extract inhibits the development of DNCB-induced atopic dermatitis in NC/Nga mice

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Vaccinium uliginosum L. (also known as Bog Bilberry or Northern Bilberry) has been reported to have anti-oxidant [1] and protective effects against UV-induced skin photoaging [2]. To investigate the anti-atopic dermatitis effects we orally administrated *Vaccinium uliginosum* L. extract (VU) dissolved in distilled water (90, 150, 250 mg/kg) to atopic dermatitis induced NC/Nga mice (n = 5) daily for 4 weeks. Prednisolone treatment group (3 mg/kg, n = 5), normal group (DNCB -, VU -, n = 5), and negative control group (DNCB+, VU-, n = 5) were compared to VU treatment groups. Oral administration of *Vaccinium uliginosum* L. extract significantly inhibited the exacerbation of AD-like skin lesions, thickness of ear and scratching behavior comparing to negative control group (DNCB +, VU -). Moreover, VU treatment decreased serum IgE level in a dose dependent manner, and significantly reduced IL-4 and IFN- γ production in concanavalin A (Con A) stimulated splenocyte. These results suggest that *Vaccinium uliginosum* L. extract may be effective therapeutic agent against Atopic Dermatitis by inhibiting IgE and cytokines production. **References:** 1. Kim YH., et al. (2009) J. Med. Food. 12(4): 885 – 892. 2. Bae JY., et al. (2009) Mol. Nutri. Food Res. 53: 726 – 738.

P584 Rosmarinic acid as the effective compound in *Cordia americana*

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In Brazil medicinal plants have been widely used for the treatment of diseases in folk medicine. However, the effective compounds responsible for the biological effects are widely unknown. The objective of this study was to characterize the phytochemical profile and to identify bioactive compounds in the leaves of *Cordia americana* (Boraginaceae) [1]. The biological activity of the constituents and the ethanolic extract were investigated for the inhibition of 5-lipoxygenase, p38α MAPK, TNFα release and fibroblast scratch assay, targeting different aspects of inflammatory and wound healing processes. The phytochemical studies (i.e., 1D, 2D NMR and MS) have led to the identification of flavonols, phenolic compounds and phytosterols. Quantification analysis showed that rosmarinic acid (RA) is the main compound with an amount of 8.44% in the ethanolic extract. RA as well as the ethanolic extract exhibited the highest inhibitory effects on 5-lipoxygenase (IC_{50}= 0.97 and 0.69 μg/ml, resp.) and p38α MAPK (IC_{50}= 1.16 and 3.25 μg/ml, resp.). Additionally, RA inhibited the release of TNFα to 36.75 ± 1.55% at a concentration of 36.03 μg/ml, which can be considered as moderately active. The ethanolic extract showed a lower inhibition of 30.3 ± 0.75% at a concentration of 100 μg/mL. RA also exhibited slight stimulatory activity on proliferation and migration of fibroblasts indicating that it may partially contribute to the wound healing effects of this plant. We demonstrated for the first time pharmacological effects of *C. americana* and we provided evidences for a crucial role of RA as the major player. **Acknowledgements:** Financial support from the government Baden-Württemberg (Zukunftsoffensive IV) is gratefully acknowledged. **References:** 1. Sobral, M. *et al* (2006). Flora arbórea e arborescente do Rio Grande do Sul, Brasil. RiMa Publisher. Porto Alegre.

P585 Aesculin from Butcher's broom reduces the permeability of endothelial cells *in vitro*

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Herbal medicinal products containing Butcher's broom (Rusci rhizoma, *Ruscus aculeatus*, Ruscaceae) are used as supportive medications against chronic venous disorders (CVD). Furo- and spirostanol glycosides have been suggested to be responsible for the efficacy of the drug [1]. The aim of this study was to identify phenolic ingredients in Rusci rhizoma which could contribute to the overall effect of Butcher's broom preparations. As a first result, aesculin was isolated from a defatted methanolic extract of Rusci rhizoma by liquid-liquid chromatography between butanol and water and further LC of the butanol fraction by Sephadex-LH20®, silica gel and RP-18. After structure elucidation by modern spectroscopic methods like 1D-, 2D-NMR techniques and ESI-MS, the potential of aesculin to affect endothelial barrier dysfunction *in vitro* was tested. Therefore, macromolecular permeability of human microvascular endothelial cells (HMECs) was measured in a Transwell® assay (0.4 μm pore size) using FITC-dextran (40 kDa, 1 mg/ml) as tracer. To induce endothelial barrier breakdown, HMECs were treated with thrombin (3 U/ml) for 60 min. The pretreatment (30 min) with aesculin concentration-dependently (0.1 – 10 μM) inhibited the thrombin-induced rise of endothelial permeability. In summary, aesculin was for the first time described as an ingredient of Rusci rhizoma. The pharmacological results indicate that phenolic compounds of Butcher's broom, such as aesculin, might also contribute to the efficacy of the whole drug against CVD. Ongoing research focuses on the identification of further compounds from Rusci rhizoma with protective activities on the human endothelium. **Acknowledgements:** Thanks are due to Prof. Dr. Heilmann for helpful discussions **References:** 1. ESCOP Monographs, 2nd edition (2003).

P586 eNOS-activity guided fractionation of vine leaves from Austria

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Polyphenol fractions of Austrian red wine samples are reported to increase endothelial nitric oxide synthase (eNOS) activity in EA.hy926 endothelial cells [1]. The present investigation aims to identify further eNOS-activating fractions from red vine leaves. Two leaf samples of the variety Blaufränkisch from the region Neusiedlersee-Hügelland were selected for bio-assay guided fractionation using EA.hy926 endothelial cells and the [14C]L-arginine/[14C]L-citrulline conversion assay measuring eNOS activity. The first leaf sample was harvested late in September together with grapes as green vine leaves (GVL), and the other sample was collected as red vine leaves (RVL) five weeks later in the same vineyard. Both vine leaf samples were dried, pulverised, and extracted consecutively with dichloromethane and methanol using an accelerated solvent extractor. Further separation of the methanol-extract of the two samples was done by liquid-liquid partition with ethylacetate and water resulting in a polar fraction (PF) and an apolar fraction (AF). The methanol-extract of both vine leaf samples (GVL, RVL) showed the same level of enhanced eNOS activity at a concentration of 600 μg/ml. The dichloromethane-extracts of GVL and RVL, tested in equal concentration, decreased the activity significantly. The AF of the samples revealed an activating effect on eNOS at 300 μg/ml, whereas the complementary PF did not increase enzyme activity at the same concentration. Thus, the compounds responsible for eNOS activation seem to reside in the AF. Therefore, ongoing bio-assay guided fractionation of the AF will show which class of compounds is responsible for the observed increased eNOS activity. **References:** 1. Donath O et al. (2008) Polyphenols Communications, 699 – 700.

P587 Anti-inflammatory effects of ethanolic extracts from *Micromeria* species

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Micromeria species are traditionally used as expectorant, antispasmodic and stimulant agents as well as condiments [1]. Our study aimed to evaluate *in vitro* modulation effects of three *Micromeria* ethanolic extracts on interleukin-6 (IL-6) production in comparison with hydroxycinnamic acids. IL-6 is a cytokine with various essential biological activities. It plays important roles in the regulation of immune response and inflammation [2]. Total contents of hydroxycinnamic acids were spectrophotometrically determined in aerial plant parts of *M. croatica* (6.8%), *M. juliana* (5.4%) and *M. thymifolia* (5.3%). TLC analysis showed the presence of caffeic, chlorogenic and rosmarinic acids in studied plant extracts. Modulation of IL-6 production in LPS-stimulated male Balb/C mice splenocytes by plant extracts (18.75 – 300 μg/mL), as well as caffeic acid, chlorogenic acid and rosmarinic acid (125 – 2000 μM) was exhibited using enzyme-linked immunosorbent assay (ELISA). None of the investigated samples showed cytotoxic effect or inhibition of cell growth of human THP-1 and HepG2 cell lines, as well as on mouse splenocytes (where we observed increased absorbance) by MTS citotoxicity test. In tested concentrations, all extracts and hydroxycinnamic acids inhibited production of IL-6 in the range of 9.5 – 100% and 30.6 – 100%, respectively. Among the extracts, the activity decreased in order *M. juliana* > *M. croatica* > *M. thymifolia*, percentages of inhibition were 56.6%, 54.3% and 42.1% at concentration of 75 μg/mL. These findings suggest that *Micromeria* ethanolic extracts possess anti-inflammatory activity, which could be attributed to the presence of hidroxycinnamic acids. **References:** 1. Vladimir-Kneevic, S. at al. (2000) Farm. Glas. 56:301 – 312. 2. Nishimoto, N., Kishimoto, T. (2004) Curr. Opin. Pharmacol. 4:386 – 391.

P588 Contribution of the components of STW 5 to its inhibitory effect on gene expression and release of the pro-inflammatory cytokine TNF-α

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STW 5 (Iberogast®) is successfully used in the therapy of gastrointestinal disorders such as functional dyspepsia and irritable bowel syndrome (IBS). Pharmacological studies have demonstrated a multi-target effect of this fixed herbal combination. Actions on nerve, smooth muscle, epithelium and intestinal inflammation are revealed. Given that recent clinical data suggest an inflammatory etiology of IBS, the influence of STW 5 and its components on the production of the pro-inflammatory cytokine TNF-α was examined. The gene expression of TNF-a was determined in rat ileum preparations by realtime-RT-PCR. TNBS was used to induce inflammation. The release of TNF-α was measured in LPS-stimulated human monocytes using a commercially available ELISA. The TNBS-induced inflammation was accompanied by an increased TNF-α gene expression. STW 5 inhibited the increased gene expression and reduced significantly the release of TNF-α to 13% in LPS-stimulated monocytes, while having no effect in untreated cells. In further experiments the single herbal components of STW 5 were tested separately in concentrations equivalent to those in the combination. Caraway (Carum carvi L.), milk thistle (Silybum marianum L.), lemon balm (Melissa officinalis) and greater celandine (Chelidonium majus L.) had no effect on the TNF-α release. Bitter candytuft (Iberis amara L.), peppermint (Mentha piperita L.), chamomile (Matricaria recutita L.), liquorice (Glycyrrhiza glabra L.) and angelica (Angelica archangelica L.) reduced the TNF-α release, though less pronounced as compared to STW 5. The results indicate that a group of five components, including Iberis amara, contributes relevantly to the distinct effect of the herbal combination STW 5.

P589 Trans-crocetin is involved in the inhibition of the glutamatergic synaptic transmission on rat cortical neurones by saffron extract

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Saffron is the dried stigmata of *Crocus sativus* L. Saffron contains a large quantity of carotenoids, the crocins, glycosides of the C_{20} apocarotenoid trans-crocetin. It has been shown that ethanolic (80 vol.-%) saffron extract (CSE) and trans-crocetin, interact with the phencyclidine binding site of the NMDA receptor [1]. The aim of the present study was to examine the influence of CSE and trans-crocetin on the glutamatergic synaptic transmission in rat brain slices. Postsynaptic potentials (PSPs) were elicited by focal electrical stimulation in pyramidal cells of the cingulate cortex and recorded using intracellular placed microelectrodes. PSPs were induced by glutamate released from presynaptic terminals which activated postsynaptic NMDA and non-NMDA receptors. In contrast, externally added glutamate induced a depolarisation of the cell membrane of the pyramidal cells. CSE (10 – 200 μg/ml) decreased the glutamate-induced membrane depolarisation and inhibited the evoked PSPs. CSE inhibited the isolated NMDA and non-NMDA component of the PSPs and decreased the NMDA and kainate induced depolarisation. This indicates an antagonistic effect of CSE on NMDA and kainate receptors. Trans-crocetin (1 – 50 μM) investigated under the same conditions as CSE showed comparable inhibitory effects on the glutamate-induced membrane depolarisation and evoked PSPs. Trans-crocetin decreased the NMDA induced membrane depolarisation, but compared to CSE trans-crocetin did not inhibit the isolated non-NMDA component of the PSPs. Therefore, it seems that trans-crocetin did not interact with the non-NMDA type of glutamate receptors. We conclude that trans-crocetin is involved in the antagonistic effect of CSE on NMDA but not on kainate receptors. **References:** 1. Lechtenberg, M. et al. 2008, Planta Med 74: 764 – 772.

P590 Acylated flavonol monorhamnosides from *Eriobotrya japonica* as XIAP BIR3 inhibitors revealed by *in silico* and HPLC-SPE-NMR techniques

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Targeting the X-linked inhibitor of apoptosis proteins (XIAP), baculoviral IAP repeat (BIR) 3 groove represents an innovative strategy for increasing the sensitivity of resistant tumour cells to conventional chemotherapeutic drugs [1]. Using a previously generated pharmacophore model [2], 3D models of 122 reported constituents from the leaves of the medicinal plant *Eriobotrya japonica* Lindl. (Rosaceae) were virtually screened against this target. Based on the predicted hits, we focused on acylated flavonol monorhamnosides (AFMR) as promising phytochemical class. AFMRs identified in the crude methanol extract by LC-MS and enriched by chromatographic methods, showed chemosensitizing potential in combination with etoposide in XIAP-overexpressing Jurkat cells (Nicoletti assay [3]). Application of the HPLC-SPE-NMR hyphenated technique enabled the structure elucidation of two new AFMRs and three known ones. By means of preparative HPLC, one of the main constituents of the AFMR mixture, the virtual hit kaempferol-3-*O*-α-L-(2″,4″-di-E-p-coumaroyl)-rhamnoside (**1**), was isolated. Compound **1** sensitizes XIAP-overexpressing Jurkat cells towards the treatment with etoposide (Nicoletti assay), binding to the XIAP BIR3 groove with a dose-dependent affinity (IC_{50} 7.69 μM, fluorescence polarization based binding assay [4]). In accordance with the predicted structural requirements within the binding site, its substructures, kaempferol-3-*O*-α-L-rhamnoside and kaempferol, were inactive (IC_{50} > 100 μM, resp.) revealing a major impact of the acid and sugar moieties of **1** on bioactivity. In conclusion, this study elucidates **1** as natural, small-molecular weight inhibitor of the XIAP BIR3 groove using a combination of *in silico* and

HPLC-SPE-NMR techniques. **References:** 1. Schimmer, A.D. et al. (2006) Cell Death Differ. 13: 179 – 188. 2. Bliem, C.B. et al. (2006) Planta Med. 72: 1008. 3. Nicoletti, I. et al. (1991) J. Immunol. Methods 139: 271 – 279. 4. Nikolovska-Coleska, Z. et al. (2004) J. Med. Chem. 47: 2430 – 2440.

P591 A prolonged protein kinase C-mediated, opioid-related antinociceptive effect of St. John's Wort in mice

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The antinociceptive profile of St. John's Wort (SJW) was investigated in mice in a condition of acute thermal and chemical pain, together with the mechanism that might underlie this effect. A standardised extract of SJW induced a prolonged antinociception that persisted for 120 minutes after administration. The thermal antinociception was prevented by naloxone and by the protein kinase C (PKC) activator PMA, whereas the chemical antinociception was prevented by PMA, remaining naloxone insensitive. A chloroform (CHL) and a methanol (MET) fractions, containing hyperforins and hypericin plus flavonoids, respectively, increased pain threshold with a time course comparable to the total extract. The CHL antinociception was prevented by naloxone, whereas the MET antinociception was antagonized by PMA. Purified hyperforin and hypericin showed an antinociceptive efficacy comparable to CHL and MET, respectively. Conversely, flavonoids were devoid of any effect. The administration of yohimbine and atropine did not modify SJW, CHL and MET antinociception. These results indicate that both CHL and MET fractions mediate the SJW-induced antinociception. In particular, the presence of hypericin was fundamental to induce both thermal and chemical antinociception through the inhibition of the PKC activity, whereas hyperforin selectively produced a thermal opioid antinociception. These findings identify important targets for a longer-acting activation of endogenous pain systems and should potentially help clinicians who seek safe, tolerable, and prolonged treatments for pain relief.

