Vegetation Ecology and Ethnobotany of *Cyphostemma digitatum* in the Western Highlands in Yemen

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Mohammed Al-Duais

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Gutachter:

1. PD. Dr. Gottfried Jetschke, Friedrich-Schiller Universität Jena
2. Prof. Dr. Matthias Hamburger, Universität Basel
3. Prof. Dr. Michael Manthey, Universität Greifswald

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CHAPTER 1: Introduction

Throughout history, Yemen, located on the southwestern Arabian Peninsula, has been known for its relatively abundant rainfall. However, considering Yemen's general geographic characteristics, its rainfall has been overestimated. Reasonable annual rainfall occurs only in the western and southern slopes of the Yemen Mountain Massif, whereas all other areas remain dry (WRAY, 1995). With these gradients in elevation, topology and continentality under different environmental combination a wealthy mosaic vegetation with a high level of biodiversity is manifested.

History of Yemen flora excavation

Yemen has a distinctive rich flora which is a reflection of the distinctive climate, topography and geology compared to the neighbouring areas in Arabian Peninsula and Africa. This fact always had attracted intellectuals and botanists. In the middle ages books dealt with Yemen vegetation including "*Kitab Al-Nabat*" for Al-Dinawari (895 A.D.) and "*Taj al'Arus*" for Al-Zabidi (1780 A.D.), two other books concerning only Yemen vegetation are "*Bughyat al Falahin*" by Al-Afdal al Abbas ibn Ali, which addressed the farmers, while the second book entitled "*Al-Mut'amad*" fofAl-Muzaffar Yusuf ibn Umer (d. 1294) is a treatise on medicaments (Wood, 1997). International excavations and plant systematics of Yemen vegetation were triggered by the Royal Danish Expedition to the Red Sea 1761–1763, led by Pehr Forsskal and Niebuhr; their work is the main source of all studies on the Yemen flora. The next visit was by botanist and plant collectors including Ehrenberg, Bove and P. E. Botta during 1820-1830. Botta came on behalf of the Paris Natural History Museum who made valuable collections (Wood, 1997). Alexandre Deflers in 1887 explored the Yemen flora and published his book "Voyage au Yemen"; his contribution also comes from his visits to areas of botanical interest like Ibb area , Manakhah, Hujariyah and Hajjaylah (Wood, 1997). In 1889 G. A. Schweinfurth visited Yemen to refine as many plants discovered by Forsskal as possible. Other flora collections of less importance include Botez (1911), de Benedictis (1926), C. Rathjens and H. von Wissmann (1927 – 1938). Their plant records were written up by O. Schwartz in the "Flora vom tropischen Arabien". Hugh Scott and E. Britton (1937-1938) wrote the book "in the High Yemen"; their collection contained for the first time some of the interesting plants of the Ibb region. Ahmed Khattab's journey was in 1945 and a checklist of his collection was published in 1971 (Wood, 1997). The collection of G. Popov and W. Zeller in 1962 was important, since they were the first who penetrated the eastern regions. Between 1970 and 1982 a great deal of botanical research has taken place in Yemen; extensive collections were made in behalf of botanical institutes among them F. N. Herpper (1975), Aradcliffe-Smith and Henchie (1977), Miller (1978, 1981), Steinberg (1979, 1980), Cuccuini (1981), Podlech (1981), Müller-Hohenstein (1980-82) and Lavranos (1974, 1976, 1977). Other collectors include Chaudhary (1976–78), Brunt, Henry and Pratt (1974–77), Wood (1978–79), Gillespie (1979), Carden (1974–76), Clissold (1982),

Christmann (1982), Ellenberg (1981), Firebrace (1980), Fleurentin (1976–81), Goodman (1980), Haj–Thomas (1977–80), Heath (1977), Kamal Ibrahim (1964), Kassasian (1982), King (1982), Larsen (1980–81), Leach (1971), Madge (1980), Ritchie (1974–76), Weber (1980) (Wood, 1997). In the last 30 years many researchers from and outside Yemen kept searching the Yemen flora with special emphasis in the hot spots of conservation priority, like Socotra archipelago and the tropical forest relicts of Jabal Bura'a.

Yemen ethnobotany situation

Especially in the south-western highlands very stable farming habits were developed where economic crops were produced intensively for thousands of years. Coffee was introduced to the world hundreds of years before from this well developed agricultural civilisation. In the vicinity of the study area Mocca, located at the Red Sea, was the first worldwide outlet to export mainly coffee, many other crops, among them different grains, legumes and fruits were produced. This region is the highest inhabited area in whole Arabian Peninsula; gathering and processing herbs for culinary and remedy use became for many families a main source of income. Consequently a very flourished market known in Arabic as ETARAH is one of the traditional activity of the peoples, the most important products are the processed discs from *Cyphostema digitatum* (Figure 1) in which high demand was observed even for consumption outside Yemen.

Fig. 1: Discs of *Cyphostema digitatum* the most consumed form.

While the fresh leaves of *C. digitatum* are very cursive when consumed directly; socio-economic significance of processed products is in continuous progress. *C. digitatum* was observed to be the most intensively gathered species from nature in Yemen. For many applications the most prominent application is the culinary use as a main component of special soup and a source of taste and aroma of many other traditional dishes: Besides, it is used as ethnic medicine for vomiting, fatigue, against malaria and headache and for general health support; other peoples used it for polishing precious metals and jewellery by pressing the fresh laves over the surface. Over-exploitation of mature *C. digitatum* by local dwellers and herbalists made the plant currently endangered; moreover, individual and family small enterprise in gathering and processing *C. digitatum* for commercial seal to the local and remote traditional food market was recently well established. Currently *C. digitatum* is exported outside its natural area, even outside Yemen.

This intensive gathering complicated with the recent dryness in some areas led to little chances for the harvested individuals to form mature seeds or even regenerate itself; as a consequence, *C. digitatum* disappeared completely from many regions in the southwest highlands. Its ecological situation became worse day after day, since large scale collectors expanded their area of gathering to remote places in Yemen where *C. digitatum* still not had been intensively used by the local people. Because of the scarcity of the species in many places in the study area, it became removed from nature and was replanted in the gardens (Fig. 2); hence, potential impairments of the ecosystem and the food web could be anticipated. Until this work nothing was done scientifically on this species in or outside Yemen.

Figure 2: Cultivated *C. digitatum* replacing grape by climbing a garden net borders.

High demand and overexploitation is a leading cause for extinction of many species like *Crassocephalum biafrae* and *Deppea splendens*. This process may cause the depletion of the resource even before it became standardised for the purpose of use. In extreme cases this leads to the extinction of the organism as genetic resource, and its role in nature is gone forever like in the case of the legendary bird Dodo. Continues clearance of the vegetation in Yemen played a large part in reducing plant species. For example, *Bersama abyssinica* had not seen since the nineteenth century, only two surviving trees are known of A*dansonia digitata, Bridelia scleroneura* and *Brucea antidysenterica*, small individual numbers of *Lannea fruticosa, Piliostigma thonningii* and *Sterospermum kunthianum* are known from one or two places, but it seems likely that they were much more common in earlier times (Wood, 1997). As plant species around the world go extinct, natural habitats become less productive and contain fewer total plants, a situation that could ultimately compromise important benefits that humans get from nature. A list of worldwide endangered plants became now available, and it is increasing more and more because of the lack of comprehensive understanding to the causes. We need to address all the interested social slices that deal with these plants which will enable different methods of solutions and different contributions that sustain the species.

Objectives and Aims

Special highlighting on the Yemen vegetation as the most important site for Arabia and northeast Africa flora beside the special global important of Socotra were done in locations where *C. digitatum* does not exist in nature or was cleared with human activities. As a biologist concern in the sustainable development, I have believed that many of the problems that encountered in the less developed countries among them my country can be solved by better scientific understanding of the culture and economical problems and its complication with nature and environment in the ruler areas. Better sustainable agriculture, implementation of productive, profitable, healthy and sustainable resources and land-use systems is urgently needed including development of safe ethnobotanical culinary and medicinal activities; to deal with nature in sustainable manner not to use nature extensively. Consequently I choose to be the first researcher who initiates the problem of overexploitation of *C. digitatum,* two main questions leading me in this effort; (1) was there any justification for this kind of high demand for a plant which is toxic before processing or was it just a legendary heritage? (2) Was there any scientific execution (other than history of the species and questioners) that could lead us to places where *C. digitatum* still intact; occupied its ecological niche? Could this ecological niche described by few environmental variables? And could a probability of existence of this variables combination given for any point of interest for possible reintroduction or restoration of *C. digitatum?*

To encompass the problem of high demand and overexploitation and to frame its application, a better understanding of the biology of *C. digitatum*, its previous and current ecological situation, its biochemical compounds and its potential usage is needed. This understanding promotes to have a wide frame for the study and research on *C. digitatum*; which differs in the techniques

applied, but share the ultimate goal to conserve this plant. For this reason different approaches are brought together in this thesis: we have to focus on the bioactive compounds of the species and the resulting utilizations of the plants products, but at the same time we want to understand the occurrence of the species in nature and its association to observable and distinctive vegetation units.

Biochemical characterisation of *C. digitatum*

When we ask why this demand on this kind of product derived from *C. digitatum* for the peoples who deals with *C. digitatum* either as consumers or handlers, the ordinary answer taste it and smell it. Consequently aroma active compound of the processed material most be identified. It is well known that priorities in standardisation of newly culinary or medicinal plants, especially for dark green leafy vegetable, are mainly given to screening for the common antioxidant activity, because this was found to have great implementation against many illnesses including cancer and heart disease, and to support a healthy life in general. If this is proved to be the case importance will be moved to the elucidation and quantification of the most important trace functional food ingredients which stand behind the antioxidant activity. Chemical and biological screening of possible bioactive other ingredients of medical application and search for the source of toxicity in the fresh plant are still within this aim.

Ecological requirements of *C. digitatum*

To understand the occurrence (or absence) of the species comprehensive observations were needed, including the vegetation that contain the species and the environmental conditions in the screened sites. There are two main ecological theories to understand the ecological niche of a species: the first concept is the individualistic concept by Gleason, (1926) who claimed that each species replies to the environment individually and independently. *C. digitatum* is an overexploited species and many of the localities were nowadays documented as absence, while essentially the species was historically present, we still need to understand in which plant communitis *C. digitatum* belongs to. This could be done by following the opposite concept, namely the holistic concept by Clements, (1916) who stated that plant associations respond to environment similarly and, therefore, plant communities are organisms of higher scale. These two concepts need to be applied, but of course, complemented with following up the plant growth and reproduction in nature, in the garden and in the green house.

Restoration of *C. digitatum*

These two previous aims mostly support the restoration of *C. digitatum*, since successful biochemical characterisation of this natural product is very promoting for restoration and sustainable use of the plant. At the gathering level it will convince the farmer to shift from gathering to cultivate the plant species in the margins of their fields instead of running in a marathon of collecting the plant from nature. The direct economical importance will be convinced governmental and local authorities to put restrictions for intensive gathering and mass

transport of the plant between different regions in Yemen. Instead they will support the idea of producing *C. digitatum* as secondary crops in the margins of their fields and sustain the plant in nature. The determination of the regions and the vegetation types that sustain or can sustain *C. digitatum* by producing prediction maps and calculating presence probability of *C. digitatum* at the finer scale will give another means of support for the restoration and conservation efforts. Meanwhile it addresses the scientist, specially the ecologist and agricultural specialist, to recognize sites of high probability in which the plant can be reintroduced or economically produced.

Organization of dissertation

This dissertation is designed into six chapters as follows:

In **Chapter 1** a general introduction to the study area, the species under investigation and the problem of the study will be given. The general framework, objectives, theoretical bases, justification of the design of the study and the intended methodology to be applied in the field and in the laboratory will be presented.

Chapters 2, 3 are already published papers and the formats of the publishing journals were kept. Both papers aimed to discover a scientific explanation behind the high culinary and medicinal demand on *C. digitatum.* In chapter 2 five well established methods for the estimation of the antioxidant activity were applied, since this interferes with many illnesses claimed by consumers and traditional healers who used this plant. A comparison of the contents found with well searched vegetables is also included. Chapter 3 will be an important consequence of chapter 2 in the sense, that the important phytonutrients compounds behind the antioxidant activity need to be identified and quantified. Processing effect in both chapters will be highlighted, too. In chapter 2 the antioxidant capacity of *C. digitatum* will be confirmed by four different widely used methods, among them the modern superior oxygen radical absorbance capacity (ORAC) assay, and their relation to the total phenolic content determined by Folin–Ciocalteu method will be estimated. In chapter 3 High Performance Liquid Chromatography (HPLC) techniques will be the method of choice which enables qualification and quantification of the examined functional food ingredients by comparison with standard reference material (SRM). In **chapters 4** we implement the solid phase microextraction (SPME) coupled with the gas chromatography techniques to qualify the aroma active components in *Cyphostemma digitatum* before and after processing. The variety of fibres used in (SPME) will insure to get all the variety of the aroma active components.

Chapters 5, 6 will be based on the ecological theories, the individualistic and holistic concepts. In **chapter 5**, to implement the holistic concept, the classical Braun-Blanquet approach along with the centralized replicate random sampling will be applied in the field to reach enough sample size in representative homogeneous vegetation. Braun-Blanquet method is the most advantageous method for such task because it gives fair weight for all the species that exist in the stand by taking the abundance data in consideration for the scarce species and the percentage

cover for the dominant species (van der Maarel, 1979). The collected species sample data matrix along with the documented site environmental conditions will be subjected to multivariate statistical analysis techniques, including cluster analysis based on indicator species analysis, to give a meaningful discrimination. Suitable ordination methods shall help to discover if there is valid grouping in the data such that they can be classified as certain plant communities harbouring *C. digitatum.*

In **chapter 6**, the individualistic concept will be directly implemented by analysing data based on presence or absence alone to build a suitable habitat model of the target species *C. digitatum*. However, the holistic concept will be indirectly implemented by modelling potential occurrence of the carrying communities, avoiding absence data being a consequence of human overexploitation. Using habitat models to predict species distributions has become a key tool in documenting and understanding biodiversity on the planet. They are very critical to understand the effect of multiple stresses caused by climate and human-induced changes (Fjeldsaå & Lovett, 1997; Pimm, 1991). Can environmental data identify areas of significance to predict species distributions and model community responses to environmental and anthropogenic changes? Around 1000 localities were visited to document whether *C. digitatum* was present and surely absent (within a certain distance). Together with suitable GIS-based environmental layers and statistical techniques a predictive model will be constructed to reproduce as output well-defined areas that contain suitable combinations of environmental variables which can sustain *C. digitatum* and where it is already present or could be cultivated in the future. Since the data of species' occurrrence alone are biased due to high human influence, a similar procedure will be applied to model the main plant communities resulting from chapter 4 to know where the suitable site conditions promote existence of the communities that could harbour *C. digitatum*. Computerised statistical techniques along with GIS give a huge enhancement that enables the researcher to get the best estimation from a large collected data set (Guisan and Zimmermann, 2000).