P592 Greek mountain tea – an herbal drug for mental enhancement

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The aerial parts of Ironwort (Sideritis ssp.) have been used for a long time, commonly known as "Mountain tea" in Greece, Turkey, Bulgaria and Albania. Mountain farmers around eastern Mediterranean region drunk beverages and classical (caffeine-free) tea preparations from Herba Sideritis traditionally after work for calming down and to remedy the common cold [1]. Newer investigations on highly lipophilic extracts [2] demonstrate reuptake-effects on serotonin metabolism in-vitro. All results were without relationship to the distinct used Sideritis species nor of their origin. Our actual investigations used this testing model as screening tool to evaluate potentials of different Sideritis species and aqueous tea-analogue extracts as well ethanolic-water extracts. Findings were used to prepare study preparation (nutrifin® mental) in an in-vivo mouse model. Herein an ElectroEncephaloGraphic Pharmacogram (EEGP) was made to detect possible psychopharmacological effects [3]. Strongest effects were seen with respect to alpha2 waves representing an activation of dopaminergic neurotransmission. Delta, theta, and especially at higher dosages also alpha1 waves were also attenuated, compatible with the view of activation of the cholinergic, norepinephrinergic and serotonergic transmission systems. All activities were located in frontal cortex and hypocampus regions responsible for cognitive performance. A clinical study on the acute effects of nutrifin® mental on healthy humans is currently in progress. **References:** 1. Dioscurides (1st century A.D.) De Materia Medica. 2. Knörle R., Schnierle P. (2005) Patent application EP 1634602. 3. Dimpfel W., Profiling of Extr. Sideritis aquos. sicc. by means of EEGpower spectra of conscious freely moving rats (electropharmacogram). unpublished data (2009).

P593 Efficacy of extracts of Thai medicinal plants as an anesthetics on carp fish (*Cyprinus carpio*)

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Anesthetics are widely used in aquaculture to prevent stress during handling. Main objective of our research is to investigate the efficacy of Thai medicinal plants in carp fish (*Cyprinus carpio*). We focused on the plant extracts from the family of Piperaceae according to their tranquilizing and sedative potential (1, 2). *Psidium guajava* was also included due to the ability to induce CNS depression (3). The ethanolic extract of *Piper betle* and *Psidium guajava* caused were significantly able to induce surgical stage (stage 4) anesthesia in concentration dependent manner. Extracts of *P. betle* at concentrations of 0.1, 0.2, 0.3 and 0.4 mg/ml were able to induce 76.67 ± 3.66, 90.00 ± 4.14, 95.00 ± 5.77 and 97.50 ± 2.89% of fish in to anesthesia state, respectively. *P. guajava* extracts caused 80.00 ± 10.00, 86.67 ± 15.28 and 100% of fish to loss pain reflex, at concentrations of 0.1, 0.2 and 0.3 mg/ml, respectively. The extract of *P. betle* significantly induced a faster anesthesia at 0.1 mg/ml when compared to *P. guajava*. The recovery time of *P. guajava* was longer than *P. betle* at the same concentration. The acute toxicity tests were performed during 20 min incubation periods. At concentrations of 0.1, 0.2, 0.3 and 0.4 mg/ml *P. betle* caused a 30.0 ± 10.0, 47.50 ± 9.57 and 75.00 ± 5.77% mortality, respectively. The mortality rates of fish exposed to *P. guajava* were 20.0 ± 10.0, 50.0 ± 10.0, 83.33 ± 5.77% at the concentrations of 0.1, 0.2 and 0.3 mg/ml, respectively. These findings suggest that the extract from *P. betle* could be an effective anesthetic for his species. **References:** 1. McFerren, M.A. et al. (2002) J. Ethnopharmacol. 83: 201 – 207. 2. Garrett, K.M. et al. (2003) Psychopharmacology (Berl). 170: 33 – 41. 3. Shaheen, H.M. et al. (2000) Phytother. Res. 14: 107 – 111.

P594 Allergy-preventive effects of flowers of *Campsis grandiflora*

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We used an *in vivo* assay system, which we had previously developed to search for allergy-preventive substances from natural sources. [1] This method monitors the decrease in blood flow (BF) in the tail vein of mice subjected to hen egg-white lysozyme (HEL) sensitization. The BF decrease is very complicated, involving various factors such as NO from iNOS, TXA_2, PGI_2, ET-1, granulocytic elastase, COX-1, 2 and cNOS. [2] Using this assay system, we found that the MeOH extract (CG) of flowers of *Campsis grandiflora* Thumb. (Bignoniaceae) significantly reduced the decrease of BF. Flowers of *C. grandiflora* have been used in traditional Chinese medicine to treat bruises, pruritus, diuresis and thrombosis. In this study, we report on the allergy-preventive effects of CG and its active compounds. The BF of HEL-sensitized mice (control group) gradually and significantly decreased, falling to about 70% of the BF of normal mice at day 9. CG could significantly the decrease of BF. Active-guided fractionation of CG led to isolation of apigenin (**1**), acteoside (**2**) from the most active AcOEt fraction and rengyoside (**3**), rengyol (**4**) and isorengyol (**5**) from a few active *n*-BuOH extracts. Among these compounds, **1** and **2** significantly reduced the decrease of BF. **1** has been reported to inhibit platelet aggregation and inhibit the expression of iNOS or COX-2, while **2** have been reported to have anti-inflammatory and antioxidant effects. Our findings show that CG could be useful for preventing allergy. Details of the mode of action are under investigation. **References:** 1. Ishiguro, K., Oku, H., Ueda, Y., Iwaoka, E., Kunitomo M., (2005) Biol. Pharm. Bull., 28;1490 – 1495. 2. Oku, H., Ogawa, Y., Iwaoka, E., Kunitomo, M., Ueda, H., Okamura, H., Ishiguro, K., (2007) Biol. Pharm. Bull., 30; 1324 – 1328.

P595 The in vivo and in vitro angiogenic evaluation of the essential oil of *Echinophora tournefortii*

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Angiogenesis is the formation of new blood vessels occuring in embryo growth and wound healing. It has a vital role in certain pathologies like chronic inflammation and cancer. The antiangiogenic approach as a new advance and target to treat or prevent such pathologies looks very promising (1). In this study, the essential oil from the aerial parts of *Echinophora tournefortii* Jaub.& Spach. was investigated for its in vivo and in vitro angiogenic/antiangiogenic properties to support its use for wound healing in folk medicine. The essential oil was obtained by hydrodistillation, which was analyzed both by GC and GC-MS. The main constituents of the oil were identified as myrcene (29%) and α-pinene (28%). Using the in vivo Chorio Allantoic Membrane (CAM) assay and in vitro toxicity (MTT), cell migration and tube formation tests (HUV-EC-C cell lines) the oil and its main constituent myrcene were tested at various concentrations. Cortisone, suramin, sodium dodecyl sulphate and thalidomide were used as standards in both assays. The oil showed a weak antiangiogenic effect with slight irritation in vivo and antiangiogenic activity in a dose dependent manner in vitro. It did not show embryotoxicity in vivo but cytotoxicity in vitro. Myrcene showed a weak antiangiogenic effect in vivo as well as a wound healing effect in a dose dependent manner but no antiangiogenic activity in vitro. **Acknowledgements:** This work was financially supported by TUBITAK Project No: SBAG-107S262 **References:** 1. Paper, D., Natural products as angiogenesis inhibitors, Planta Med., 64, 686 – 695 (1998).

P596 Genome-scale microRNA identify small RNA modulators of the effect of berberine on pancreatic cancer cell growth

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MicroRNAs (miRNAs) are newly identified small RNA molecules up to 22 nucleotides in length. miRNAs have emerged as important regulators of genes involved in many biological processes, including development, cell proliferation and differentiation, apoptosis and metabolism. Disturbance of microRNA expression may play a role in the initiation and progression of certain diseases including cancer. Human cancers commonly exhibit an altered expression profile of miRNAs with oncogenic (miR-21) or tumor-suppressive (miR-34a) activity. Pancreatic cancer is one of the most aggressive human malignancies with an incidence rates almost identical to mortality rates. This discouraging information is due to the lack of improvement in detection and diagnosis strategies and the paucity of breakthroughs in treatment regimens. A successful drug development in this disease continues to be a major challenge. Recently, a microRNA expression signature has been identified that is associated with pancreatic cancer. Berberine is an isoquinoline alkaloid, isolated from *Rhizoma coptidis*, and reported to have anti-cancer effects in different human cancer cells. There is however, no available information on the effect of berberine on global miRNA expression pattern in pancreatic cancer. Here we carried out a global miRNA-based expression profiling study in order to identify novel molecular miRNA targets mediating the growth inhibitory effects of berberine on pancreatic cancer cells. Among the 59 significantly expressed genes, 14 were significantly up-regulated and 45 were significantly down-regulated in their expression. Our results showed, for the first time, that berberine was able to modulate the miRNA signature of pancreatic cancer cells.

P597 Two novel phloroglucinols from *Hypericum empetrifolium* with antiproliferative activity on endothelial cells

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Acylphloroglucinols are often accumulated in the familiy Clusiaceae. They seem to contribute to the antidepressant efficacy of St. John's wort and have antibacterial activities. More recently, a potent anti-angiogenic activity has been described [1]. Aim of the present study was to identify compounds from the endemic greek *Hypericum* species *H. empetrifolium* (Clusiaceae) with antiproliferative activity on endothelial cells to identify possible new anti-angiogenic substances. As a first result, two novel acylphloroglucinol derivatives were isolated from a petrol ether extract of *H. empetrifolium* after fractionation by LC on silica gel, RP-18 and purification by preparative HPLC (RP-18), namely 1-O-(p-1-menthen-8-yl)-4-geranyl-2-(2-methylpropionyl)-phloroglucinol (**1**) and 1-O-(p-1-menthen-8-yl)-4-geranyl-2-(2-methylbutyryl)-phloroglucinol (**2**). Their structures were elucidated by 1D-, 2D-NMR techniques and ESI-MS. To determine a possible anti-angiogenic activity, the inhibition of endothelial cell proliferation was measured. Subconfluent grown HMEC-1 cells (human microvascular endothelial cell line) were treated with (**1**) and (**2**) for 3 days before cells were stained with crystal violet. Both substances showed a concentration dependent activity by inhibiting the cell proliferation between 1 and 20 µM.

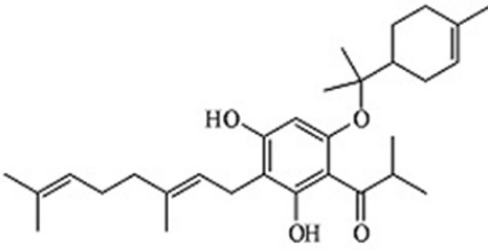

Fig. 1: 1-O-(p-1-menthen-8-yl)-4-geranyl-2-(2-methylpropionyl)-phloroglucinol

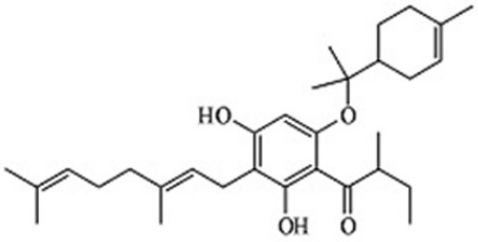

Fig. 2: 1-O-(p-1-menthen-8-yl)-4-geranyl-2-(2-methylbutyryl)-phloroglucinol

Acknowledgements: Thanks are due to Prof. Theophanis Constantinidis, University of Athens, Faculty of Biology, for botanical classification as well as to Dr. E. Ades and Mr. F. J. Candal of CDC (USA) and Dr. T. Lawley of Emory University (USA) for providing the HMEC-1 cells **References:** 1. Martinez-Poveda, B. et al. (2010) PLoS One 5(3): e9558.

P598 Influence of hyperoside and hyperforin on the regulation of β1-adrenergic receptors on living C6 glioblastoma cells: A new postsynaptic approach for the mode of action of St. John's wort

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In depressive disorders hyperactivity of the hypothalamic-pituitary-adrenal axis is one of the most consistent findings which can be corrected with standard antidepressant drugs. One of the receptors in-

volved in mediating the effects of antidepressants is the β1-adrenergic receptor (β1-AR). It was shown that pretreatment of C6-cells (overexpressing the β1-AR-GFP) with 1 μM of hyperforin or hyperoside from St. John's wort extract, led to β1-AR downregulation [1], the same holds true for desipramine (DMI) and was explained by inhibition of receptor recycling [2]. Here we investigated the effects of preincubation with 1 μM of DMI, hyperforin and hyperoside on cAMP-levels of C6-cells. Compared to non-treated control cells, both hyperforin and DMI decreased the basal cAMP-level, while hyperoside did not show any effect. In contrast, under stimulating conditions hyperoside reduced the dobutamine (selective β1-agonist) mediated increase in cAMP formation significantly, whereas stimulation of the remaining β1-AR seemed to be unaffected after both DMI and hyperforin preincubation. Although pretreatment of C6-cells with DMI, hyperforin and hyperoside under stimulating conditions led to a similar reduction in cAMP formation, apparently different modes of action are responsible for this effect. Remarkably, reduction in β1-adrenergic sensitivity of C6-cells with hyperforin and hyperoside from St. John's wort extracts was found without the need of increased presynaptic neurotransmitter release and downstream regulatory processes. Thus, the direct influence of hyperforin and hyperoside on the regulation of postsynaptic β1-adrenergic receptors is a novel contribution to the mode of action of St. John's wort extracts in the treatment of mild to moderate cases of depression. **References:** 1. Prenner L., (2006) Untersuchungen zum Einfluss von Inhaltsstoffen aus Hypericum perforatum L. auf das β-adrenerge Rezeptorsystem am postsynaptischen Modell lebender Zellen, Thesis, Bonn. 2. Bürgi, S. et al. (2003) Antidepressant-induced switch of beta 1-adrenoceptor trafficking as a mechanism for drug action, J. Biol. Chem. 278 (2), 1044 – 1052.

P599 Effect of parsley (*Petroselinum crispum*) extract on intra-abdominal fat deposition in mice

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In this study the effects of non toxic concentrations of parsley (*Petroselinum crispum*) decoction (0.5%) on fat deposition in pregnant mice was assessed. **Methods:** 40 adult female mice were mated overnight and checked daily for vaginal plaque until yielding 20 pregnant mice. The vaginal plaque observation was considered as the1st day of pregnancy. The remaining females were considered as non pregnant. Both pregnant and non pregnant animals were randomly and equally divided into 2 groups. Animals of both control groups received tap water while the animals in test groups received parsley decoction (0.5%) in the whole period of gestation ad libitum. 18 days after the onset of the experiments animals were sacrificed by deep anesthesia and striping fats deposited in the abdominal cavity from diaphragm to the genitalia were precisely removed and weighted. **Result:** There was no significant difference between the fat deposition in pregnant and non pregnant animals and the intra abdominal fat deposition in all test groups. **Conclusion:** In the literature it is reported that parsley decreases blood cholesterol in some patients[1]. However, our data indicate that parsley has not any effect on the intraabdominal fat deposition. **References:** 1. Suido, H. et al. (2002) J Agric Food Chem. 50: 3346 – 50.

P600 Prevention effect of *Matricaria recutita* L. on withdrawal syndrome in morphine dependent female rats

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Addiction to opiates such as morphine is in one of the major public health problems. Some reports show that Matrivaria chamomilla extract contains flavonoids, which exert Benzodiazepine – like activity and inhibited the expression of abstinence syndrome in morphine dependent animals. **Objective:** to determined and comparison the effect of Matricaria chamomilla extract injection on morphine withdrawal syndrome signs (MWS) in rats. **Material and Methods:** 32 male rats (200 – 300gr) (n = 8) were tested in 2 groups: control and morphine groups (twice daily for 10 days) and divided in 4 sub groups: (1) saline only group, (2) morphine group were received 10 mg/kg morphine for 10 days, (3) Matricaria chamomilla group – 25 and 50 mg/kg I.P 30 min before Morphine administration and (4) Methadone group. In day 10, 30 min before naloxan administration. In the end of training day all groups were received naloxane (5 mg/kg I.P) 3 h after last injection of morphine and then the frequencies of withdrawal behavior were assessed for 30 min. **Results:** Our results show that I.P administration of MC extract dose dependently attenuates most of morphine withdrawal syndrome. **Conclusion:** These result suggested that ip injection of Matricaria chamomilla might be helpful in the treatment of morphine withdrawal syndrome. There is a need for additional research and study of the medicinal plants and herbal medicine with respect to the treatment of addiction.