In **chapter 7**, a general discussion will be given to evaluate our findings from all different approaches. Recommendations based on the results highlighting the current situation of *C. digitatum* will be given to conserve the species and to allow a reasonable economic use of the natural product while sustaining the species in its natural habitat.

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CHAPTER 2: Antioxidant capacity and total phenolics of *Cyphostemma digitatum* before and after processing: use of different assays

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ORIGINAL PAPER

Antioxidant capacity and total phenolics of *Cyphostemma digitatum* before and after processing: use of different assays

Mohammed Al-Duais · Lars Müller · Volker Böhm · Gottfried Jetschke

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Abstract In the framework of standardisation of new healthy food sources, this paper aimed to study the total phenolics and the antioxidant power of *Cyphostemma digitatum* (Vitaceae) in water and ethanol extracts, using 96-well micro plates with BMG FLUOstar Optima micro plate reader. Total phenolics by Folin–Ciocalteu method in the water extracts were significantly lower after processing, decreasing from 1.41 ± 0.06 g GAE/100 g in the raw leaves to 0.80 ± 0.08 g GAE/100 g in the processed sample; the ethanol extract revealed the same trend with higher values, decreasing from 1.95 ± 0.03 to 1.56 ± 0.12 g GAE/100 g. The antioxidant capacity was elucidated by four methods: TEAC, DPPH, FRAP and ORAC. No or very weak correlations were found between antioxidant assays and total phenolics; this confirms that the antioxidant capacity could be attributed to other molecules. The ORAC assay proved to be more powerful than the other assays; it showed 103.3 ± 2.5 mmol/100 g Trolox equivalents in the raw leaves ethanol extract and 91.9 ± 3.0 mmol/100 g in the processed sample. ORAC assay showed the opposite for the water extract where the antioxidant capacity increased from 16.7 ± 0.2 to 41.7 ± 2.7 mmol/100 g Trolox equivalents after processing, which could be attributed to new water-soluble compounds generated in the consumed form.

M. Al-Duais (✉) · G. Jetschke
Institute of Ecology, University of Jena,
Dornburger Straße 159, 07743 Jena, Germany
e-mail: b5almo2@uni-jena.de; dassssan@yahoo.com

M. Al-Duais
Department of Biology, Ibb University, Ibb, Yemen

L. Müller · V. Böhm
Institute of Nutrition, University of Jena,
Dornburger Straße 25-29, 07743 Jena, Germany

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Introduction

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [1]. The potential of the antioxidant constituents of plant materials for the maintenance of health and protection, e.g. from coronary heart disease and cancer, is also of raising interest among scientists and food manufacturers as consumers move towards functional foods with specific health effects [2]. Some secondary plant products had up to 16 times higher antioxidant potential compared to well known food additives. It has been recommended to the food industry to use these natural antioxidants instead of synthetic ones to get storage stability for processed food items, which according to recent surveys, are in the interest of consumers [3].

Many medicinal plants contain large amounts of antioxidants other than vitamins C, E and carotenoids [1] and the assessment of the contribution of culinary and medicinal herbs to the total intake of dietary antioxidants was a target of many investigations [4–6]. A normal dietary intake of herbs may therefore contribute significantly to the total intake of plant antioxidants and would be even a better source of dietary antioxidants than many other food groups, such as fruits, berries, cereals and vegetables. In addition, herbal drugs in application, e.g. Stronger Neo-Minophagen C, a glycyrrhizin preparation used as an intravenous injection for the treatment of chronic hepatitis, boost total antioxidant intake [7]. It is tempting to speculate that

several of the effects due to these herbs are mediated by their antioxidant activities [6]. Quantitative and qualitative determination of phenolic compounds with strong antioxidant capacity has been done for many plant extracts [4]. Especially plants belonging to the Lamiaceae family, such as sage, oregano and thyme, showed strong antioxidant capacity [8]. Furthermore the contribution of total phenolics for certain culinary herbs was determined as a percentage of the whole antioxidant capacity for those species [9].

The demand for raw material of natural origin for the production of food supplements, nutraceuticals and cosmetic products is growing. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are of increasing interest in the food industry, because they hinder oxidative degradation of lipids and thereby improve food quality and nutritional value [9]. Among the various medicinal and culinary herbs, the species *Cyphostemma digitatum* is of particular interest because there is a high demand on this species in central Yemen. Usually dry food discs (8–12 cm in diameter with irregular 1–5 mm thickness) are commercially produced which are regularly used to prepare many traditional dishes or as a source for food flavouring. However, fresh leaves are highly toxic and corrosive to the mouth and palate mucosa. Interestingly, no herbivore was found feeding on this plant in more than 1,500 sites, which were visited to evaluate the ecological situation of the species in central Yemen (to be published elsewhere).

Herbal medicine represents one of the most important fields of traditional medicine in Yemen. Thus, phytotherapy is practiced by a large proportion of the population for the treatment of several physical, physiological, mental and social ailments. According to information from old farmers and traditional healers, *C. digitatum* has been also used in traditional medicine for vomiting, against malaria and headache and for general health support, which led to intensive gathering of this plant from nature and for replantation in gardens and field margins. This bad ecological situation caused us to start a comprehensive investigation and standardization of this species.

Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. In this paper, four common methods used widely all over the world, namely Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) were applied on samples of *C. digitatum*. Measured antioxidant capacities of biological samples depend on the type of free radical or oxidant used in the assay. However, recently the ORAC method adopted the $ROO^{\bullet}$ as standard radical since it is the most common in biological systems [10]. Meanwhile new methodology adopted a new fluorescent substance (fluorescein) to replace the target protein B-phycoerythrin (B-PE) that was used until that time as a probe in the ORAC assay [11]. Data on the modified method normally gave values that are two to three times higher than with B-PE. From the huge variety of aromatic, medicinal or spicy plants, only a few have found a niche for application as antioxidants in food products, hence, information about plants that are less widely used in food or as medicines is scarce. For this vast source of potentially useful plants to be available, the assessment of their properties is a necessary first task. Therefore, this paper is aimed to establish a suitable rapid method for estimating the antioxidant capacity of *C. digitatum* before and after processing and also to estimate the total phenolic content by Folin–Ciocalteu method and its possible contribution to this capacity in water and ethanol extracts.

Materials and methods

Sample preparation

Fresh leaves were harvested from nature in August 2006 in Baddan countryside in the southwest highlands in central Yemen. This area was selected for sample gathering because it is away from any substantial human impact. A voucher sample was given to the Agricultural Research Centre in Taiz, Yemen. Part of the clean leaves was boiled for 30 min under pressure, then the water was removed and the leave mass was mixed with wood spoon. The thick homogeneous stature baste was thinned into disks (8–12 cm in diameter) and dried in the sun in clean plates covered with tiny mesh, changing upside down each day until complete dryness; this was called the "processed" sample. Other parts of the freshly cut leaves were dried in an oven at 40 °C, and this sample was called "raw material". Both processed sample and raw material were used for the tests as dry materials. Heat treatment was used to reduce the content of antinutritional substances and to diminish their effects [12]. Both samples were packed in plastic bags and stored at ambient temperature (<30 °C) for 3–5 months before use.

Sample dry mass (DM) determination

Approximately 30 g sea sand were given in a cup with a glass rod and put for 1 h into an oven at 103 °C. After cooling off in the desiccator the sand was weighted (weight empty), then 2 g sample (original weight) were added to the sea sand and put for 4 h at 103 °C. After 2 h the sample was mixed again by using the glass rod, cooled in the desiccator and finally weighed out. The sample was returned for further 1 h in the oven, cooled and weighed

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again until it was completely dried, i.e. no more mass loss was registered (net weight). DM was calculated from the following equation:

$$\text{dry mass (DM) (g/100 g)} = \frac{\text{net weight (g)} - \text{weight empty (g)}}{\text{original weight (g)}} \times 100. \quad (1)$$

Extraction procedures

Both raw material and processed samples of *C. digitatum* were milled with a cyclotec mill (UDY Corp., Fort Collins, CO) (1 mm mesh) prior to extraction and six aliquots were taken from each sample. Three aliquots were extracted with 70% ethanol and three aliquots with HPLC water. Each aliquot was dissolved in 5 ml of the corresponding solvent with vortex for 1 min, and then the solutions were centrifuged at 5,000 rpm for 5 min. This was done for each aliquot three times. Each time the supernatant was collected and the volume was completed to 20 ml by the corresponding solvent. Then 1 ml was taken from each sample solution into a 1.5 ml reaction tube and centrifuged at 10,000 rpm for 5 min. From each of the twelve samples five dilutions were obtained (1:1, 1:5, 1:10, 1:25, 1:50) to be scanned for the suitable concentration in order to be detected within the standard curve of different tests applied.

Reagents

The following reagents were used for the assessment of total phenolic content and antioxidant capacity: DPPH, Folin–Ciocalteu reagent, TPTZ, $K_2S_2O_8$, $FeSO_4$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2′-azobis(2-amidinopropan) hydrochloride (AAPH), fluorescein, HCl were from Sigma (Taufkirchen, Germany); ethanol, methanol, Na_2CO_3, $CH_3COONa{\cdot}H_2O$, KH_2PO_4, $NaH_2PO_4{\cdot}2H_2O$, $Na_2HPO_4{\cdot}2H_2O$, NaCl, NaOH were from VWR (Darmstadt, Germany), $FeCl_3{\cdot}6H_2O$ was from Roth (Karlsruhe, Germany). For calibration gallic acid·H_2O and Trolox were from Sigma (Taufkirchen, Germany). HPLC grade water (18 MΩ) was prepared using a Millipore Milli-Q purification system.

Analytical procedure

Total phenolic content, TEAC, DPPH, FRAP and ORAC were measured by using 96-well micro plates (Sarstedt, Nürnbrecht, Germany) and BMG FLUOstar Optima micro plate reader (BMG Labtech, Offenburg, Germany). Gallic acid standard solutions were used for calibration of total phenolics determination. Trolox, a water-soluble analogue of vitamin E, was used as standard for antioxidant capacity determinations.

Antioxidant capacity assays

Total phenolics determination

Total phenolic content of both aqueous and ethanol extracts were quantified using the Folin–Ciocalteu method [13]. For the preparation of the calibration curve the following dilutions of gallic acid standard solutions were prepared by dilution with HPLC water: 2.100, 4.200, 6.000, 8.077 and 10.500 mg/100 ml. Upon the initial screening 1:5 dilution was used for the raw material, while the processed *C. digitatum* was used undiluted. 20 µl of each sample, each standard solution and blank (HPLC water) were pipetted to the corresponding well of the 96-well plate. To each well 100 µl Folin–Ciocalteu reagent (diluted tenfold) and 75 µl of sodium carbonate solution were added. The absorbance of blue solutions was read after 2 h incubation in dark at 740 nm, the calibration curve was constructed and the content of total phenolic compounds in plant extracts was calculated as gallic acid equivalents (GAE).

TEAC assay

The TEAC method is based on the performing of $ABTS^{\bullet+}$ by mixing 6.62 mg $K_2S_2O_8$ in 10 ml HPLC water with 38.4 mg ABTS in 10 ml HPLC water. After 24 h at room temperature in the dark $ABTS^{\bullet+}$ radical will be at optimum concentration in the reaction mixture [14]. The $ABTS^{\bullet+}$ solution was then diluted with pH 7.4 phosphate buffer solution to obtain an initial absorbance of 0.70 ± 0.10 at 730 nm. For the preparation of the calibration curve from fresh 2.5 mmol/L Trolox standard solutions the following dilutions were prepared with phosphate buffer: 0.0125, 0.050, 0.100, 0.150, 0.200 mmol/L.

Sample dilution 1:5 was used for the processed samples and 1:25 dilution was used for the raw material. 20 µL of each sample, Trolox standard solution and HPLC water as blank were mixed with 200 µL fresh $ABTS^{\bullet+}$ solution. Total volume for each reaction mixture in each well was 220 µL. $ABTS^{\bullet+}$ emission reduction (y) was measured at 730 nm and plotted against the Trolox concentrations (x). A linear regression ($R^2 = 0.998$) was used to calculate the antioxidant capacity of the samples.

FRAP assay

The FRAP assay measures the ferric reducing antioxidant power. At low pH, when a ferric (Fe^{3+}) TPTZ complex is reduced to the ferrous (Fe^{2+}) form, an intense blue colour with an absorption maximum at 595 nm developed [15]. For the preparation of the calibration curve from fresh 5 mmol/L $FeSO_4$ standard solutions the following dilutions were prepared by dilution with HPLC water: 0.25, 0.50,

1.00, 1.43, 2.00, 2.5 mmol/L. Samples were used undiluted. 10 µL from water or ethanol extracts of the samples, standard solutions and blank (HPLC water) were mixed with 200 µL of the FRAP reagent, which consisted of 10 volumes of 300 mmol/L acetate buffer (pH 3.6), 10 volumes of 20 mmol/L $FeCl_3$ and one volume of 10 mmol/L TPTZ in 40 mmol/L HCl. The resulting FRAP value was estimated as mmol Fe^{2+}/100 g sample; the total volume for each reaction mixture in each well was 210 µL, FRAP antioxidant emission was measured at 595 nm. The absorbance values (y) were plotted against the Fe^{2+} concentrations (x). A linear regression with $R^2 = 0.996$ was used to calculate the antioxidant capacity of the samples.

DPPH radical scavenging assay

Radical scavenging capacity of plant extracts against the stable DPPH radical was determined by spectrophotometry according to the method of [16]. For the preparation of the calibration curve from fresh 2.5 mmol/L Trolox standard solutions the following dilutions were prepared with HPLC water: 25, 50, 100, 200, 250 µM. 100 µL of standard or undiluted sample extracts were mixed with 100 µL of the DPPH solution, 200 µL methanol was used as control and 100 µL methanol with 100 µL DPPH was used as blank. The total volume for each reaction mixture in each well was 200 µL, the colorimetric change (from deep violet to light yellow), when DPPH was reduced, was measured at 510 nm. Radical scavenging capacity was calculated by using the formula

$$\%\mathrm{DPPH}^{\bullet}_{\mathrm{remaining}} = \frac{A_{\mathrm{sample}} - A_{\mathrm{control}}}{A_{\mathrm{blank}} - A_{\mathrm{control}}} \times 100, \quad (2)$$

where A_{sample}, A_{blank} and A_{control} stand for the absorbance of sample, blank and control. To estimate the total $\mathrm{DPPH}^{\bullet}$ scavenging capacity of a selected antioxidant sample at 510 nm and the reaction time t, the % $\mathrm{DPPH}^{\bullet}$ quenched was determined according to the following equation:

$$\%\mathrm{DPPH}^{\bullet}_{\mathrm{quenched}} = \left(1 - \frac{A_{\mathrm{sample}} - A_{\mathrm{control}}}{A_{\mathrm{blank}} - A_{\mathrm{control}}}\right) \times 100. \quad (3)$$

The values of % $\mathrm{DPPH}^{\bullet}$ quenched (y) obtained from Eq. 3 were plotted against the Trolox standard concentration (x). A linear regression ($R^2 = 0.997$) was used to calculate the radical scavenging capacity of the samples (expressed as mmol Trolox equivalents/100 g sample).