P601 *Cryptolepis sanguinolenta* (Lindl.) Schltr. root extract inhibits prostaglandin production in IL-1b stimulated SK-N-SH neuronal cells

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Cryptolepis sanguinolenta (Lindl.) Schltr. is a shrub used in West Africa for the treatment of fevers and inflammatory conditions. In the present study the effect of the root extract of *C. sanguinolenta* on prostaglandin E2 (PGE2) release from IL-1β-stimulated SK-N-SH neurons was investigated. The effects of the extract on COX-2 and p38 MAP Kinase proteins were also investigated. *C. sanguinolenta* (2.5 – 10 μg/ml) produced a dose-dependent inhibition of IL-1β-induced PGE2 release from SK-N-SH cells. Western blot experiments revealed that the extract (5 – 20 μg/ml) inhibited IL-1β-induced COX -2 and p38 expressions in these cells. The present work provides evidence that *C. sanguinolenta* root extract inhibits the production of PGE2 in IL-1β-stimulated neuroblastoma cells through inhibition of COX-2 protein. It is suggested that the observed effects may be dependent on the inhibition of p38 MAP Kinase activation. **Acknowledgements:** The Alexander von Humboldt Foundation funded this study through a research fellowship to Dr Olajide

P602 Cartilage regeneration effect of orally administered *Zingiber officinale* Roscoe against MIA-induced osteoarthritis in rats

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Osteoarthritis (OA) is developed when the cartilage that protects the bone is gradually destroyed within the synovial joint [1]. *Zingiber officinale* Roscoe (ZO) is potential to reduce pain and symptoms caused by OA by suppressing cyclooxygenase, lipooxygenase metabolites, and arachidonic acid [2]. This study compares and quantifies the histopathological changes of cartilage between ZO treated rats and control rats in Monosodium idoacetate (MIA)-induced OA. Rats were treated by administration of the suggested therapies using feeding catheter for 28 days. At day 28, rats were sacrificed. Whole right and left knee joints were dissected, processed, and stained with H&E or Safranin-O. Histopathological scores [3] were quantified. ZO group showed significantly low degeneration of the cartilage and significantly less severity of the subchondral bone compared to control group. This study concluded that oral administration of ZO revealed the curative effects of the extract on cartilage of OA joints. **Acknowledgements:** Ministry of Science, Technology and Innovation, Malaysia and Universiti Putra Malaysia. **References:** 1. Moreland, LW. (2003). Arthritis Res. Ther. 5:54 – 67. 2. Tjendraputra, E. et al. (2001). Bioorg. Chem. 29(3):156 – 163. 3. Kobayashi K. et al. (2003). J. Vet. Med. Sci. 65(11): 1195 – 1199.

P603 Evaluation of the diurectic activity of an isolated flavonol of the aqueous extract from *Boldoa purpurascens* Cav.

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Boldoa purpurascens Cav. (1) is a wild species which grows in Latin America and the Caribbean; used in several countries as diuretic for affections of the urine tract. The phenol compounds here are in larger proportion in the bioactive fraction. From the aqueous extract of the leave one flavonoid glycoside (2,3) with group benzodioxalane (4′,5-dihydroxy-6,7-methylendioxyflavonol-3-O-α-L-rhamnopyranosil-(1®2)-β-D-xylopyranoside was isolated. This compound could be related with the attributed effect in the investigation. (4). The new flavonoid was evaluated to discern its effect, for the experiment, male rats were used, Sprague Dawley weighing between 160 y 220 g. Assay. In the experimental test doses of 100, 50, 25 12, 6, 3 mg/Kg of weight were used, the volume of administration being of 10 mL/Kg; as positive control a solution of Furosemide was used (4 mg/Kg) and as negative control, water distilled. The statistical analysis was carried out by the test of Kruskal-Wallis with an interval of trust of 99%. The flavonol; showed significant diuretic effect. The activity of the flavonol was most important than that of the standard reference drug, Furosemide but without modifications in the excretions ions, it allowed to suggest that the compound can be included within the group of diuretic compounds called uncovered acuaretics in 2003. The results of the present investigation indicated that the flavonol isolated of B. purpurascens shows profound diuretic activity **Keywords:** Boldoa purpurascens, flavonol glycosides, Furosemide **References:** 1. Roig, J. T,(1988). Dictionary of Cuban Common Yams, pp 225 – 226. 2. Niassy, B.; Um, B. – H, et al, (2004). C. R. Chimie 7, 993 – 996. 3. Jpn. Kokai Tokkyo Koho,(1988), JP63203682 A2. 4. Magalhães AF, Tozzi AM, et al,(2007) Acad Bras Cienc; 79,351 – 675.

P604 *Reseda luteola* extract RF-40 displays antioxidative, antiinflammatory and photoprotective activities in vitro, ex vivo and in vivo

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The flavone luteolin is present in higher amounts in the dyers weld, *Reseda luteola* L. During the last few years antimicrobial, anticancer, antiinflammatory and antioxidative activities of luteolin have been described [1, 2]. Reactive oxygen species play a major role in ultraviolet-induced skin inflammation, photoaging and photocarcinogenesis. Therefore, we were interested in the antioxidant and ultraviolet-absorbing properties of a dry extract from *Reseda luteola* (RF-40) [3] rich in flavones (40% w/w), mainly luteolin. Spectrophotometric measurements with 1% (v/v) solution revealed similar extinction maxima in the UVB and UVA range for RF-40 and luteolin. Ultraviolet transmission below 370 nm was < 10%. The free radical scavenging activity of RF-40 was assessed using cell-free and cell-based assays. In the cell-free DPPH assay the IC_{50} of RF-40 was 37 µg/ml (Luteolin:12 µg/ml, Trolox: 25 µg/ml; N-acetylcystein: 34 µg/ml). In UVB irradiated (60 mJ/cm^2) HaCaT cells the formation of DCFH was reduced by RF-40 and luteolin in a concentration-dependent manner. Luteolin (IC_{50} 3 µg/ml) and RF-40 (IC_{50} 4 µg/ml) were more effective compared to Trolox (IC_{50} 12 µg/ml) and N-acetylcystein (IC_{50} 847 µg/ml). Furthermore RF-40 inhibited the expression of inducible cyclooxygenase-2 *ex vivo* in UVB irradiated skin explants, and *in vivo* in suction blister roofs from human volunteers. In the suction blister fluid the UVB induced, cyclooxygenase-2 catalyzed synthesis of prostaglandin E2 was reduced by RF-40. These data suggest that due to its ultraviolet absorbing, antioxidant and antiinflammatory properties RF-40 is an interesting active ingredient for topical sun-protecting and anti-aging dermocosmetics. **References:** 1. Seelinger G, Merfort I, Schempp CM (2008) Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin. Planta Med 74:1667 – 1677. 2. Seelinger G, Merfort I, Wölfle U, Schempp CM (2008) Anti-carcinogenic effects of luteolin and other flavonoids. Molecules 13:2628 – 51. 3. Wölfle U, Simon-Haarhaus B, Merfort I, Schempp CM. *Reseda luteola* L. extract displays antiproliferative and pro-apoptotic activities that are related to its major flavonoids. Phytotherapy Research (in press).

P605 Inhibitory effect of kaempferol isolated from Semen Cuscutae on dendritic cell activation

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Semen Cuscutae has been using as a tonic for nourishing the liver and kidneys. However, the immunoregulatory effect of Semen Cuscutae is rarely studied. Dendritic cells (DCs) play a critical role in initiating immune response. Thus, DCs are regarded as a major target of immunomodulator for controlling harmful immune responses. In this study, we examined the effect of Semen Cuscutae on mouse bone marrow-derived DC activation. We found that the n-butanol and methanol partitions of Semen Cuscutae potentially suppressed LPS-induced DC activation. HPLC chromatography showed that several flavonoids might be responsible for this inhibitory activity. Then, we identified that kaempferol was the major flavonoid to inhibit LPS-induced DC activation by reducing the production of pro-inflammatory cytokines and maturation. The inhibitory ability of kaempferol was tested at the concentration of 10 ug/ml. Importantly, consistent to the *in vitro* results, the recall assay *in vivo* showed that kaempferol significantly inhibited the T cell proliferation, indicating that kaempferol abrogated the ability of LPS-stimulated DCs to induce Ag-specific T cell activation, both *in vitro* and *in vivo*. Therefore, we demonstrate that Semen Cuscutae has immunosuppressive activity for the first time. In addition, we are the first group to report that kaempferol attenuates the DC activation and could potentially be applied in the therapy for inflammatory and autoimmune diseases. **References:** 1. Yu YL, et al., 2009. Eur J Immunol. 39(9):2482 – 2491.

P606 Effects of *Passiflora incarnata* L. on rat hippocampal G protein-coupled receptors (GPCRs)

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Passiflora has potential effects for treatment of some diseases like anxiety, insomnia, attention-deficit hyperactivity disorder, cancer, and may be helpful in the treatment of substance addictions [1], [2]. However, very few pharmacological studies have been undertaken on the activity of *P. incarnata*; most of these investigations have been carried out with different *Passiflora* species, such as *P. edulis*, *P. alata*, or *P. coerulea* and with insufficient phytochemical characterization of the extracts. The goal of the present study was to search for possible new targets of the extract contained as active ingredient in the herbal drug Pascoflair® 425 mg on rat hippocampal GPCRs. The following agonists evoked an increased [35S]-GTP? S signal and it can therefore be concluded that the respective receptors are present in the membrane preparation (in brackets): acetylcholine (muscarinic M2 or M4), DAGO (µ-opioid), ADP (P2Y12 or P2Y13), HU-210 (cannabinoid CB1 > CB2), CCPA (adenosine A1), serotonin (serotonin 5-HT1all subtypes) and glutamate (metabotropic glutamate receptors, mGlu2,3,4,6,7,8). An antagonistic effect of Passiflora on Hu-210 and CCPA-evoked [35S]-GTP? S binding was observed. No other agonistic/antagonistic action of *Passiflora* could be detected. Thus, our findings suggest that the *Passiflora* extract is an antagonist of the adenosine A1 and the cannabinoid receptors. This would be consistent with the observed effects of *Passiflora*, because adenosine A1 receptor antagonists display anxiolytic activity [3] and cannabinoid receptor antagonist have therapeutic effects for the treatment of substance dependence [4]. **References:** 1. Dhawan, K. et al. (2004) J Ethnopharmacol. Sep;94(1):1 – 23. 2. Patel, S. et al. (2009) Int J Green Pharm 3:277 – 80. 3. Maemoto, T. et al (2004). J Pharmacol. Sci. 96, 42 – 52. 4. Schindler et al. (2010) Eur J Pharmacol. May 10;633(1 – 3):44 – 9.

P607 Lipolysis effects of lancemaside A and aglycon from *Codonopsis lanceolata* water extract in primary cultured adipocytes from obese rat fed high calorie high fat diet

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We examined the effects of Lancemaside A and aglycon, its acid hydrolysis product from Lancemaside A, isolated from *Codonopsis lanceolata* water extract on triglyceride and free fatty acid in primary cultured adipocytes from obese rats fed high calorie high fat diet. Animals (6-week old male Sprague-Dawley rats) were administered a 60% high-fat diet for 6 weeks and adipocyte isolated from apididymal fat [1]. Adipocytes cultured with samples for 4 day. Lipids were extracted by Folch method [2]. Lancemaside A and aglycon significantly reduced in lipid droplets, TG and FFA in a concentration-dependent manner. These data suggest that Lancemaside A and aglycon were active compounds of *Codonopsis lanceolata*, and these samples improves lipid dysregulation. It could be widely utilized in the therapeutic and functional food for obesity patients. **References:** 1. Rodbell M. (1964) J. Biol. Chem. 239: 375 – 380. 2. Folch, J. (1957) J. Biol. Chem. 26:497 – 509.

P608 Spathulenol inhibit the human ABCB1 efflux pump

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Since multidrug resistance (MDR) is a major cause of failure in cancer chemotherapy, the search for new compounds that can be used as adjuvants of chemotherapy is urgent. This study focuses on the evaluation of cytotoxicity and MDR reversal activity of eleven compounds representing diverse structural types of Asteraceae sesquiterpenes. Xanthatin, 4-epixanthanol, sintenin, cnicin, 4′-acetylcnicin, 3b-hydroxycostunolide, desacetylmatricarin, paulitin, isoalantolactone, chrysanin and spathulenol were tested, by flow cytometry, for their activities as modulators of the efflux of rhodamine123 by the human ABCB1 (commonly known as P-gp) pump. Two cell lines were used: a L5178 mouse T-cell lymphoma cell line (PAR cell line) and the L5178 mouse T-cell lymphoma cells transfected with pHa MDR1/A retrovirus (MDR cell line). It was observed (figure 1) that spathulenol highly promoted the accumulation of rhodamine123 (substrate of the ABCB1) by the MDR cells, which over-expresses the ABCB1 efflux pump. Spathulenol, sintenin and desacetylmatricarin presented moderate or low cytotoxicity with IC50 higher than 6 μM, while the remaining compounds presented higher cytotoxicity against the two cell lines tested. The results with spathulenol suggest that this compound is a good candidate to be used in combination chemotherapy of MDR cancer and therefore is worthy for further in vivo studies.

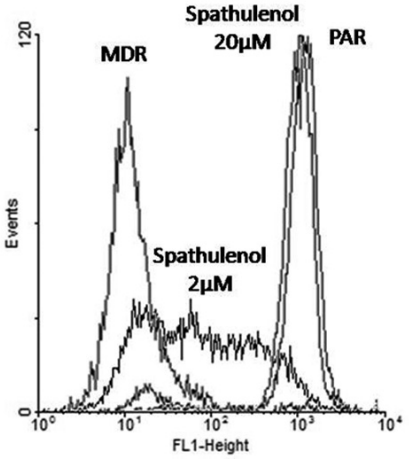

Fig. 1: Histogram of activity of 2 and 20 μM of spathulenol on the retention of rhodamine 123 by the MDR mouse lymphoma cells

Acknowledgements: This work was supported by Hungarian Research Fund (OTKA K72771) grant. The author thanks Dr. Imre Ocsovszki for the flow cytometry measurements. A. Vasas acknowledges the János Bolyai Scholarship of the Hungarian Academy of Sciences.

P609 The *in vivo* angiogenic evaluation of betulin

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Betulin is a pentacyclic triterpene alcohol with a lupane skeleton mostly found in bushes and trees like the bark of Betula species. Betulin and its derivatives have been reported for their biological activities like anti-HIV, antimicrobial, antiinflammatory properties and moreover betulinic acid was found to selectively to inhibit tumor cells. Betulin shows anticancer activity in a similar mechanisms such as betulinic acid (1). Angiogenesis is the formation of new blood vessels which has a role in the development processes of embryo growth as well as in such diseases like cancer and chronic inflammation. The antiangiogenic approach for the treatment or the prevention of such pathologies looks very promising (2). In this present study, betulin was evaluated both for its angiogenic and antiangiogenic properties using the in vivo chicken chorioallantoic membrane assay (2). The assay performed in three concentrations 5 – 50 μg/pellet. As a result, betulin showed medium-strong anti-angiogenic effect at the concentration of 50 μg/pellet in the CAM assay, in a scoring system when compared to the standards suramine, thalidomide and cortisone. Furthermore, betulin showed neither toxicity nor membrane irritation at the tested concentrations. **Acknowlegement:** This work was financially supported by Badebio Biotechnology, Eskisehir, Turkey. **References:** 1. Sami A., Taru M., Salme K., Jari Yli-Kauhaluoma, 2006, Pharmacological properties of the ubiquitous natural product betulin, European Journal of Pharmaceutical Sciences, 2 9, 1 – 13. 2. Demirci F., Paper D.H., Franz G., Baser K.H.C., 2004, Investigation of the Origanum onites L. Essential Oil Using the Chorioallantoic Membrane (CAM)-Assay, J. Agric. Food Chem. 52 (2), 251 – 254.