ORAC assay

Oxygen radical absorbance capacity (ORAC) assay was performed as described in detail in [11]. Analyses were conducted in physiological pH (phosphate buffer pH 7.4) at 37 °C. Peroxyl radical was generated using AAPH and fluorescein was used as fluorescent probe. Fluorescence conditions were as follows: excitation at 490 nm, emission at 520 nm, 1:5 dilutions were used for the processed sample, 1:10 dilution for the ethanol extract from the raw material and 1:25 for the water extract from the raw material. For the preparation of the calibration curve from fresh 2.5 mmol/L Trolox standard solutions the following dilutions were prepared by dilution with HPLC water: 0.1, 0.5, 1.0, 1.5, 2.0 mmol/L. 10 µL of sample, Trolox standard or blank (HPLC water) were added to the wells, then 25 µL fluorescein working solution (1.2 µmol/L) and 100 µL phosphate buffer were added to each well. The reaction mixture was left for 10 min, then 150 µL AAPH solution (129 mM) was added to the reaction mixture. To control the photostability of fluorescein, 25 µL fluorescein, 10 µL HPLC water and 250 µL phosphate buffer were mixed and measured under same conditions.

Samples and Trolox calibration solutions as well as blank and control were always analysed in four trails in a "forward" order as follows: blank, control, 0.1, 0.5, 1.0, 1.5, 2.0 mmol/L Trolox, sample extracts 1–4 as triplicate. The control was necessary to get the relative fluorescence for samples, standard and blank.

The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescein decay curve ($R^2 = 0.995$) and were expressed as Trolox equivalents with mmol/100 g. The normalised area under curve (AUC) was calculated as

$$\mathrm{AUC} = (f_0 + f_1 + f_2 + \cdots + f_n)/f_0, \quad (4)$$

where f_0 is the initial fluorescence reading at 0 min and f_k is the fluorescence reading at time k and n is the total numer of time steps. The $\mathrm{AUC}_{\mathrm{net}}$ was obtained by subtracting the AUC of the blank from that of the sample or standard:

$$\mathrm{AUC}_{\mathrm{net}} = \mathrm{AUC}_{\mathrm{sample/standard}} - \mathrm{AUC}_{\mathrm{blank}}. \quad (5)$$

Statistical analysis

All five performed tests were prepared and measured in triplicates for the processed sample water extract, i.e. the consumed form (prWat), the processed sample ethanol extract (prEth), the raw material water extract (rWat), and the raw material ethanol extract (rEth). Moreover, accuracy and precision were ensured in the pipetting step in which four wells from each sample, from the blank, from the standard and from the negative control, if any, were done. The results were averaged, standard deviation and coefficient of variation were calculated for each sample. Correlation coefficients (R) and linear regression equations were calculated to determine the relationship between different variables (for antioxidant capacity assays and content of total phenolics). The significance of differences

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among treatment means was tested by one-way analysis of variance (ANOVA) at the $P < 0.05$ level.

Results and discussion

The overall results for the five tests are shown in Table 1.

Total phenolics content and antioxidant capacity of *C. digitatum* in water and ethanol extract before and after processing

Total phenolics content of *C. digitatum* was significantly higher ($P < 0.05$) in the raw material than in the processed sample (see Table 1), but also significantly higher in the ethanol extracts compared to the water extracts in both raw material and processed sample. This could be attributed to the consequences of processing on the phenolic compounds and the solubility criteria of these classes of compounds respectively.

Besides polyphenols, the Folin–Ciocalteu method may also determine other reducing compounds as e.g. ascorbic acid. However, ascorbic acid also reacts within the tests to determine the antioxidant capacity. Thus, the correlations between the methods are not directly affected. Reducing sugars only interfere in the Folin method if they are present in high concentrations [17]. In addition, the Folin–Ciocalteu reagent also reacts with some nitrogen compounds as amino acids (e.g. tyrosine, tryptophan) and amines [18], [19]. However, the Folin–Ciocalteu assay is used by several authors as one possible sum parameter to characterise the antioxidant potential of plant materials.

The correlation coefficient between FRAP and TEAC was relatively high ($R = 0.82$); moreover the antioxidant capacity measured by TEAC and FRAP methods (cf. Table 1) were in accordance with the elucidated phenol contents. Antioxidant capacity was significantly higher before processing. The ethanol extract had significantly higher ($P < 0.05$) antioxidant capacity than the water extract, except the raw material ethanol extract in FRAP assay, where there were no differences in antioxidant capacity between the ethanol extract and the water extract.

Contrarily, the antioxidant capacity of *C. digitatum* measured by DPPH method was significantly higher ($P < 0.05$) after processing (see Table 1).

The correlation coefficient between DPPH and ORAC was relatively high ($R = 0.75$). In accordance with DPPH assay, ORAC assay also confirmed a significantly higher ($P < 0.05$) antioxidant capacity in the processed sample water extract (i.e. the consumed form) compared to the raw material water extract. This result strongly suggests that there could be other important classes of phytochemicals of high antioxidant capacity generated by the household processing. ORAC and DPPH assays confirmed the previous approach by the other three assays, in which antioxidant capacity was significantly higher ($P < 0.05$) in the ethanol extract than in the water extract in both samples (Table 1).

Correlations between total phenolics content and antioxidant capacity

As a promising healthy food source *C. digitatum* contained a reasonably high total phenolics content of 1.41 g/100 g in the raw and 0.80 g/100 g in the processed water extracts, respectively, compared to 0.49 and 0.45 g/100 g in the water extract of asparagus and broccoli measured by the same method (Folin–Ciocalteu method) [20]. Despite this good result the total phenolics of the *C. digitatum* samples did not correlate with their antioxidant capacity measured by all four methods used.

Correlations are expressed through the following correlations coefficients: total phenolics content versus DPPH, $R = 0.11$; total phenolics content versus ORAC, $R = 0.66$; total phenolics versus TEAC, $R = 0.59$; total phenolics versus FRAP, $R = 0.64$. This confirms that the phenolic

Table 1 Results of the five tests applied to the four samples

Test/sample	Total phenolics GAE (g/100 g)	TEAC TE (mmol/100 g)	FRAP TE (mmol/100 g)	DPPH TE (mmol/100 g)	ORAC TE (mmol/100 g)
rWat	1.41 ± 0.06a	10.75 ± 0.73a	30.36 ± 1.57a	1.02 ± 0.08a	16.65 ± 0.15a
rEth	1.95 ± 0.03b	8.57 ± 0.65b	30.10 ± 1.39a	1.63 ± 0.06b	103.27 ± 2.54b
prWat	0.80 ± 0.08c	5.38 ± 0.45c	12.18 ± 0.91b	1.64 ± 0.03b	41.66 ± 2.71c
prEth	1.56 ± 0.12d	7.86 ± 0.46d	13.60 ± 0.75b	2.18 ± 0.08c	91.91 ± 2.99d

The total phenolics content was quantified in gallic acid equivalents (GAE) (g/100 g), all other tests were quantified in Trolox equivalents (TE) (mmol/100 g)

Values in the table are means of three replicates (four measurements each) ± standard deviation ($n = 12$). Different letters denote pairwise significant differences (ANOVA, $P < 0.05$) within each column

rWat raw material water extract, *rEth* raw material ethanol extract, *prWat* processed sample water extract, *prEth* processed sample ethanol extract

compounds are only weakly responsible for the antioxidant capacity of the raw and processed samples of *C. digitatum*. This remarkable capacity could also be attributed to other constituents. Antioxidant capacities of *C. digitatum* water extracts were 1.02 and 1.64 mmol/100 g of the raw material and processed samples respectively, compared to 1.09 and 0.48 mmol/100 g in the water extract of asparagus and broccoli measured by the same method (DPPH) [20].

The antioxidant capacities measured by TEAC for the raw material and processed *C. digitatum* water extracts were higher (10.75 and 5.38 mmol/100 g respectively) compared to only 2.62 and 2.51 mmol/100 g in the water extract of asparagus and broccoli [20]. FRAP and ORAC assay confirmed these results, the high antioxidant capacity even more (see Table 1), compared to the values of 18.5 mmol/100 g by FRAP and 9.7 mmol/100 g by ORAC in the water:acetone extract 1:1 of red pepper [21]. This capacity was even much higher than in white onion, snap bean, tomato, white cabbage, carrot and pea investigated in the same study by both ORAC and FRAP methods. Blueberries, which were considered as an excellent source of antioxidants, had ORAC values of 90–870 mg TE/g, estimated on DM basis [5]. Hence, there is a potential to easily obtain high antioxidant *C. digitatum* fractions that may be used in food and other applications, taking in consideration the high yield and the easy cultivation, processing and preservation of *C. digitatum*.

Processing effect on antioxidant capacity and total phenolics of *C. digitatum*

The variability in the antioxidant capacity between methods is very high, as it was anticipated. The antioxidant values were examined for prominent standard antioxidants including gallic acid, Trolox and urea by using six methods, but different results were obtained for the same pure antioxidant when using different methods [22]. The different mechanisms of action for each assay and the variety of the compounds that exert antioxidant capacity may also contribute to this variability. For example, there was a remarkable antioxidant capacity for fractionated tea phenols, tea proteins and tea polysaccharides; however, the ranking of this three fractions according to their antioxidant capacities was completely different between the two methods used, ORAC and DPPH [23].

Due to the circumstances mentioned above it was necessary to get a trend in the data to facilitate the comparison of antioxidant capacity between different methods, therefore, the data from each method were transformed to a relative antioxidant capacity index (RACI) by assuming the maximum value in each method as 100% and the others expressed as percentage of the maximum value (Table 2).

Table 2 Relative antioxidant capacity index (RACI) of the samples assuming the maximum value in each method as 100% and the others expressed as percentage of the maximum value

RACI	Total phenolics	TEAC	FRAP	DPPH	ORAC
rWat	72.3	100.0	100.0	46.8	16.1
rEth	100.0	79.7	99.1	74.8	100.0
prWat	41.0	50.1	40.1	75.2	40.3
prEth	80.0	73.1	44.8	100.0	89.0

rWat raw material water extract, *rEth* raw material ethanol extract, *prWat* processed sample water extract, *prEth* processed sample ethanol extract

Percentages of capacity retention in ethanol and water extract after processing were also calculated for the five test methods (Table 3). When *C. digitatum* was processed, reasonable amounts of the antioxidant capacities measured by different methods and total phenolic contents of the raw *C. digitatum* were retained in both water and ethanol extract (cf. Table 3). Several household food-processings methods including thermal processing can be used to enhance the bioavailability of micronutrients in plant-based diets, which aim to increase the physicochemical accessibility of micronutrients and decrease the content of antinutrients [24]. This implies that *C. digitatum* can be processed into foods that are functional by just the simple traditional way, a result being in accordance with previously conducted research on sorghum and its products [25].

The remarkably high retention and regeneration of the antioxidant active compounds measured by DPPH and ORAC systems in both water and ethanol extracts and by TEAC and total phenolics in the ethanol extract could be attributed both to the processing and the subsequent deterioration of some compounds (to be more potent antioxidant) and to the sensitivity of this methods to the new compounds resulting from processing. Hence, particular antioxidant molecules could be more efficient at quenching certain radicals than others [26]. Since FRAP assay does not measure SH-group containing antioxidants and is less relevant to chain-breaking antioxidants activity [27], the lower retention of the antioxidant by FRAP assay after processing in both samples compared to the other methods could give us a hint about the nature of the generated antioxidant. More analysis in this area has to be done to determine how different alternative processing conditions affect the phytochemical components in *C. digitatum* fractions.

Table 3 Percentages of antioxidant capacity retention in ethanol and water extract after processing calculated for the five test methods

Capacity retention (%)	Total phenolics	TEAC	FRAP	DPPH	ORAC
Water extract	56.7	50.0	40.1	160.8	250.2
Ethanol extract	80.0	91.7	45.2	133.7	89.0

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Epidemiological studies have consistently shown that a regular consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases such as cancer and cardiovascular disease by exhibiting strong antioxidant and antiproliferative activities. The individual antioxidants studies do not appear to have consistent preventive effects, since the major part of total antioxidant capacity comes from the combination of phytochemicals action commonly known as the synergistic effects of phytochemicals in fruits and vegetables [28]. In this context due to the high level of phenolic compounds and antioxidant capacity of *C. digitatum*, this species seems to be an important new source of such functional food ingredients since this plant has been highly consumed regularly for generations without any noticeable side effects.

Comparison of ORAC with TEAC, DPPH and FRAP methods

Indeed, there are situations in which knowledge of the individual levels of specific antioxidant components might be more useful than the total antioxidant potency of the medium concerned. These assays give a wide range of results; they should never be used in isolation and be interpreted with caution. A true measure and a golden standard of total antioxidant capacity is not yet available [27].

The ORAC values (ethanolic + hydrophilic) were remarkably very high compared to TEAC or DPPH values (Table 1). However, because individual antioxidant molecules are more efficient at quenching certain radicals than others [26], the relative ranking in capacity of different samples across methods is more relevant than absolute values for comparing activities. Since *C. digitatum* showed highly variable antioxidant capacities in all methods, a logarithmic scale was chosen for better comparison and explanation of the results (Fig. 1).

This result is in accordance with a comprehensive study comparing the ORAC to the FRAP of different vegetables [21]. No correlation was observed between the two methods among most of the tested vegetables, concluding that ORAC was a better indicator of antioxidant capacity of vegetables than FRAP based on the reaction mechanisms involved. Moreover, the low pH (3.6) used for the FRAP assay versus the ORAC assay (pH 7.4) as well as colour interference with vegetable extracts in the FRAP assay would seriously compromise antioxidant activities measured among different samples. ORAC assay demonstrated a very clear enhancement of the antioxidants content in the processed sample water extract (the consumed form) compared with the raw material water extract. Moreover this sample showed a steady increase in antioxidant capacity for the five tests in accordance with the individual

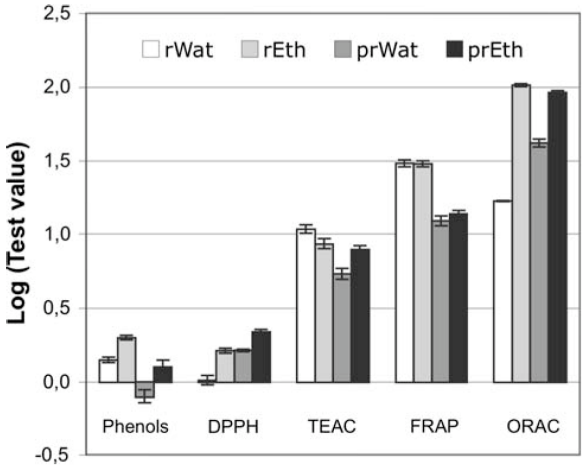

Fig. 1 Logarithm of test values, quantified in GAE (g/100 g) for total phenolics and in TE (mmol/100 g) for the four antioxidant tests. The tests are arranged according to increasing antioxidant capacity, where the processed samples (*prWat*, *prEth*) show a consistent pattern through all tests

test capacity. The processed sample ethanol extract confirmed this tendency, too (see Fig. 1).