P610 Betulin in formulation with ramified gamma type cyclodextrin targeting tumour cells and angiogenesis

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Betulin is a pentacyclic triterpenic compound, found in important quantities in the bark of birch trees, widespread in northern latitude areas. It is known to possess multiple biological activities, including anti-inflammatory, antiviral and anticancer effects [1] and acting as an antimelanoma agent by binding to the melanocortin receptor subtype 1 (also expressed by endothelial cells) [2], which represented an indication to a possible angiogenic – modulator activity. This study aimed to evaluate the effect of betulin on tumor cell lines – A431 (skin epidemoid carcinoma), HeLa (cervix crcinoma), and MCF7 (breast adenocarcinoma) – using the MTT assay, and on the angiogenesis process by performing the *in ovo* chick chorioallantoic membrane (CAM) assay [3]. The formulation with cyclodextrin (Oktakis (2,6di-O-pentyl)-gamma cyclodextrin) was prepared applying a specific technique: dissolution with an organic solvent (chloroform) and polysorbate 20, stirring and evaporation under vacuum. Different dilutions of a 0,01 mg/ml betulin stock solution showed on tumor cells antiproliferative properties. Inhibition rate ranged for A431 between 72 – 80%, for MCF7 between 35 – 55% and for HeLa between 75 – 82%. We applied nanoformulations of cyclodextrin complexed with betulin onto the rapid growing CAM. Histological and morphometric evaluation of treated specimens showed modifications both of the CAM epithelium, mesenchyme and of the capillaries, indicating an indirect inhibition of the angiogenic process. The *in vitro* and *in vivo* results suggest that betulin can represent a possible antitumor as well as an antimetastatic compound. **Acknowledgements:** This work was supported by CNCSIS-UEFISCSU, project number PN II- IDEI code 1257/2007. **References:** 1. Yogeeswari P., Sriram D. (2005) Curr Med Chem. 12(6): 657 – 666. 2. Muceniece R. et al. (2007) Cell biochem funct. 25(5): 591 – 596. 3. Ribatti D. et al. (2006) Nat protoc. 1(1): 85 – 91.

P611 Purinergic agents of hydroalcoholic *Agaricus brasiliensis* extracts: identification, quantification and biological assays

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In the present work the effects of an hydroalcoholic extract of *Agaricus brasiliensis* (formerly known as *A. blazei*) on metabolic parameters in the perfused rat liver have been examined with emphasis on its content on nucleotides and nucleosides, which act as purinergic agents. Several nucleosides and nucleotides were identified in the *A. brasiliensis* extract. Consistently, the extract is active on several liver functions in a relatively complex way. It increased perfusion pressure, oxygen consumption, glycogenolysis, glycolysis, ureogenesis, gluconeogenesis, and shifted the redox state of the cytosolic NAD-NADH couple toward a more reduced state. A significant part of these effects seems to be the result of purinergic action, as indicated mainly by experiments with inhibitors and antagonists: pressure changes, glycogenolysis control (glucose and lactate release) and the redox state changes. Another set of phenomena, namely the increased gluconeogenesis and ureogenesis and especially the ubiquitous stimulation of oxygen consumption, are more likely the consequence of metabolic transformation of several substrates contained within the extract, especially amino acids. The results of the present work have implications for both the consumer of *A. brasiliensis* as a functional and nutraceutic food and for the experimentator interested in the physiologic and pharmacologic effects of the mushroom. It seems apparent that consumption of *A. brasiliensis* represents not only the ingestion of metabolic precursors, but also the ingestion of substances that, even at low concentrations, can exert important signalling functions not only in the liver but in the organism as a whole. **Acknowledgements:** Financial support: CNPq and Fundaçao Araucaria.

P612 Comparative pathological study of *Matricaria recutita* L. and methadone effects on liver and kidney of morphine dependent rats

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Introduction Addiction to opiates such as morphine is one of the major world health problems. Some reports show that Matricaria chamomilla extract contains flavonoids, which exert Benzodiazepine-like activity and inhibited the expression of abstinence syndrome in morphine-dependent animals. The objective of this study was to determined and compare the effect of injection of Matricaria chamomilla extract on morphine withdrawal syndrome signs (MWS) in rats. In addition to compare the pathological effects of Matricaria chamomilla and methadone on the liver of morphine dependent rats. **Material and Methods**: 32 male rats (200 – 300 gr) (n = 8) were tested in 4 groups: (1) saline only group, (2) morphine group were received 10 mg/kg morphine for 10 days, (3) Matricaria chamomilla group – 20 mg/kg I.P 30 min before Morphine administration and (4) Methadone group. At the end of the training day all groups received naloxane (5 mg/kg I.P) 3 h after last injection of morphine and then the frequencies of withdrawal behavior were assessed for 30 min using the Open field test. In addition hepatic and renal pathology was studied. **Results**: Microscopically there were severe pathological effects, such as necrosis and hemorrhage, acute pre-portal necrosis, atrophy of pre-portal hepatocytes, hyperplasia of bile ducts and infiltration of lymphocytes and granulocytes, in the liver of morphine – treated group. No hepatic lesions were observed in the Matricaria chamomilla – treated group. No kidney lesions were observed in either group. **Discussion:** These result suggested that ip injection of Matricaria chamomilla might be helpful in the treatment of morphine withdrawal syndrome and appears to be safer than methadone. There is a need for additional research and study of the medicinal plants and herbal medicine with respect to the treatment of addiction. Acknowledgments: Dr. Sharif Azadeh (Head of Students Scientific association of islamic Azad University) Dr. Rahim vakilzadeh, Miss rana mokhtarzadeh.

P613 Effect of *Hypericum perforatum* aqueous extract on formalin induced pain and inflammation in mice

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Introduction: Hypericum perforatum is a medical plant species containing many polyphenolic compounds, specially flavonoids and phenolic acids. A number of studies have shown that polyphenolic compounds have high antioxidant potential and anti inflammatory effects in some of the animal models (but not all the animals). Also in traditional medicine this herb has been used to decrease pain and inflammation. The aim of this study was to investigate the effect of Hypericum perforatum aqueous extract on formalin induced pain and inflammation in mice. **Material & Methods:** Animals were pretreated with Hypericum perforatum aqueous extract (0.5 g/kg) orally before induction of pain by intrapadly formalin (20 µl, 0.5 %) injection. The time of licking and biting of injected paw was measured as pain response at 5 min intervals for an hour. **Results:** The results have shown that formalin induces a biphasic pain response (first phase; 0 – 5 min & second phase; 15 – 45 min after injection) and oral administration of Hypericum perforatum aqueous extract before formalin injection, reduces the second phase of pain response significantly ($p < 0.05$) but it didn't have significant effect on first phase ($p \geq 0.05$). **Conclusion:** Our results suggest that the effect of Hypericum perforatum aqueous extract is probably because of its high antioxidant, antinociceptive and anti inflammatory properties. **Keywords:** Hypericum perforatum, aqueous extract, pain, inflammatory, mice

Pharmacology

P614 Anti-inflammatory effect of an ethanol extract from rhizomes of *Stahlianthus involucratus* in rats

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Stahlianthus involucratus (King) Craib ex Loes, a perennial herbaceous plant of the Zingiberaceae family, is widely distributed in Asia [1]. Since no pharmacological and phytochemical studies have been reported, this study aimed to investigate the anti-inflammatory activity of an ethanol extract from its rhizomes in rats using the ethyl phenylpropiolate (EPP)-induced ear edema model [2] and the carrageenin-induced hind paw edema model [3]. The topical application of the extract (5 mg/ear) significantly inhibited EPP-induced ear edema, at all evaluation time points, with comparable percentages of inhibition to those of diclofenac (5 mg/ear), the reference nonsteroidal anti-inflammatory drug. The extract (75, 150 and 300 mg/kg p.o.) as well as diclofenac (10 mg/kg p.o.) also significantly inhibited carrageenin-induced hind paw edema, at all evaluation time points. Moreover, the latter effect of the extract appeared to occur in a dose-dependent manner. The present study revealed an anti-inflammatory effect of the ethanolic extract from *S. involucratus* rhizomes in rats possibly through inhibition of cyclooxygenase similar to diclofenac. **Acknowledgements:** This work was supported by the Faculty of Medicine Endowment Fund, Faculty of Medicine, Chiang Mai University. **References:** 1. Chaveerach, A. et al. (2007) Taiwania 52:315 – 319. 2. Brattsand, R. et al. (1982) J. Steroid. Biochem. 16:779 – 786. 3. Winter, C.A. et al. (1962) Proc. Soc. Exp. Biol. Med. 111:544 – 547.

P615 Influence of *Ginkgo biloba* extract EGb 761® on NADH levels in the brain of Mapt-transgenic mice

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Impaired mitochondrial function can be improved by *Ginkgo biloba* extract EGb 761® [1]. NADH is an indicator for the course of the mitochondrial respiratory chain. We investigated the influence of *Ginkgo biloba* extract EGb 761® on NADH levels in the brains of Mapt-trasgenic mice, using the newly designed OMC 02 (mfd Diagnostics). OMC 02 causes fluorescence of NADH by means of a nitrogen laser and detects the fluorescence over time. The long-term objective of the OMC 02 is the noninvasive measurement of cerebral energy metabolism state in the brain. Wild type mice (n = 6) and Mapt-transgenic mice (n = 6) received *Ginkgo biloba* extract (30 mg/kg/week) through the drinking water on 3 – 4 days/week over a 4 week period. The identical composed control group received water without any additives. Water maze test was used to verify the results obtained from laser-induced NADH fluorescence measurements. The results from the water maze test point to an improvement of retentivity in *Ginkgo biloba*-treated mice. However this result does not seem to be reflected in the LIF-measurement. **References:** 1. Abdel-Kader R., Hauptmann S., Keil U. et al. (2007) Stabilization of mitochondrial function by *Ginkgo biloba* extract (EGb 761®) Pharmacol. Res. 56: 493 – 502.

P616 Effects of the special *Cimicifuga racemosa* extract BNO 1055 on hot flashes on ovariectomized rats

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Hot flashes occur due to the lack of estrogens and are the most common climacteric complaint. Hormone replacement therapy is the gold standard treatment but now its use is limited due to severe side effects. It is well established that extracts of *Cimicifuga racemosa* (CR) ease climacteric complaints but solid animal experimental data supporting such effects are not available. The availability of temperature sensitive transponders enables experiments in rats to establish whether they have hot flashes following ovariectomy (ovx) and if so, whether they can be influenced by the extract CR BNO 1055. Intact Sprague Dawley rats (n = 16) were implanted with transponders under the skin of the nape. Subcutaneous temperature was measured in 5 min intervals for 3 h. Thereafter, the rats were ovx and fed either with soy free (sf) or CR BNO 1055 (11.3 mg/animal/day) food. Temperature was recorded again after acute and sub-acute application of CR. In intact animals temperature was stable over the 3 h recording period. Following ovx temperature pulses appeared with peaks occurring every 20 – 40 min. These fluctuations were not seen in intact or CR BNO 1055 treated animals resulting in significantly higher mean temperatures. The reduction of hot flashes by BNO 1055 outlasted the experimental period of 3 weeks. These results suggest that the ovx rats and the new temperature sensitive transponders may be useful for the study of hot flashes. Furthermore they prove that the CR BNO 1055 exerts hot flash reducing effects.

P617 Effects of echinacea supplementation on alkylamide pharmacokinetics

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Echinacea preparations are widely used for the prevention and treatment of colds and flu. Studies have shown that alkylamides are present in plasma after oral dosing with echinacea preparations [1,2]. It is well known that continued use of certain drugs can result in changes to their clearance rate and hence their pharmacokinetic profiles and therapeutic effects. Pharmacokinetic data such as this does not exist for the prophylactic use of echinacea. In this study, the effects of supplementation with echinacea on alkylamide bioavailability and pharmacokinetics were examined before and after prophylactic use of echinacea. Six healthy volunteers aged 24 to 66 years with a body mass index ranging from 22.7 to 24.6 participated in the study. They consumed 2 echinacea tablets twice a day for 14 days. On days 0 and 15, participants consumed 4 echinacea tablets (a total of 17.67 mg of alkylamides). Blood samples were taken at 0, 30, 90, 120, 180, 240 and 360 minutes post dose and alkylamide levels were determined as previously described [1]. There was no evidence for either the induction or inhibition of alkylamide metabolism as evidenced by consistent elimination half life data. There was a trend towards more rapid alkylamide absorption and achievement of a higher Cmax before the prophylactic use of echinacea but these changes were not statistically significant and there was no difference to the extent of absorption (AUC). Plasma ratios of the tetraene alkylamides were also examined. These were similar to those found in the tablets used in the study and were not altered after prophylactic use of echinacea. In conclusion, prophylactic use of echinacea for 2 weeks does not appear to alter the pharmacokinetics of the alkylamides in echinacea. **References:** 1. Matthias, A. et al. (2005) Life Sci 77:2018 – 29. 2. Woelkart, K. et al. (2005) J Clin Pharmacol 45: 683 – 9.

P618 *Iberis amara* L. constituents reduce excitatory cholinergic and inhibitory purinergic neurotransmission

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An extract of *Iberis amara* L. (extraction solvent ethanol 50%, 1:1.5 – 2.5) is, together with eight other herbal extracts, component of STW 5 (Iberogast®), a phytomedicine with clinically proven efficacy and safety in functional gastrointestinal diseases (1, 2). The objective of this study was to evaluate the pharmacological effects of *Iberis amara* and some if its characteristic constituents (glucoiberin, kaempferol, cucurbitacin E and I, and kaempferol-3,4′-diglucopyranoside-7-*O*-rhamnopyranoside) on cholinergic neurotransmission in the enteric nervous system of rat and mouse. Cholinergic contractions of isolated muscle strips of ileum were studied in an organ bath. Myenteric reflex responses elicited by EFS were studied in a perfused organ bath using a four inch long ileum segment. Intracellular recordings of EJP and IJP in smooth muscle cells of the circular muscle layer of the proximal colon were performed. *Iberis amara* and its components kaempferol and cucurbitacin E reduced cholinergic contractions in organ bath studies in a concentration dependent manner. Kaempferol and the cucurbitacins E and I reduced the amplitude and latency of ascending and descending myenteric reflex contractions in a concentration dependent manner. Kaempferol reduced cholinergic EJP and increased the purinergic fast part of the IJP. Thus, *Iberis amara* and its characteristic components interact with mechanisms of excitatory cholinergic and inhibitory purinergic neurotransmission in the enteric nervous system. Characterization of these distinct effects on gastrointestinal neurotransmission helps to understand how the combination phytomedicine STW 5 alleviates patient symptoms. **References:** 1. Roesch W et al.: Phytotherapy for functional dyspepsia: A review of the clinical evidence for the herbal preparation STW 5. Phytomedicine 2006, 13, 114 – 121. 2. Schmulson, MJ: How safe and effective is the herbal drug STW 5 for patients with functional dyspepsia? Nature Clinical Practice Gastroenterology & Hepatology 2008, 5, 136 – 137.

P619 The effect of rice bran extract on beta cells of rats fed with high-fat diet

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Both water extract of rice bran (RBW) and rice bran oil (RBO) were previously shown to reduce fat deposition and area under the glucose-clearance curve in Sprague-Dawley rats fed with high-fat diet (65% of total calories) for four weeks [1]. To elucidate the effect on pancreatic

islets, the study on the histology of these rat tissues was conducted. Rats in group 2 to 9 were high-fat fed. Group 3 to 6 were separately either co-treated daily with three doses (22.05, 220.5, 2205 mg/kg) of RBW or 9.55 mg/kg of metformin, twice daily. The high-fat feed in group 7 to 9 contained 20, 40 and 50% of total calories as cold pressed RBO, respectively. After four weeks of treatment, the insulin staining showed significant amount of vacuoles within beta cells (arrow head) as well as the dilated blood vessel (arrow) in tissues of rat fed with high-fat diet alone. RBW at the minimum daily dose of 220.5 mg/kg, metformin and cold pressed RBO (group 8 and 9) were able to abolish these abnormalities. There was no positive Ki-67 staining in any tissue indicating no cell proliferative effect. It was concluded that rice bran extract ameliorates insulin resistance partly by protecting beta-cell function [3, 4]. **Acknowledgements:** Research Unit, Faculty of Medicine, Thammasat University, The National Research Council of Thailand. **References:** 1. Kandee N., et al (2009) Thamm Med J 9:140 – 7. 2. Sone H., Kagawa Y. (2005) Diabetologia 48:58 – 67. 3. Karger C., Ktorza A. (2008) Diabetes Obes Metab 10 (suppl 4):43 – 53. 4. Bonora E. (2008) Nutr Metab Cardiovasc Dis 18:74 – 83.