In this study, fluorescein was used as a probe for the ORAC assay as recommended in previous research [11]. Thus, some of the problems for the fluorescent protein B-PE were eliminated, which was reported to give poor reliability due to significant variability, poor repeatability, lack of photostability and interaction with polyphenols due to nonspecific protein binding. Although the TEAC assays were conducted at pH levels comparable to that for the ORAC assay, hence reducing the pH effect, TEAC absorbance was measured at 730 nm, a wavelength that may be far away from the absorbance bands of plant extracts [25].

The validity of TEAC and DPPH methods has been questioned as they use oxidants (free radicals) that are not necessarily prooxidants and are of unknown biological value, unlike ORAC, which uses oxidants that are actually prooxidants (e.g. $ROO^{\bullet}$, $OH^{\bullet}$) and are of pathological significance [9]. Among other factors a major advantage of ORAC is that the method can be automated and largely standardised, hence, values can be easily compared across laboratories. Also, the ORAC method is reported to mimic antioxidant capacity of phenols in biological systems better than other methods since it uses biologically relevant free radicals and integrates both time and degree of capacity of antioxidants [29, 9, 21].

Comparison of TEAC and DPPH antioxidant methods on *C. digitatum* extracts

The TEAC and DPPH methods showed no correlation with respect to both *C. digitatum* samples, while minimal

differences were reported between the TEAC and the DPPH values. The two methods were equally good at measuring antioxidant capacity of sorghum and sorghum products [25], although the TEAC was a better choice than DPPH. The TEAC method has the extra flexibility in that it can be used at different pH levels (unlike DPPH which is sensitive to acidic pH) and thus is useful when studying the effect of pH on antioxidant capacity of various compounds [25]. C. *digitatum* is highly acidic and rich in organic acids, among them vitamin C and acetic acid. Additionally, TEAC is soluble in aqueous and organic solvents and is thus useful in assessing antioxidant capacity of samples in different media and is currently most commonly used in simulated serum ionic potential solution (pH 7.4 phosphate buffer containing 150 mmol/L NaCl) (PBS). Another advantage of TEAC method was that samples reacted rapidly with ABTS in the aqueous buffer solution (PBS) reaching a steady state within 30 min while the $DPPH^{\bullet}$ reacted very slowly with the samples reaching steady state after 8 h [25]; similar slow reactions of most antioxidants with the DPPH were reported [14].

In summary, *C. digitatum* is the first member of the Vitaceae family investigated and showed a high antioxidant capacity compared to the intensive research in the members of other families, like Lamiaceae, Asteraceae, Fabaceae, Geraniaceae and Rosaceae. Although there is a wide research in various plants, food application examples of antioxidants from less known plants are very few. This is caused by the fact that complex and expensive research is needed in order to create a new, effective and available product. Another important question is, whether the term "natural product" automatically means "safe product". This is obviously not always the case; therefore the assessment of safety aspects is a necessity, which is still expensive. However, for *C. digitatum* most of the safety questions are answered, since it has been consumed daily as food and medicinal plant in some regions without any reported chronic or acute toxicity. *C. digitatum* could thus be an important source of ingredients for use in functional foods, food additives and other applications. Such information will be necessary, since *C. digitatum* might become a competitive source of phytonutrients, being quickly accessible, reliable and cost effective. Before new natural antioxidants can be utilised as food additives still many technological and related questions should be answered. We have to know which oxidation mechanisms are going to take place, whether antioxidants will be soluble in the used media, whether they will be stable, whether there is any impact on the product flavour or colour and what is the proper concentration needed. These and a number of related questions are important for food producers. For the moment, it is of great interest to investigate the antioxidant contributions of fractioned polyphenols, polysaccharides and proteins obtained from *C. digitatum*, also to estimate possible functional food ingredients other than phenolic compounds being responsible for the remarkable antioxidant capacity in *C. digitatum* like carotenoids and vitamins.

There is a need to standardise the antioxidant measuring methods to allow for data comparison across laboratories. The ORAC method is generally regarded of high standard due to its use of biologically relevant free radicals and due to its integration of both degree and time of inhibition. It also offers advantage in terms of predicting the overall antioxidant capacity, when compared with the more common TEAC and DPPH methods. However, it is difficult to predict "actual overall antioxidant value" of samples based on any single in vitro assay. Additionally, antioxidant capacity alone does not explain the potential effects of compounds in vivo since other properties such as modification of enzyme capacity or cell signalling pathways can be involved.

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CHAPTER 3: Contents of Vitamin C, Carotenoids, Tocopherols and Tocotrienols in the Subtropical Plant Species *Cyphostemma digitatum* as Affected by Processing:

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Contents of Vitamin C, Carotenoids, Tocopherols, and Tocotrienols in the Subtropical Plant Species *Cyphostemma digitatum* as Affected by Processing

MOHAMMED AL-DUAIS,*,† JULIANE HOHBEIN,‡ SUSANNE WERNER,‡ VOLKER BÖHM,‡ AND GOTTFRIED JETSCHKE†

†Institute of Ecology, Dornburger Strasse 159 and ‡Institute of Nutrition, Dornburger Strasse 25-29, Friedrich Schiller University Jena, 07743 Jena, Germany

The subtropical plant species *Cyphostemma digitatum*, Vitaceae, is used in central Yemen in traditional medicine, as a culinary herb, and as a source of food flavoring. The contents of vitamin C, vitamin E, and carotenoids and changes caused by common processing were investigated. Carotenoids were determined by reversed phase C30-high-performance liquid chromatography (HPLC) with diode array detection at 470 nm, while tocopherols and tocotrienols were analyzed by using normal phase HPLC with fluorescence detection (excitation, 292 nm; emission, 330 nm). Ascorbic acid was determined spectrophotometrically after reaction with DNP by measuring the absorbance at 520 nm. For the raw material and for the processed commercial food product, both in dried form, reasonable quantities of carotenoids were found in the raw material as follows: lutein, 18.89 ± 0.73 mg/100 g; zeaxanthin, 9.46 ± 0.30 mg/100 g; canthaxanthin, 0.21 ± 0.01 mg/100 g; β-cryptoxanthin, 0.67 ± 0.03 mg/100 g; and β-carotene, 14.60 ± 0.46 mg/100 g. Household processing reduced the carotenoid contents dramatically; only β-carotene sustained the processing. Likewise, vitamin C, 49.50 ± 0.01 mg/100 g in the raw material and 20.30 ± 0.02 mg/100 g in the processed material, was affected negatively by processing; only 41% was retained after processing. In contrast, the outstanding high content of vitamin E, 82.74 ± 0.63 mg/100 g in the raw material, was increased by processing to 101.20 ± 1.38 mg/100 g; it was found in different forms, some of which were rare in other sources.