P620 Preclinical investigations of possible drug interactions with WS® 1375, a proprietary dry extract from *Rhodiola rosea* roots

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WS®1375 is the pharmaceutically active ingredient in Vitango®, a traditional herbal medicinal product used as adaptogen for the treatment of fatigue and other stress associated symptoms. The extract is prepared from the roots and rhizomes of Rhodiola rosea L. The aim of the investigation was to determine potential drug interaction effects of WS®1375. Of the 57 cytochrome P450 isoenzymes encoded by the human genome only 5 enzymes are responsible for the oxidative metabolism of 95% of all drugs (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4).1 We investigated the inhibitory/stimulatory effects of WS®1375 in microsomes, prepared from freshly isolated human hepatocytes. To get comprehensive information on the inhibitory effects the activity of all five major CYP isoenzymes was measured whereas the possible induction potential of WS®1375 was investigated only on the inducible isoenzymes CYP1A2 and CYP3A4. The IC50 values for inhibition were 1A2 = 63 µg/ml, 2C9 = 77 µg/ml, 2C19 = 75 µg/ml, 2D6 = 25 µg/ml and 3A4 = 104 µg/ml. A more than 7-fold induction was observed for positive controls in the induction experiments confirming the functional state and the validity of the test system. The extract did not induce the catalytic activity of the cytochrome isoenzymes tested up to a concentration of 100 µg/ml. **Conclusion:** The IC50 values for all CYP isoenzymes in the inhibition experiments were between 25 µg/ml and 104 µg/ml and thus far away from clinical relevant plasma concentrations. These data in combination with the lack of induction potential clearly demonstrates that WS®1375 is devoid of a clinical relevant interaction potential. **References:** 1. Johnson, W.W. (2008) Drug Metabolism Reviews, 40:101 – 147.

P621 Influence of the herbal component of a commercial feed additive on serum parameters, fertility and longevity of dairy cows

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Fertility dysfunction is a main culling reason for dairy cows [1]. A randomized placebo controlled trial investigates a herbal mixture containing mainly Urtica dioica L. (herba), Trigonella foenum-greacum L. (semen), Silybum marianum (L.) Gaert. (fructus) and Achillea millefolium L. (herba). It was fed cows daily (50 g per cow and day) about 60 d from dry off to calving date. 63 dairy cows (32 in the herb-(h)- and 31 in the placebo-(p)-group) of one Swiss organic dairy farm were included in the study. Cows were differentiated depending on their lactation number (L); L1: first lactating cows (h: n = 10, p: n = 9), L2 – 4: cows of second to fourth lactation (h: n = 11, p: n = 12) and L> 4: cows with more than four lactations (h: n = 11, p: n = 10). Blood samples were taken and a rectal palpation according to Rosenberger [2] of uterus and ovaries was done once between day 21 and 35 after calving. Furthermore all cows were observed until culling or next calving. Table 1 shows the significant differences ($p < 0.05$) between the herb and placebo group. The fed herbal mixture seems to have an impact on the postpartal metabolic status of dairy cows which is a main factor influencing fertility [3]. The herbs seem to prolong the postnatal anoestrus but also to decrease the culling rate particularly for fertility reasons. Overall the prolonged intercalving period had a lower economic effect than the decreased culling rate.

Table 1: Significant differences ($p < 0.05$) between herb and placebo group

	herb fed group	placebo group
abnormalities in at least one of 7 tested serum parameters (glucose, bilirubin, aspartate aminotransferase, glutamate dehydrogenase, calcium, phosphate, magnesium) in the lactating group L1	0%	44%
serum phosphate contents in the lactating group L > 4	7.2 mg/dl	5.9 mg/dl
ovaries with palpable follicles in the lactating group L > 4	18%	63%
uterus dimension score in the lactating group L > 4	3	2
intercalving period	387 days	344 days
overall culling rate	17%	50%
culling rate for fertility reasons	3%	23%

References: 1. Seegers, H. et al. (1998) Prev Vet Med. 9:257 – 271. 2. Rosenberger, G. (1990) Die klinische Untersuchung des Rindes. Verlag Paul Parey. Berlin und Hamburg. 3. Westwood, C. T. et al. (2002) J. Dairy Sci. 85:3225 – 3237.

P622 Identification of γ-aminobutyric acid type A ($GABA_A$) receptor modulators: HPLC-based activity profiling of *Asarum heterotropoides*

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γ-Aminobutyric acid type A ($GABA_A$) receptors are the major mediators of fast synaptic inhibition in the central nervous system and thus play a crucial role in controlling the excitability of the brain. Diverse types of these ligand-gated chloride ion channels are a target for several therapeutically relevant drugs, such as benzodiazepines, barbiturates, neurosteroids and various general anaesthetics. However, these drugs interact in a non-selective way with several $GABA_A$ receptor subtypes and hence cause side effects such as reduced coordination, cognitive impairment, increased accident proneness, and development of dependence and abuse liability [1, 2]. In a search for new natural product derived scaffolds for $GABA_A$ receptor modulators we screened a plant extract library for $GABA_A$ receptor activity by means of a two-microelectrode voltage clamp assay using *Xenopus laevis* oocytes, which transiently express the target receptors of desired subunit composition. With the aid of an HPLC-based activity profiling approach [3], an active dichloromethane extract of *Asarum heterotropoides* herb (Aristolochiaceae) was fractionated in a time-based manner and submitted to the assay. In a next step, some of the compounds of the active time-based fractions were isolated and identified by on-line high-resolution mass spectrometry and off-line microprobe 1D and 2D NMR spectroscopy, using only milligram amounts. Dose-response experiments were carried out with these compounds in order to determine EC_{50} values and maximum potentiation of GABA-induced chloride influx, with the aid of the oocyte functional assay. **References:** 1. Möhler, H. (2006) J. Recept. Signal Transduct. 26:731 – 740. 2. Sieghart, W. (2000) TiPS. 21: 411 – 412 3. Kim, H. et al. (2008) Planta Med. 74:521 – 526.

P623 Genome wide expression analysis of the effect of Pinelliae Rhizoma extract on psychological stress

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Psychological stress is known to induce many physiological changes including the induction of stress-related hormone and the reduction of immune function in humans and animals [1, 2]. Pinelliae Rhizoma has traditionally been used as an anti-depressant in Asian Traditional medicine [3]; therefore, in this study, the effect of Pinelliae Rhizoma extract (PRe) on psychological stress was investigated in mice. The results of an elevated plus-maze experiment revealed that application of psychological stress to mice using the communication box method led to the development of an abnormal behavioral pattern. However, oral administration of PRe significantly reduced the abnormal behavior of mice with recovery rate of 75.5%. To elucidate the molecular mechanism by which PRe reduced psychological stress, a microarray analysis of the brains of mice was conducted. The results of this analysis revealed that 456 genes were up-regulated and 392 genes were down-regulated in response to psychological stress. A comparison of the genes that were altered in response to physical or psychological stress revealed a significant correlation between the two types of stress. Furthermore, the expression of most of the genes that were altered in response to stress was restored to normal levels in PRe treated mice, with a recovery rate of 81.5% and 85.2% being observed for up- and down-regulated genes, respectively (Fig. 1).

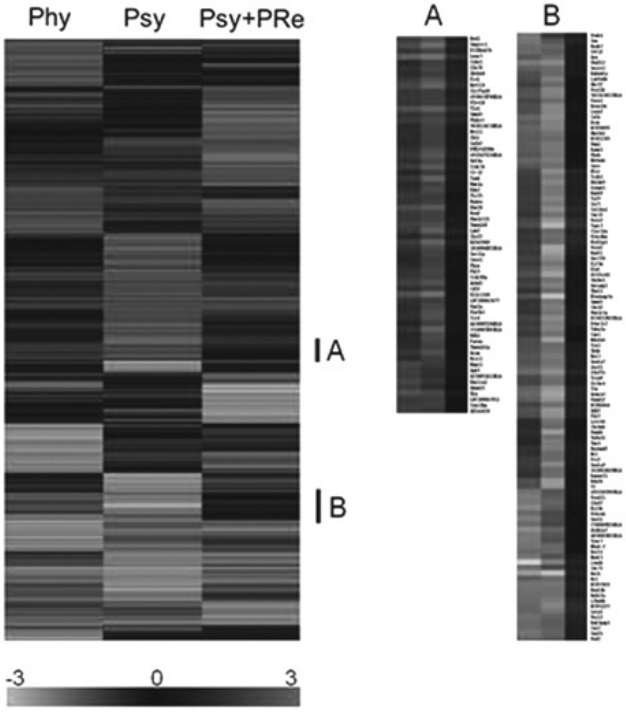

Fig. 1

References: 1. Oishi, K. et al. (2003) Stress 6(1):33 – 40. 2. McEwen, BS. (2008) Eur J Pharmacol 583(2 – 3):174 – 85. 3. Yoon, JS. et al. (2006) Am J Chin Med 34(3):401 – 8.

P624 Enhanced skin delivery of polyphenols by microemulsion and prevention against UV irradiation-induced erythema formation

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Polyphenols have been applied for topical purposes such as photoprotection against UV-induced skin damage. However, intradermal delivery of most polyphenols is inefficient. To improve the efficiency, we tried to clarify the usefulness of microemulsion using excised guinea pig dorsal skin and Yucatan micropig skin. We used two types of microemulsions consisting of isopropyl myristate (IPM), 150 mM NaCl solution, Tween 80 and ethanol. Weight ratio of IPM, 150 mM NaCl solution, Tween 80 and ethanol was 8:25:20:47 in microemulsion A, and 33:7:30:30 in microemulsion D. By using these microemulsions solubility and skin delivery of polyphenols such as genistein and chlorogenic acid were markedly improved. For example, solubility of genistein in 150 mM NaCl was only 0.059 mM, it increased to 80 mM in o/w-type microemulsion A, and further increased to 140 mM in w/o-type microemulsion D. Skin delivery of genistein also increased 36 times using microemulsion A and further increased about 60 times using microemulsion D at saturated concentration. On the other hand, enhancement effect of microemulsion A was larger than that of microemulsion D for skin accumulation of hydrophilic chlorogenic acid. Genistein and chlorogenic acid retained in the skin significantly inhibited lipid peroxidation dose-dependently *in vitro*. When genistein was applied with microemulsion D at saturated concentration (140 mM), lipid peroxidation tested by ammonium iron (II) sulfate and sodium citrate decreased to less than 10%. We furthermore revealed that pretreatment of guinea pig dorsal skin with microemulsion gel which contains either genistein or chlorogenic acid prevented UV-irradiation-induced erythema formation. These findings revealed the potential use of microemulsion for the delivery of these polyphenols to protect skin against UV-irradiation-induced damage.

P625 *Salvia officinalis* water extract: a potential hypolipidemic, hypoglycemic and anti-ulcerogenic remedy

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Herbal medicine, an aspect of folk medicine, is nowadays practiced extensively in the community and most often without control when it comes to third world countries. Sage (*Salvia officinalis* L., Lamiaceae), widely used in folk medicine [1], is one of the medicinal herbs commonly used in the Middle East. The present investigation explores and sheds light on possible medicinal effects of the aqueous extract of sage leaves upon lipemia, glycemia and gastric ulcer in rats. After one month of extract intake via drinking water (50, 200 and 500 mg/kg body weight), the highest dose showed significant improvement in serum HDL cholesterol, LDL/HDL cholesterol, and glucose. All doses used caused significant decreases in stool water content indicating that the extract is a possible remedy for patients with diarrhea. Assessment of liver enzyme activities (AST, ALT, LDH, ALP) revealed no hepatotoxic effects. Protection against ethanol-induced gastric ulcer [2] was investigated using 100 and 500 mg/kg body weight doses. Results showed significant protection with both doses used. A maximum protection (46%) was observed at 100 mg/kg body weight dose compared to the reference drug cimetidine (34%) at a dose of 10 mg/kg body weight. The assessment of antibacterial activity against 11 bacterial hospital isolates using disk diffusion technique showed no potential in this respect. In conclusion, *Salvia officinalis* leaves water extract showed no liver toxicity, and exhibited a positive effect on lipemia, glycemia and gastric ulcer protection. **Acknowledgements:** Mr. Jean Karam. **References:** 1. Newall, C. et al. (1996). Herbal Medicine, A Guide for Health-Care Professionals. (pp. 330 – 334). 2. Gurbuz, I. et al. (2005) J. Ethnopharmacol. 101: 313 – 318.

P626 Potential anti-inflammatory, anti-ulcerogenic and antioxidant activity of *Nasturtium officinalis* water extract

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Nasturtium officinalis is a widely used edible plant in the Mediterranean region and well known for its medicinal value in folk medicine. The present study explores the effects of the water extract of *N. officinalis* upon rat blood lipid profile, glycemia, liver enzymes, gastric ulcer, inflammation and antioxidant activities. One month of aqueous plant extract intake (100, 250 and 500 mg/kg body weight) via drinking water, showed no significant changes in the serum lipids and glucose level. Liver enzyme activities (AL T, ALP, AST) were not negatively affected thus assuring that the extract has no hepatotoxic effects over the study period. Doses of 100, 250, and 500 mg/kg body weight exhibited substantial anti-inflammatory effects against carrageenan induced inflammation (60.0, 51.1 and 46.11%) compared to 73.33% for diclofenac [1]. Similar doses caused respectively 4.7, 51.5 and 57.1% anti-inflammatory effects against formalin induced inflammation [2]. For the anti-ulcerogenic activity, pre-treatment of fasted rats with100 and 250 mg/kg body weight demonstrated significant protection (32.1% and 51% respectively) against ethanol-induced gastric ulcer, compared to 10 mg/kg cimetidine (50.24%) and 3 mg/kg omeprazole (47.81%)3. The extract also exhibited a strong scavenging activity against DPPH radicals (55%)4. In conclusion, our data demonstrates the beneficial anti-ulerogenic, anti-inflammatory and antioxidant activities of *Nasturtium officinalis*. **Acknowledgements:** Mr. Jean Karam. **References:** 1. Winter, C. et al. (1962) Proc Soc Exp Biol Med 111:544 547. 2. Northover, B. et al. (1961) Brit J Pharmacol. 16:163 – 169. 3. Gurbuz, I. et al. (2005) J. Ethnopharmacol. 101: 313 – 318. 4. Pieroni, A. (2000) J. Ethnopharmacol. 70: 235 – 273.

P627 Cardiovascular effects induced by *Camellia sinensis* in experimental diabetes

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Diabetes mellitus is a metabolic disorder syndrome characterized by high glucose blood levels because of a reduced insulin secretion by pancreas beta cells and/or reducing biologic activity of this hormone. This syndrome affects carbohydrates, fat and protein metabolism. Diabetic patients exhibit important endothelial alterations associated with the oxidative stress, being one of the most important cause of cardiovascular disorder in these people. Green tea (leaves of *Camellia sinensis*) is a popular beverage in East Asia, also used as an herbal remedy in Europe and North America. Green tea is being widely studied for its beneficial effect in the treatment and prevention of human diseases. It is considered to be anti-inflammatory, anti-oxidative, anti-mutagenic and anti-carcinogenic and can prevent cardiac disorders. The aim of this study was to evaluate the effects of *C. sinensis* tea on cardiovascular parameters in diabetic animals induced by a single injection of streptozotocin (STZ, 50 mg/kg, i.v.) administered 21 days before the experiments. Induction of diabetes mellitus in animals was confirmed by blood glucose value above 250 mg/dl 48 h after STZ induction. STZ promoted attenuation in mean BP (~10% of reduction) as well as in HR (~20% of reduction) when compared with control animals. Green tea intake prevented the reduction of both parameters, MAP and HR in diabetic animals. These results suggest that although green tea presents important antioxidant action, the presence of caffeine in its extract may have a sympathomimetic action determining changes in blood pressure in animals. **Acknowledgements:** MackPesquisa, PIBIC/CNPq

P628 Mechanisms involved on the hypotensive effect of a standardized fraction from *Hancornia speciosa* leaves

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Hancornia speciosa Gomes is popularly known in Brazil as "Mangaba". The barks, roots and leaves of the species are traditionally used to treat several diseases, including hypertension and diabetes. Previous works of our group demonstrated that the crude ethanolic extract from *H. speciosa* leaves was able to inhibit the activity of angiotensin-converting enzyme (ACE) and to induce vasodilatation in large and small arteries [1,2,3]. In the present work, we evaluated the hypotensive effect and its mechanism of action for a standardized fraction from *H. speciosa* leaves (SFH) in normotensive Swiss mice. SFH (10 mg/Kg) reduced the systolic blood pressure (SBP), measured by tail pletismography, in 32.3 ± 11.7 and 28.2 ± 4.9 mm Hg, after single administration by ip and po routes, respectively. The plasmatic activity of ACE as well as the plasmatic level of angiotensin II was strongly inhibited by SFH (1 mg/Kg; po). Meanwhile, the plasmatic level of nitrite, an indirect indicator of NO production, was significantly increased by SFH (1 mg/Kg; po). The hypotensive effect was significantly inhibited by L-NAME (20 mg/Kg; po), whereas nitrite contents were reduced. In mouse mesenteric arteries, SFH induced a concentration-dependent vasodilatation (IC_{50}= 32.0 ± 0.2 μg/mL), partially dependent on functional endothelium and on production of NO. The present results demonstrate that the hypotensive effect induced by SFH involves the inhibition of the renin-angiotensin system, the increase on production or bioavailability of NO and a direct vasodilator effect in normotensive mice. **Acknowledgements:** CNPq and PAPPE/FAPEMIG (Brazil) **References:** 1. Serra, CP. et al. (2005) Phytomedicine 12:424 – 432. 2. Ferreira, HC. et al. (2007) Phytomedicine 14:473 – 478. 3. Ferreira HC. et al. (2007) J Ethnopharmacol 109:161 – 164.

P629 *Echinacea* induced hsp70 alterations in leukocytes

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Heat shock proteins (hsp) are expressed constitutively as well as induced in response to mild stress. These stress factors include inflammation, viral and bacterial infection, oxidative stress and cytotoxins. Altered hsp expression has been found in a number of disease states [1]. Echinacea is used as a prophylactic to boost the immune system and has been previously shown to enhance hsp70 expression in leucocytes after mild heat shock [2]. In this study, the effects of echinacea supplementation on hsp70 expression in the different leucocyte subpopulations has been examined. Twenty four healthy volunteers aged between 20 and 66 years with a body mass index ranging from 20.2 to 30.7 participated in the study. They consumed 2 echinacea tablets (4.42 mg total alkylamides per tablet) twice a day for 14 days. Blood samples were taken on days 1 and 15 and hsp70 expression was determined by flow cytometry in whole blood with and without in vitro heat shock. Significant differences were observed in the percentage of several white cell subsets that expressed hsp70. CD4 and CD8 lymphocytes expressed significantly more hsp70 in both males and females after supplementation but in natural killer cells and B lymphocytes, expression of hsp70 were only significantly increased in the male participant cohort. These results indicate that Echinacea may play a role in activating the immune system when the body encounters a challenge such as a virus. **References:** 1. Srivastava, PK. (2002) Ann Rev Immunol 20: 395 – 425. 2. Agnew, LL. (2006) J Clin Pharm Ther 30: 363 – 9.

P630 Acute toxicity study of the crude extract of the fruit rind of rambutan (*Nephelium lappaceum* L.) in male Wistar rats

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Rambutans (*Nephelium lappaceum* L.) have various uses in medicine and the fruit rind of rambutan can be considered as an easily accessible source of natural antioxidant and antibacterial agent [1]. Since toxicological data of this extract have not been reported, this study aimed to investigate acute toxicity and liver function effects of crude extract from the fruit rind of rambutan in male Wistar rats. Acute toxicity was stu-

died in 42 male rats that received distilled water, 1, 2, 3, 4 or 5 g/kg of rambutan rind extracts by oral gavage. The number of deaths in each group was recorded within 24 h. Survived rats were further investigated for 14 days. For liver function test, 24 male rats received distilled water, 1 g/kg tannin or 1 g/kg rambutan rind extract by oral gavage. Five hours later, trunk bloods were collected and the plasmas were measured for GPT (Glutamic-pyruvic transaminase) and GOT (glutamic-oxaloacetic transaminase) by automatic analysis apparatus. The rambutan rind was extracted by 85% ethanol and the extract had total phenolic content of 18.69 ± 0.2 mg gallic acid/g dry sample extract and 50% inhibitor concentration (IC_{50}) values of 0.288 ± 0.04 mg/ml sample extract. Oral acute toxicity study revealed that there was no toxicity found at doses up to 5 g/kg and there was no abnormal clinical signs. Tannin and rambutan rind extract didn't have effect on plasma GPT and GOT levels. The results suggest that rambutan rind extract should be safe to be used in cosmestic, nutraceutical and pharmaceutical applications. **References:** 1. Palanisamy, U. et al. (2008) Food Chem. 109:54 – 63.

P631 Some therapeutic effects of herbal based ointment "Dermoplant G"on venous ulcers

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Venous ulcers (*ulcus cruris venosum*) are a common chronic disease requiring continuing treatment, significantly influencing patients' way of life. After the first signs like heavy and achy legs, ankle and leg swelling, the disease progresses, and the patient frequently develops leg ulcers, which can become infected. We tested the therapeutic effects of the ointment "Dermoplant G"on the epithelization (reducing the affected area, ulcers score and microbial flora) of venous ulcers in 10 human patients (4 men and 6 women) during 7 weeks. The major components of the ointment were dry water extract of *Allii bulbus*, dry ethanol extract of *Hyperici herba* and oil extract of *Calendulae flos*. The patients were older than 18 years, with ulceration no longer then 2 months or with recurrent ulcers during last 6 months, localized in lower leg. The involved patients did not use any other phytomedicine or supportive therapy. The parameters were evaluated before the treatment (after clinical diagnosis of venous ulcers), and every 2 weeks during the period of 7 weeks. The ulcers score was evaluated on the basis of epithelization, granulation (0-very marked, 1-moderate marked, 2-little marked, 3-no effect), fibrin deposits, exudation and eczema (0-no effect, 1-little expressed, 2-good expressed, 3- very expressed). The percentage of epithelization of venous ulcers was 61,53 ± 28,26 after 7 weeks, without significant effects on microbial flora (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). After 7 weeks of treatment, the ulcers score and symptoms score improvement was almost maximal-99,0%. The total score of treatment with "Dermoplant G" was 86,37% after 7 weeks.

P632 Beneficial effects of carrots (*Daucus carota*) on adipocyte differentiation, glucose uptake, and fat accumulation

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Consumption of carrots (*Daucus carota* L.) is in general believed to be beneficial for human health and several bioactive compounds have been identified e.g. the polyacetylene falcarinol, which exhibits anticancer activities both in vitro and in vivo [1,2]. Here we report a range of results suggesting a potential new field of application for carrots towards conditions associated with the metabolic syndrome e.g. insulin resistance and abdominal obesity. Dichloromethane (DCM) and methanol (MeOH) extracts of two varieties of carrots (var. bolero and purple haze) were made and tested in a number of different bioassays and one in vivo model system. DCM extracts of both carrot varieties were found to activate peroxisome proliferator-activated receptor (PPAR)γ without stimulating adipocyte differentiation, suggesting that they can have a positive effect on insulin sensitivity. At low concentrations (< 0.5 μg/mL) the DCM extract was able to enhance glucose uptake (GU) in porcine myotubes but at higher concentrations this was impaired. Biphasic concentration-dependent bioactivity has previously been reported for e.g. falcarinol [2]. The nematode *Caenorhabditis elegans* is a good model system for studying lipid metabolism and fat accumulation in vivo. At 200 μg/mL, the DCM extract of var. Bolero was able to reduce fat accumulation, as measured by lipophilic dye Nile red, in *C. elegans* by 50%. The carrot polyacetylenes have structures similar to both the endogenous ligands of PPARγ as well as recently identified alkamides able to activate PPARγ and enhance insulin-stimulated GU in adipocytes [3], indicating that these could be responsible for the observed effects. **References:** 1. Kobaek-Larsen M et al. (2005) J. Agric. Food Chem. 53: 1823 – 1827. 2. Hansen SL et al. (2003) J. Sci. Food Agric. 83: 1010 – 1017. 3. Christensen KB et al. (2009) J. Nat. Prod. 72: 933 – 937.

P633 Elderflowers (*Sambucus nigra* L.) have a significant impact on cellular mechanisms related to lipid storage and insulin resistance

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Preparations of elderflowers (*Sambucus nigra* L.) have traditionally been used as diuretics however recent studies suggest that they also have a potential use in the treatment/prevention of conditions associated with the metabolic syndrome. Extracts of elderflowers are known to activate the key regulator of adipocyte differentiation, peroxisome proliferator-activated receptor (PPAR)γ, without stimulating adipocyte differentiation, and furthermore to enhance insulin-stimulated glucose uptake (GU) in adipocytes [1]. The flavanone naringenin was identified as one of the bioactive components whereas major elderflower metabolites were not active [2]. In the present study, we have further investigated the effects of elderflower extracts and metabolites on mechanisms associated with lipid storage and insulin resistance in relation to the metabolic syndrome. Both dichloromethane (DCM) and methanol (MeOH) extracts of elderflowers were found to be able to enhance GU in pig myotubes both with and without insulin-stimulation corresponding with previous studies on mouse abdominal muscle [3]. The nematode *Caenorhabditis elegans* provides an excellent model system for studying lipid metabolism and fat accumulation *in vivo*. Extracts of elderflowers were able to significantly reduce fat accumulation as measured by the lipophilic dye Nile red, in *C. elegans* at concentrations of 200 μg/mL, and in particular the DCM extract was one of the most potent plant extracts with more than a 50% reduction of Nile red fluorescence [4]. However, on individual basis major elderflower metabolites do not seem to possess the same magnitude of bioactivity in the tests performed as the extracts. Hence, synergistic effects might be at play warranting further investigations. **References:** 1. Christensen KB et al. (2009) Phytother. Res. 23: 1316 – 1325. 2. Christensen KB et al. (2010) Phytother. Res. in press. 3. Gray AM et al. (2000) J. Nutri. 130: 15 – 20. 4. Boelt SG et al. (2008) Chem. Physics Lipids 154: S32-S32.

P634 Evaluation of muscarinic M_3-receptor antagonism of *Solenostemma argel* leaves

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Solenostemma argel (Del.) Hayne is a perennial shrub widely distributed in the deserts of North Africa. The decoction of the leaves was used in folk medicine as a antispasmodic for the treatment of various colic [1]. Continuing our researches on *S. argel* [2], in the present study we investigated the implication of colinergic system, in particular of muscarinic receptors, in the spasmolytic action of *S. argel* leaves decoction and two kempferol glycodides (**1, 2**) isolated from methanol extract. Detailed phytochemical investigations were carried out on the decoction by HPLC-MS^n. The evaluation of spasmolytic activity of the extract and two compounds has been performed on the guinea-pig ileum smooth muscles contracted by cholinergic agonist carbachol. In the same experimental conditions, papaverine has been tested for comparison. The decoction reduces the contraction to carbachol in a concentration-dependent manner. Moreover, the decoction reduced the maximum response to carbachol, defining a non-competitive antagonism. The flavonoid **1** has not elicited any antagonistic M_3 activity, while **2** has shown a non-competitive and concentration-dependent antagonism toward M_3 muscarinic receptors. The effects of **2** is similar to those induced by papaverine, but at a concentration about two order of magnitude lower. Concluding these preliminary results suggest that the spasmolytic and antispasmodic effects of *S. argel* phytocomplex might be mediated by M_3-receptor antagonism and justify its use in folk medicine in several intestinal diseases. **References:** 1. El Tahir, K.E.H. et al. (1987) Int. J. Crude Drug Res. 25: 57 – 63. 2. Innocenti, G. et al. (2005) J. Ethnopharmacol. 102: 307 – 310.

P635 Cholinergic and anticholinergic activities of steroids from marine alga *Turbinaria conoides*

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Nature gifted us with oxygenated steroids 3, 6, 17-trihydroxy-stigmasta-4, 7, 24(28)-triene (**A**) and 14, 15, 18, 20-diepoxyturbinarin (**B**) to quench our thirst of isolating steroids from the highest antimicrobial active cyclohexane-soluble extract of *Turbinaria conoides* (family: Sargassaceae), which were characterized by spectral analyses. Cytotoxic oxygenated fucosterols have been reported from the ethyl acetate extract of *Turbinaria conoides* [1]. The purpose of this investigation was to evaluate the isolated steroids for their response to acetylcholine-induced contraction on frog rectus abdominus muscle by *in vitro* standard method [2] as certain steroids have been reported for cholinergic, anticholinergic activity as well. Compound **A** significantly inhibited acetylcholine-induced contraction with EC_{50} (50% effective concentration) of 16 µg/mL ($P < 0.05$) and produced a shift to the right on the dose-response curve as exhibited by Pancuronium a positive control. The potency and affinity were exhibited by significant increase ($P < 0.05$) in the mean EC_{50} values of 1.66 ± 0.02 M (acetylcholine alone) to 2.52 ± 0.15 M (acetylcholine with compound **A**) and decrease in the mean pA_2 values of 0.82 ± 0.08 M (acetylcholine alone) to 0.47 ± 0.11 M (acetylcholine with compound **A**) respectively. Compound **B** potentiated acetylcholine response by 2-fold at EC_{50} level of 20 µg/mL, signifying estrogenic nature of cholinergic activity. Hence *Turbinaria conoides* has joined the band of elite species that possess both cholinergic and anticholinergic activities, thus proving to be an exquisite treasure chest for further research.

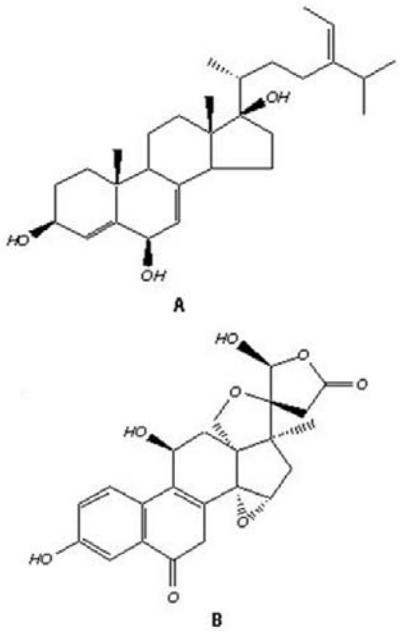

Fig. 1

Acknowledgements: We thank Dr.K. Eswaran for authentifying the Brown alga, *Turbinaria conoides*. **References:** 1. Sheu, JH. et al. (1999) J. Nat. Prod. 62: 224 – 227. 2. Kulkarni SK. (1999) Handbook of Experimental Pharmacology. Vallabh Prakashan. Delhi.

P636 *Haemonchus contortus*: *in vivo* anthelmintic activity of *Eugenia dysenterica* DC. and *Caryocar brasiliense* Cambess leaves in sheep

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Internal parasites represent the largest threat to productivity and economic gain for Brazilian sheep producers. The incidence of resistant populations to available anthelmintics have increased. In this work, "santa inês" breed sheep were fed with powder extract of E. *dysenterica* and C. *brasiliense* leaves, which were dried at 37 °C, grounded and mixed together. These leaves were added to animal feed (dose of 1,2 g/kg). The sheep weight on the 1th, 7th and 14th day was measured; as well the fecal egg counts per gram (epg) and blood tests were undertaken. To provide nutritional information, the powder extract was analyzed. A reduction of 81% in epg was observed on the 14th day. The blood test showed considerable decrease on eosinophils levels in treated animals. Bromatology analysis of powder leaves added to animal feed in comparison with pattern animal feed, showed no significant variation on the level of fatty acids. The levels of protein and minerals decreased 7% and 17%, respectively; however, the final protein concentration was 22%, whereas 12% represents the normal concentration used by most producers. An increase of 37% of fiber was observed, but no feed consumption decrease was noticed. Despite the variations of chemical composition, after 14 days, no significant weight loss was observed (0,6%) in treated animals. In conclusion, the powder extract used here, could represent an alternative natural source for anthelmintic compounds.

P637 Apocynin reduces dextran sulfate sodium-induced colonic inflammation in mice

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Apocynin, a naturally occurring 4-hydroxy-3-methoxyacetophenone, is used experimentally as an inhibitor of NADPH-oxidase. It can decrease the production of superoxide from activated neutrophils and macrophages, leaving their ability to undergo phagocytosis unaffected [1]. Its anti-inflammatory activity has been demonstrated in a variety of cell

and animal models [2]. In this study, we investigated whether apocynin exhibits anti-inflammatory activity on a model of inflammatory bowel disease (IBD) using dextran sulfate sodium (DSS)-induced colitis in mice. Acute colitis was induced in the mice through administration of 5% DSS in water for 7 days. The mice were fed a control diet or a diet supplemented with 2% apocynin or 2% rutin (a flavonoid used as reference) [3]. The animals were sacrificed on day 8 and their colons were removed, immediately snap-frozen on liquid nitrogen, and stored until use. The disease activity index score (DAI) was analyzed taking weight loss, stool blood, and rectal bleeding into account. The removed colons were subjected to extraction of proteins and COX-2 expression was analyzed with the aid of a Western blot assay. Treatment with apocynin ameliorated the course of colonic inflammation with results similar to those of rutin, as can be seen by reductions in the DAI scores (60% reduction for both compounds), colon length (47% and 42% reduction vs. blank, respectively), and COX-2 levels (65% and 75% inhibition, respectively). These results are promising for further experimental studies on treating gastrointestinal diseases and the potential protective effects of apocynin. **Acknowledgements:** Spanish Government, MICIIN (SAF 2009 – 13059-C03 – 01). **References:** 1. Simons, J.M., et al. (1990) Free Rad. Biol. Med. 8:251 – 258. 2. Stefanska, J., Pawliczak, R. (2008) Mediators Inflamm. ID 106507. 3. Xu, L., et al. (2009) Phytomedicine 16:989 – 995.

P638 Effects of the crude extract of the fruit rind of rambutan (*Nephelium lappaceum* L.) on blood pressure, heart rate and respiratory rate in anaesthetized male rats

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The bark of rambutan (Nephelium lappaceum L.), which is normally discarded was found to contain extremely high antioxidant activity [1]. Since polyphenols found in plants are widely known to affect the cardiovascular system, there is no evidence suggesting how polyphenols found in the crude extract of the fruit rind of rambutan did. We, therefore, investigated acute effects of rambutan on cardiovascular and respiratory responses in male rats. The ethanolic rambutan bark extract with a total phenolic content of 18.69 ± 0.2 mg gallic acid/g dry sample extract were used in this experiment. Invasive arterial blood pressure, heart rate and respiratory rate were recorded in pentobarbital-anaesthetized male rats for 2 hours after single i.p. injected with vehicle (n = 8) and 1 g/kg rambutan bark extract (n = 8). Intragroup comparison showed that rambutan rind extract markedly increased mean arterial blood pressure (MABP) and systolic blood pressure ($p < 0.05$, compared to baseline). In comparison between groups, significant increases in MABP, systolic blood pressure, diastolic blood pressure and heart rate can be observed 60 minutes after rambutan bark extract ($p < 0.05$, two way repeated measures ANOVA). Significant increases in those parameters were found over the rest period of investigation. However, there were no changes in respiratory rate In conclusion, this study provided the first evidence of prolonged cardiovascular response (increases in MABP, systolic blood pressure and heart rate) following acute rambutan rind extract in anesthetized male rats. The mechanism of which rambutan rind extract regulating blood pressure in normotensive, hypotensive and hypertensive conditions should be further investigated. **References:** 1. Palanisamy, U. et al. (2008) Food Chem. 109:54 – 63.

P639 Naringin protects against dextran sodium sulfate-induced colitis in mice

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Naringin (4′,5,7-trihydroxyflavanone 7-rhamnoglucoside) is a well-known natural flavonoid that acts as a potent anti-oxidant, superoxide scavenging, anti-apoptotic, anti-atherogenic and metal chelating agent [1]. Orally administered naringin is metabolized to its aglycone, naringenin, which has a wide range of pharmacological properties, including an anti-inflammatory effect through inhibition of nitric oxide and prostaglandin E2 production [2]. The aim of this study is to determine the effect of naringin on a model of inflammatory bowel disease (IBD), namely dextran sulfate sodium (DSS)-induced colitis in mice. Female Balb-C mice were randomized to receive either normal water or 5% DSS drinking water to induce colitis. The mice were fed a control diet or a diet supplemented with 2% naringin or 2% rutin for 7 days [3,4]. On day 8, the mice were sacrificed and their colons were removed, immediately snap-frozen on liquid nitrogen and stored until use. The disease activity index score (DAI) was analyzed taking weight loss as well as stool blood and rectal bleeding into consideration. The removed colons were subjected to protein extraction and COX-2 expression was analyzed with the aid of Western blot techniques. Naringin was found to significantly reduce the extent and severity of injury to the colon as shown by the DAI score (49% reduction), colon length (40% reduction vs. blank) and COX-2 levels (78% inhibition). Rutin, a polyphenolic flavonoid used as reference, also diminished these parameters by percentages of 60, 42 and 75, respectively. These results indicate that naringin may prove to be a useful agent for treating IBD. **Acknowledgements:** Spanish Government, MICIIN (SAF 2009 – 13059-C03 – 01). **References:** 1. Jagetia, G.C., Reddy, T.K., (2005) Life Sci. 77:780 – 794. 2. Raso, GM., et al. (2001) Life Sci. 68:921 – 931. 3. Claire Billerey-Larmonier, M.S., et al. (2008) Inflamm. Bowel Dis. 14:780 – 793. 4. Xu, L., et al. (2009) Phytomedicine 16:989 – 995.

P640 Isolation, structure determination and cannabinoid receptor activating effect of new metabolites of *Echinacea purpurea* root

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Extracts of *Echinacea purpurea* are extensively used in the therapy of common cold and upper respiratory tract infections as immunomodulators and anti-inflammatory agents. The extracts of the root and herb of *E. purpurea* have a complex chemical composition, including alkamides, caffeic acid conjugates, and polysaccharides. Alkamides, the main lipophilic compounds, are the modulators of the endocannabinoid system in consequence of their structural similarity to the endogenous ligand anandamide. Recently, receptor binding assays demonstrated the CB2 receptor affinity of Echinacea alkamides, proving by this the CB2-receptor-dependent immunomodulatory effect [1,2]. The aim of the present study was to reinvestigate the chemical composition of the roots of *E. purpurea* and to gain new information about the affinity of the isolated compounds to the cannabinoid system. The *n*hexane-soluble extract of the root was subjected to multiple chromatographic separations affording 19 compounds. The structures were analysed by extensive NMR and MS studies resulting the identification of four new natural products (3 alkamides and nitidanin-diisovalerianate). In addition, five compounds were detected for the first time in this species, and ten known *E. purpurea* metabolites were identified. Cannabinoid receptor activation of thirteen isolated compounds has been studied by [^{35}S]GTPγ*S* binding assays in rat brain membranes. This test measures (i) receptor-mediated G-protein activation induced by agonists and (ii) inhibition by antagonist ligands. A number of *E. purpurea* compounds turned out to be weak-to-moderate partial agonists, while others displayed inverse-agonist actions. In the presence of a reference CB1 receptor agonist, both sort of compounds exhibited concentration-dependent competitive antagonist effects. **References:** 1. Woelkart, K., Bauer, R. (2007) Planta Med. 73:615 – 623. 2. Raduner, S., et al. (2006)J. Biol. Chem. 281:14192 – 14206.

P641 High-dose St. John's wort extract STW 3-VI as a daily single-dose is safe and effective in the treatment of mild to moderate depression in different age-groups – results of a re-evaluation

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Objectives: In a post-marketing surveillance study (1) 4188 patients of 793 general practitioners were included. The patients suffered mainly from mild to moderate depression and dysthymia according to ICD 10 and were treated with St. John's wort extract (STW 3-VI, 900 mg extract) 1 x 1 tablet daily for 12 weeks. This revaluation assessed the change of the Hamilton Depression Rating Scale (HAMD) scores over time to different age-groups. **Methods:** An analysis of variance (ANOVA) for repeated measures (SPSS 15.0) was conducted. Only patients with completed data for all measurements were revaluated. **Results:** 1701

patients with mild depression, 1433 patients with moderate depression and 194 patients with dysthymia were revaluated (913 male, 2415 female patients). The 3 main ICD-10 disease classes showed a significant and comparable change of the HAMD-scores over time with very similar scores in moderate depression and dysthymia (mild depression: HAMD-means: 13.5 at baseline vs. 3.3 after 12 weeks; moderate depression: HAMD-means: 18.2 at baseline vs. 5.5 after 12 weeks). In a 2. analysis the patients were separated into 7 age-groups (from < 18 years to > 65 years). All age-groups, including the elderly, showed the above mentioned significant and comparable change of the HAMD-scores over time. **Conclusion:** This revaluation indicates that there is neither an influence of the disease classification (i. e. mild or moderate depressive disorders) nor of the age to the time course of the treatment outcome in patients treated with St. John's wort extract (STW 3-VI). **References:** 1. Demling et al., Nervenheilkunde 2004, 23:160.

P642 Extract and fractions from birch bark affect stimulation of human dendritic cells and their activation of allogeneic CD4+ T cells *in vitro*

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Birch bark has been used in traditional medicine to treat skin disorders and rheumatism. Several components have been isolated from birch bark with only few being tested for their effects on the immune system. Dendritic cells play a major role in the regulation of an immune response, e. g. by directing the differentiation of naive T cells. Therefore, the effect of extract and fractions from the bark of *Betula pubescens* was tested in a human monocyte-derived dendritic cell model. Dendritic cells cultured with ethanol extract from birch bark secreted less IL-6, IL-10 and IL-12p40 than cells cultured with stimulation factors alone. These effects were also observed for fractions III and IV obtained with VLC using dichloromethane:methanol gradient. Allogeneic CD+ T cells co-cultured with dendritic cells treated with fraction IV secreted less IFN-γ than T cells co-cultured with dendritic cells treated with stimulation factors alone. Further fractionation revealed that the active fractions are mainly composed of triterpenes and further characterization of these compounds is in progress.

P643 Toxicological studies on the *Eugenia dysenterica* DC and *Caryocar brasiliense* Cambess leaves in rats

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Powder extract of *E. dysenterica* and *C. brasiliense* leaves proved to be active against *Haemonchus contortus*, in vivo, in sheep, according to previous work performed by our group. Therefore, toxicology studies become necessary. To this aim, twenty male rats received a powder extract of *E. dysenterica* and *C. brasiliense* leaves for 15 days. The leaves were dried at 37 °C, ground, mixed and added to animal feed at concentration of 10%, 20% and 30%. A control group was fed only with diet without the mixture of leaves. After 15 days, only rats that received the feed with 30% of powder leaves showed significant weight loss, probably due to nutritional deficit since no feed reduction consumption was observed. A reduction on thymus weight, a discrete degeneration of liver and decrease on creatinin levels were observed on rats that received the major dose after 15 days. No important variation on hemoglobin, hematocrit, leukocytes and total protein was observed. According to these data we can suggest some toxicity when we use a mixture of 30% of powder leaves added to rat feed, and although a reduction of 81% in fecal egg counts per gram has been observed when we used the dose of only 1,2 g/kg in sheep, we intent to perform new experiments to analyze the treatment during a extend time period.

P644 In vitro and in vivo evidence of synergy between *Hypericum* and *Passiflora* in antidepressant pharmacological models

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Neurapas®balance is a combination of special extracts of *Hypericum perforatum* (St. John's wort), *Passiflora incarnata* (passion flower) and *Valeriana officinialis* (valerian); it is used for the treatment of mild depression, mild anxiety and sleep disorders. Since the daily dose of Hypericum in Neurapas® balance is lower than the doses of *Hypericum* in single extract phytomedicines, we were interested whether a combination of extracts of *Hypericum* and Passiflora exerts comparable effects to Neurapas® balance. We used two well-established models for investigating extracts for their anti-depressant activity, namely the effects on synaptic uptake of serotonin and the forced swimming test; in both tests *Hypericum* has previously been shown to exert pharmacological effects. We show here for the first time, that the *Passiflora* extract significantly enhances the pharmacological potency of *Hypericum* in both models. The potency of Hypericum to inhibit serotonin uptake depended on the hyperforin content of the test batches. The dose-dependent enhancement of the *Hypericum* effect by *Passiflora* (50 µg/ml) resulted in an IC50 of 14 µg/ml (*Hypericum* containing 1% hyperforin) and 9.7 µg/ml (*Hypericum* containing 2.1% hyperforin). A similar synergy between *Passiflora* and *Hypericum* extracts was observed in the in vivo model. A greater maximal effect (60% reduced immobility) was observed with *Hypericum* in a fixed (2:1) combination with *Passiflora* (90 mg/kg) than with higher concentrations (180/360 mg/kg) of *Hypericum* alone (maximal 45% reduced immobility). Our data prove that the anti-depressive therapeutic effects of *Hypericum* are possible with lower doses, when it is combined with *Passiflora*, than with mono-preparations of *Hypericum*.

P645 Gemmotherapy-complementary treatment in juvenile rheumatoid arthritis

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Rheumatoid arthritis is a chronic disease that damagesandeventually destroys the joints of the body. Juvenile rheumatoid arthritis (JRA) is not a single disease, but a group of diseases with symptoms and signs developed in children younger than 16 years. There are three major forms: pauciarticular disease, poliarticular disease, and systemic disease. The aim of treatment is to stop or slow down the progress of inflammation, improving function, and preventing joint damage. The most usefull drugs are: 1) nonsteroidal anti-inflammatorydrugs (NSAIDs) reduce inflammation, swelling, and pain, 2) disease-modifying antirheumatic drugs (DMARDs), in children Methotrexat is the most used drug, it interferes in the immune processes that cause inflammation and JRA. 3) Biologic response modifiers are a newer, specialized type of immunosuppressive drugs, carefully designed to block the actions of natural substances that are part of the immune response, such as tumor necrosis factor. A variety of complementary approaches can be very effective in relieving pain and improving the outcome of the disease. One of these complementary therapy is gemmoterapy, a scientific use of a special glycerin extracts from plant buds. In our study,19 pacients with diagnosed JRA (15 with pauciarticular disease and 4 with poliarticular form) was treated with specific antirheumatic drugs associating gemmotherapy. It has been used three types of bud extracts (Ribes nigrum, Buxus sempervirens, Vitis vinifera) with proved antirheumatic effects, during a period of 3 months. In all the pacients we observed a clinical amelioration, permitting a slight reduction of the dose of the NSAIDs and the DMARDs.

P646 Topical anti-inflammatory agents from the alpine flavouring plant *Artemisia umbelliformis* Lam.

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Artemisia umbelliformis Lam. (Asteraceae) is an alpine flavouring plant used to produce the bitter liqueur genepy. The lipophilic flavonoid eupatilin, a series of sesquiterpenes lactones and the sesterpene lactone genepolide, isolated from this plant [1,2], were investigated for their topical anti-inflammatory activity (inhibition of the Croton oil-induced mouse ear dermatitis) in comparison to the steroidal and non steroidal anti-inflammatory drugs hydrocortisone and indomethacin [3]. Six hours after dermatitis induction, the anti-oedema potency of eupatilin was comparable to that of indomethacin (ID_{50}= 0.30 and 0.26 μmol/cm^2), and only one order of magnitude lower than that of hydrocortisone (ID_{50}= 0.03 μmol/cm^2). A slightly lower effect was observed for genepolide and the sesquiterpenes anhydroverlotorin, santamarin, 5-deoxy-5-hydroperoxy-telekin, 5-deoxy-5-hydroperoxy-epitelekin and costunolide (ID_{50} ranged from 0.35 to 0.73 μmol/cm^2), the most active being anhydroverlotorin and genepolide (ID_{50}= 0.35 and 0.40 μmol/cm^2). The overall effect of eupatilin (0.3 μmol/cm^2), anhydroverlotorin (0.4 μmol/cm^2) and genepolide (0.4 μmol/cm^2) on oedema development up to 48 h was intermediate between those of equimolar doses of indomethacin and hydrocortisone at one order of magnitude lower doses, but their activity profile was similar to the latter. Eupatilin reduced also the leukocytes infiltrate in the ear tissue, similarly to the reference drugs. The effect of anhydroverlotorin and genepolide on inflammatory cells infiltration will also be discussed. **References:** 1. Appendino, G. et al. (2009) J. Nat. Prod. 72: 340 – 344. 2. Rubiolo, P. et al. (2009) J. Agric. Food Chem. 57: 3436 – 3443. 3. Tubaro, A. et al. (1985) Agents Actions 17: 347 – 349.

P647 Analgesic effects of *Stachys turcomanica* extract in mice

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Pain and inflammation are the complex biological responses to harmful stimulants. In fact, they are a protective attempt by the organism to remove the injurious stimulant. Natural products have long been recognized as an important source of therapeutically effective medicines. Different approaches have been developed to analyze analgesic and anti-inflammatory potential of plants in the past years. *Stachys* species (Lamiaceae) have been used as medicinal plants because of their biological activities. Some *Stachys* have been used as remedy for painful or inflammatory conditions in folk medicine [1,2]. In this study, the antinociceptive property of *S. turcomanica* was investigated by formalin test in mice. Aerial parts of *S. turcomanica* were collected from its growing area and identified. Dried and milled plant material was extracted by using methanol 80% and different concentrations of the dried extract were prepared. During the test, formalin solution (0.5%) was injected into the plantar surface of the right hind paw of the mice, 15 minutes after peritoneal injection of the plant methanol extract (100, 200 and 400 mg/kg). In order to elucidate the probable mechanism of action of the plant extract, naloxone was administered with extract. The results showed that injection of the plant extract at the doses of 100, 200 & 400 mg/kg significantly inhibited the chronic phase of formalin- induced pain. Intraperitoneal injection of opioid antagonist (naloxone) significantly reversed the analgesic effect of the extract in chronic phase. This result suggests that analgesic activity of this plant may be partly mediated by opioid system. **References:** 1. Rezazadeh, Sh. et al. (2009) J. Med. Plants Res. 3: 368 – 376. 2. Hajhashemi, V. et al. (2007) Res. Pharmac. Sci. 2: 92 – 98.

P648 Antiproliferative effect of *Bryonia aspera* Stev. ex Ledeb

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This study was designed to evaluate antiproliferative effects from the roots of *Bryonia aspera* Stev. ex Ledeb which is used traditionally in the treatment of gastrointestinal disorders, cardiac disorders and cancer [1]. Methanol, chloroform and petroleum ether fractions were prepared and investigated for antiproliferative activity against MCF7 (human breast adenocarcinoma), HepG2 (human hepatocellular carcinoma), WEHI (mouse fibrosarcoma) and MDBK (normal kidney epithelial cell) cell lines by MTT assay and cisplatin and tamoxifen were used as positive control [2]. All the fractions showed significant antiproliferative activity on MCF7 whereas they were inactive on HepG2 and WEHI cell lines (IC_{50}> 50 μg/ml). Our study showed that the $CHCl_3$ fraction was the most potent one against the MCF7 cell line. In our previous study, we isolated some triterpene cucurbitane-type compounds from the $CHCl_3$ fraction [3]. Further studies are in progress to evaluate the antiproliferative activity of these pure compounds. **References:** 1. Ghorbani A. (2005) J. Ethnopharmacol. 102:58. 2. Carmichael J.et al (1987) Cancer. Res. 47:936 – 42. 3. Sahranavard S. et al (2010) Planta Med. 76: 1 – 4.

P649 Pharmacological and histological study of *Centaurea bruguierana* ssp. *belangerana* on indomethacin-induced peptic ulcer in rats

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The species *Centaurea bruguierana* (DC.) Hand.-Mzt. ssp. *belangerana* (DC.) Bornm. (CBB) (Asteraceae) [1], namely "Baad-Avard" in Bushehr province, south of Iran, is used in folk medicine as hypoglycemic in diabetes and remedy for peptic ulcer disorders. The inhibitory effects of total extract and fractions of dried flowering samples of CBB collected from Borazjan, Bushehr Province, Iran on indomethacin-induced peptic ulcer in rats were studied. The 80% EtOH extract and petroleum ether, $CHCl_3$, EtOAc, *n*-BuOH, and remaining MeOH fractions obtained by solvent-solvent fractionation of dried flowering samples of CBB was investigated for anti-ulcer activity against indomethacin-induced ulcerogenesis in rats. The percentage inhibition for total extract with dose of 100 mg/kg (99%) and $CHCl_3$ fraction with dose of 42 mg/kg (98.95%) were found to be more prominent (***$p < 0.001$) compared to the reference group (cimetidine 100 mg/kg, 87.62% ulcer inhibition). A dramatic decrease in ulcer index was observed following the administration of total extract (100 mg/kg, ***$p < 0.001$) and CHCl3 fraction (42 mg/kg, ***$p < 0.001$) compared to control group. Pharmacoilogical and histological results of the present study proved that the aerial flowering parts of CBB possess preventive activity against peptic ulcer supporting the folkloric assertion in southern Iranian folk medicine. However, these effects are not limited to this species and previous studies have revealed that some other Centaurea species are active as well [2,3,4]. **Acknowledgements:**Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences grant number: 6091 – 33 – 03 – 86, Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran. **References:** 1. Rechinger, K.H., 1980. Flora Iranica, No. 139b. Akademische Druck-u. Verlagsanstalt, Graz. 2. Yesilada E. et al. (1995). J Ethnopharmacol 39: 31 – 38. 3. Yesilada E. et al. (2004). J Ethnopharmacol 95: 213 – 219. 4. Yesilada E. et al. (1995). J Ethnopharmacol 46: 133 – 152.

P650 Anti-spasmodic properties of *Matricaria recutita* L. cultivated in Golestan province of Iran: potential role of nitrergic and cholinergic pathways

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Introduction: Previous studies revealed anti-inflammatory and anti-spasmodic effects of Matricaria recutita. The objective of present study is to determine: 1) the effect of spray dried total extract of M. recutita collected in Golestan province of Iran on contractile phase of isolated jejunum of rabbit 2) the role of Cholinergic and Nitregic systems on anti-spasmodic effects of M. recutita. **Material &Methods:** In first groups (N = 6), the effects of increasing concentrations of plant extract on the contractile responses of jejunum of rabbits induced by cumulative addition of extracellular calcium and high K+ were studied. The role of the nitrergic and cholinergic systems was assessed in the presence of various concentrations of M. recutita (0.003 – 0.013 mg/ml) and cholinerhic and nitrergic modulators at the second and third groups (N = 12). **Results:** Significant concentration-dependet inhibitory effects of M. recutita on contraction of smooth muscle caused by cumulative concentrations of calcium (0.03 – 3 mM) and to 50 mM Kcl was observed. Pretreatments with L-Name (100 μm) and atropine (3 μm) abrogated effectively the spasmolytic effect of M. recutita. Although, the M. recutita effect was significantly reduced in preparations pretreated with L-Argening (100mM), a precursor of Nitric Oxide production. Interestingly, contractile response of smooth muscle to Ach was completely reverted by M. recutita **Conclusion:**Our results indicate that the anti-spasmodic effects of Matricaria recutita arise from its direct inhibitory effects on Ca2+ channels. however, modulation of cholinergic and nitregic pathway could be involved. These observations suggest that Matricaria recutita inhibit contractile elements of peristalsis by facilitating inhibitory role of nitrergic pathway.

P651 Adjuvant potentials of AcF1, an immunostimulant fraction of *Alchornea cordifolia* extract

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As a result of strong experimental data supporting effectiveness and safety, herb-based immunomodulators are paving way as alternative sources of potent adjuvants for vaccines. In this study, the immunostimulatory and adjuvant properties of *AcF1*, a flavonoid-rich fraction of Alchornea cordifolia extract, were evaluated. *AcF1* was shown to activate total splenocytes, CD4+ T cells, and B cells; inducing remarkable increases in CD69 expression, proliferation, cytokine (IL-4 and IFN-γ) expression by naûve splenic cells in a concentration-dependent manner. Lympho-activation and proliferation induced by *AcF1* was partly inhibited by U0126, a selective mitogen activated protein kinase kinase (MKK) inhibitor. Additionally, AcF1 was shown to induce structural and functional maturation of bone-marrow derived dendritic cells (BM-DCs) and also increased their specific-antigen presentation functions in vitro. When employed as an adjuvant in a homologous prime-boost OVA immunisation in C57BL/6 mice, *AcF1* significantly ($P < 0.05$) increased the level of OVA-specific IgG1 and IgG2a titres in the sera of immunised mice, compared to the control group immunised with OVA alone. The antigen-specific CTL responses, measured by intracellular staining for CD8+/IFN-γ+, did not show any significant difference. The improvement in antibody responses and strong immune cells activating properties observed in the study show that *AcF1* could be employed as a potential vaccine adjuvant and requires further study in combination with other vaccine antigens capable of eliciting strong CTL responses. **Acknowledgements:** The Alexander von Humboldt Foundation is gratefully acknowledged for postdoctoral fellowship award to Dr C.S Nworu **References:** 1. Allison AC, Byars NE (1991). Mol. Immunol 28: 279 – 284 2. Petrovsky N, Aguilar JC (2004). Immunol and Cell Biol 82: 488 – 496.

P652 Attenuation of stress-induced excitatory behaviors in mice by valerena-4,7(11)-diene from spikenard

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Valerena-4,7(11)-diene (VLD) is a sesquiterpenoid obtained from spikenard (*Nardostachys grandiflora DC., syn. Nardostachys chinensis*). Volatised spikenard oil had shown sedative effects on mice in the open field test, and VLD was determined to be the principal active constituent [1]. The volatised VLD at the concentration of 300 μg/cage reduced mice locomotor activity by 78% and prolonged the continuous sleep time of pentobarbital-treated mice by 2.7 times. In addition, VLD showed antidepressant effect comparable to imipramine in the forced swimming test. Immobilized time of VLD group was reduced by 33% in comparison with that of control group. Considering the following results, these effects of VLD were seemed to be expressed through both olfactory stimulation and direct CNS stimulation. In the experimentally induced anosmic mice, the effects of VLD were reduced by 52%. The intra venous injection of 0.1 mg/ml VLD reduced mice locomotor activity by 53%, and the VLD concentration in the blood was 3.0 ng/ml. On the other hand, the mice kept for 60 min with volatised VLD had shown much less spontaneous activity, but the VLD blood level was 1.0 ng/ml. In addition, VLD had dose dependently reduced the stress-induced behavioural changes in the mice subjected to immobilisation stress. This stress shortened sleeping time in the pentobarbital sleeping test by 47%, and immobilised time in the forced swimming test by 43%. The vaporised VLD completely suppressed these stress-induced excitatory behaviours. Moreover, vaporised VLD reduced this stress-induced rise of serum cortisol level. Our data suggest that valerena-4,7(11)-diene would be useful as an anti-stress agent. **References:** 1. Hiroaki T et al. (2009) Journal of Natural Medicines (63)4: 380 – 5.

P653 Topical application for treatment of atopic dermatitis with flavononol galloyl glycoside from the leaves of *Acer ginnala* in NC/Nga mice

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Acer ginnala (Korean maple) have been used as folk medicine for eye diseases related to inflammatory and infection. As part of our continuing research for new anti-atopic natural products based on their traditional therapeutic usages, we isolated several components and evaluated their anti-atopic activities in vivo. Activity guided isolation of 80% methanol extract from the leaves of *Acer ginnala* (AGL) yielded two flavononol galloyl glycosides and three unusual gallotannins including a new component (2,4,6-trigalloyl-1,5-anhydroglucitol). In order to evaluate anti-atopic activities, 80% methanol extract from the AGL and quercetin 3-O-(2"-galloyl)-α-L-rhamnopyranoside, which was the main compound of the AGL, were applied to atopic dermatitis-like skin lesion in Nc/Nga mice which have recently been recognized to be a model for atopic dermatitis [1,2]. As a result, clinical skin severity score decreased by the treatment of them. They also lowered eosinophils, IgE and Th2 cytokines levels in serum, significantly ($p < 0.05$). In addition, both COX-2 and iNOS in mouse skin tissues and their mRNA expressions were suppressed by them, significantly ($p < 0.05$). These results demonstrate that AGL and its main component, quercetin 3-O-(2″-galloyl)-α-L-rhamnopyranoside may be useful for treatment to skin allergies as a novel immunomodulator. **References:** 1. Simon, D. et al. (2004) Allergy. 59:561 – 570. 2. Thepen, T. et al. (2006) J. Allergy. Clin. Immunol. 97: 828 – 837.

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Effects of intraperitoneal administration of Silexan, an essential oil from flowers of *Lavandula angustifolia* on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex of freely moving rats

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Silexan is an essential oil of selected quality produced from Lavandula angustifolia flowers by steam distillation. The essential oil is the active pharmaceutical ingredient of Lasea®, a new phytochemical preparation, which is approved in Germany for the treatment of restlessness and mild anxiety. Native preparations of lavender oil have traditionally been used in aromatherapy as the scent has a calming effect. The aim of the present study was to investigate the effects of acute treatment with Silexan on extracellular levels of noradrenalin (NA), dopamine (DA) and serotonin (5-HT) in the prefrontal cortex of freely moving rats applying microdialysis technique. The microdialysis probe was inserted into the medial prefrontal cortex as described elsewhere (1). Following a stabilization period, the samples were collected every 60 minutes. The first sample was taken for determination of basal levels. Thereafter, Silexan was given at doses of 3,10 or 30 mg/kg intraperitoneally and additional fractions were collected 60 and 120 min after Silexan administration. Silexan at doses of 10 and 30 mg/kg, significantly increased the NA concentrations by 30% and 36%, respectively, whereas the DA concentrations increased already at the dose of 3 mg/kg by 34% and by 43% and 44%, respectively at the two higher doses. The 5-HT levels increased by 34% only at the highest dose tested. These data demonstrate that Silexan after a single acute administration increases the extracellular levels of monoamines in the prefrontal cortex of awake rats and this activity may contribute to the clinically observed relaxing and anxiolytic action of Silexan. **References:** 1. Kehr, J. (1999).

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Evaluation of anti-inflammatory activity of *Cymbopogon citratus* (DC) Stapf on an *in vivo* acute inflammation model

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Lemongrass (*Cymbopogon citratus* (DC) Stapf.), an Indian native herb belonging to the family of Poaceae, is used in popular medicine with a wide range of indications such as digestive and nervous disorders, inflammation, fever, diabetes [1,2]. In a previous study it was proved that, under inflammatory conditions, an essential oil-free infusion of *C. citratus* leaves allowed the cell viability and conferred significant reduction of the nitric oxide production in dendritic cells stimulated with lipopolysaccharide [3]. Based on these findings, the aim of this work was to evaluate the anti-inflammatory activity of an essential oil-free infusion of *C. citratus* leaves (CcE) in an animal model of acute inflammation. The carrageenan-induced rat paw oedema model was used [4], employing diclofenac as reference drug (10 mg/kg). The test groups received the aqueous extracts at the doses of 200 mg/kg (D1) and 400 mg/kg (D2) p.o. The anti-inflammatory potency of the drugs was calculated at 4 h after carrageenan administration and was expressed as percentage of oedema inhibition for the treated animals, with respect to the carrageenan control group. The results obtained were 39.00% for D1, 73.10% for D2 and 75.88% for positive control group, suggesting that oral treatment with CcE significantly prevents carrageenan-induced swelling in a dose-dependent manner, the potency of the higher CcE dose tested being comparable with that of the reference drug used. **Acknowledgements:** FCT and POFC/FEDER for financial support. Research supported by FCT PhD fellowship SFRH/BD/41283/2007 and the project FCOMP-01 – 0124-FEDER-011096 (ref FCT PTDC/SAU FCF/105429/2008). **References:** 1. Carvajal D, et al. (1989) J Ethnopharmacol. 25: 103 – 107. 2. Lorenzetti, BB et al. (1991) J Ethnopharmacol. 34: 43 – 48. 3. Figueirinha, A. et al. (2010) J Med Food 13 (in press). 4. Green, AY et al. (1971) Br J Pharmac 41: 132 – 139.

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Willow bark extract STW 33-I in the long term treatment of osteoarthritic and back pain

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The efficacy and safety of willow bark extract in the therapy of painful disorders of the musculosceletal system has been shown in a large number of clinical studies, but only for shorter treatment periods. The aim of this long term postmarketing surveillance study with STW 33-I (Proaktiv®, extraction solvent water; drug extract ratio 16 – 23:1) was the documentation of the treatment of patients suffering mainly from osteoarthritic and back pain for a period of up to 6 month. An extensive case report form with pain questionnaires and patient diary was used to evaluate the outcome of the treatment. 350 patients were included. 62% of them were treated with STW 33-I as a mono therapy, 28% with a combination of STW 33-I and NSAIDs, and 5% with opioids in addition. A first evaluation of the pain intensity (measured by Visual Analogue Scale VAS 0 – 100) over the treatment period shows mean improvements of resp. 23.5 for STW 33-I only, 18.8 for STW 33-I and NSAIDs, 21.2 for STW 33-I, NSAIDs and opioids simultaneously. Pain intensity was also evaluated with other scales for pain intensity at rest and in motion and for pain duration, and with respect to further subgroups and correlations. Adverse events were registered as well. Within the limitations of a pragmatic design of postmarketing surveillance, these data give a hint to an effectiveness of willow bark extract over 6 months, as a monotherapy and even in combination with conventional analgetics.

Authors' Index

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