KEYWORDS: ***Cyphostemma digitatum*; antioxidant activity; carotenoids; provitamin A; vitamin C; vitamin E; processing effect; Yemen**

INTRODUCTION

Cyphostemma digitatum (Vitaceae) is a perennial, climbing, succulent undershrub with compound fleshy leaves and tendrils. The leaves are petiolate, digitately 3−5 foliolate; leaflets are ovate and dentate. It flowers in pedunculate axillary cymes, and the fruits are one-seeded, red fleshy berries (**Figure 1**). *C. digitatum* usually occurs between 1400 and 2500 m a.s.l., often on cliffs and with preference for shaded stony places such as gullies and terraces walls. It is usually associated with *Acacia* spp., *Agava* spp., *Senecio hadiensis*, *Clematis* spp., and *Euphorbia* spp. (*1*).

The species is strongly declining in its natural habitats, because it becomes a commercial food product after processing. Therefore, it has disappeared completely from many regions in the southwestern highlands of Yemen because of intensive gathering. The leaves and fleshy young stem branches are used in dried form after processing. People started to cultivate it by removing the plant completely from its original sites and replanting it in gardens. The plant is used mainly as a food flavoring, but it is also a main constituent of traditional Yemeni soup (Marak). Besides that, it has been described to be used as a medication for gastroenteritis, fatigue, vomiting, and headache, against malaria, and for general health support.

In general, carotenoids are lipid-soluble pigments found in many vegetable crops that are reported to have health benefits against cancer and eye diseases when consumed in the diet (*2*). Structurally, carotenoids are a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). As carotenoids are responsible for the colors of many plants, fruits, and flowers, more than 700 carotenoids have been isolated from natural sources. They serve as light-harvesting complexes (with proteins) in photosynthesis (*3*). Functionally, some carotenoids are important in human nutrition as a source of vitamin A (e.g., β-carotene) and as a prevention agent for cancer and heart disease (e.g., lycopene) (*3*). A high intake of β-carotene also might decrease the risk of cancer in humans (*4*). In addition, carotenoids are the precursors of many important chemicals responsible for the flavor of foods and fragrance of flowers (*3*). Vitamin C, being a water-soluble antioxidant, may reductively regenerate oxidized vitamin E (*5*).

Vitamin E became important clinically to be determined; age-dependent reference intervals were constructed; in a cross-sectional survey, the influence of age, sex, and season of sampling on vitamin E plasma concentrations in 208 Swiss individuals aged

*To whom correspondence should be addressed. Tel: +49 3641 949403. Fax: +49 3641 949402. E-mail: b5almo2@uni-jena.de.

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Figure 1. *C. digitatum* mature plant before harvesting, showing the fleshy leaves and different flowering and fruiting stages.

from 0.4 years to 38.7 years was studied (*6*). Age was a significant predictor of plasma vitamin E concentrations; no sex-related differences were observed. The season of sampling affected α-tocopherol, γ-tocopherol, and cholesterol concentrations; they were higher in winter and spring than in the other seasons. The ratios of plasma α-tocopherol to cholesterol were not affected by age (*6*).

Vitamin C, vitamin E, and β-carotene all displayed antioxidant activity and thus provided a cellular defense against reactive oxygen species, which could damage the DNA (*7*). Serum β-carotene and vitamin E levels showed a strong protective association with lung cancer and suggestive protective associations with melanoma and bladder cancer (*8*). Additionally, cooperative interactions between vitamin E and vitamin C in protecting against lipid peroxidation in liposomes have been examined (*9*). Barclay et al. (*9*) briefly discussed the mechanism of this synergism between vitamin E and vitamin C. Simultaneous determination of vitamins C and E and carotenoids became a target for many clinical screening studies in different samples associated with many illnesses (*10*).

In a duration-dependent manner, vitamin E and β-carotene provide protection against erythema in humans and may also be useful for diminishing sensitivity to ultraviolet light. The supplementation with carotenoids or a combination of carotenoids and vitamin E for 12 weeks at dosages exceeding dietary intakes of these antioxidants increased the basal protection of skin against erythema (*11*). Nowadays, the effects of β-carotene and vitamins C and E sets of supplementation are a hot topic of research (*12*). Consequently, the simultaneous determination of this set of functional food ingredients was done for many food products (*5*, *13*). The chloroplasts contain carotenoids (e.g., β-carotene), vitamin E (tocopherols), and vitamin C (L-ascorbic acid), which cooperatively act as antioxidants. This led to a study of biomass production by the aerial microalga *Trentepohlia aurea* in liquid culture for the simultaneous production of β-carotene, vitamin E, and vitamin C under various conditions (*5*).

Multichannel, flash kinetic spectroscopy with microsecond time resolution has been used for investigation of the photochemical and photophysical behavior of vitamin E forms and its interaction with carotenoids (*14*). Although β-carotene, vitamin E, and vitamin C are antioxidants, it has been suggested that they may also serve as pro-oxidants in certain situations. They may promote oxidation via their action upon certain molecules, such as cupric (Cu^{2+}) and ferric (Fe^{3+}) ions, by the failure to regenerate after serving in their role as antioxidants (*12*).

It is well-known that vitamin A deficiency (VAD), xerophthalmia, and age-related macular degeneration (AMD) are primarily due to an inadequacy of provitamin A and macular pigments in the diet. This is the reason why VAD and AMD are well-known as serious public health problems among children and adults in Yemen and many developing countries (*15*). Consequently, much research has been concentrated on the identification of carotenoids and provitamin A in common and in less familiar green leafy vegetables (GLVs) and on its way of processing in different cultures to illustrate its valuable importance for human health and nutrition among them. It is known that vitamin C, vitamin E, and β-carotene were more decreased the higher the temperature and pressure were in the processing of hot and sweet pepper (*13*). The bioavailability of carotenoids seems to be affected by postharvest and processing activities; relevant studies emphasized the importance of carotenoids enhancement in vegetable crops and the need to characterize potential changes in carotenoids composition during cultivation, storage, and processing before consumer purchase (*2*).

The additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for these potent antioxidant and anticancer activities, and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (*16*). In our previous paper, the remarkably high antioxidant capacity of *C. digitatum* was quantified by four different methods in the context of standardization of food products derived from this species (*17*). Therefore, this paper is aimed to investigate the contents of vitamin C, vitamin E, and carotenoids in *C. digitatum* as a suspected cause for the above-mentioned antioxidant capacity together with the possible influence of household processing conditions.

MATERIALS AND METHODS

Chemicals. All chemicals for extraction were analytical grade, and solvents for the chromatography were high-performance liquid chromatography (HPLC) quality. The carotenoid standards (*all-E*)-lutein, (*all-E*)-zeaxanthin, (*all-E*)-canthaxanthin, (*all-E*)-β-cryptoxanthin, (*all-E*)-β-carotene, (9*Z*)-β-carotene, (13*Z*)-β-carotene, and (*all-E*)-echinenone as an internal standard were purchased from CaroteNature (Lupsingen, Switzerland) and were used for identification and quantification. They were dissolved in cyclohexane/toluene (4+1, v/v) and stored in the dark at −30 °C. The concentration of stock solutions was calculated periodically using their absorption maxima and appropriate extinction coefficients. For preparing working solutions, stock solutions were diluted daily 1:50 with methanol (MeOH)/tetrahydrofuran (THF) (1+1; v/v) containing 0.1% 2, 6-di-*tert*-butyl-4-methylphenol (BHT). A mixed carotenoid standard solution was also prepared to check the peak separation. Magnesium hydroxide carbonate, sodium sulfate anhydrous, and *tert*-butyl methyl ether (TBME) were also used in carotenoids analysis.

The following reagents were used for the assessment of vitamin E: *n*-hexane/TBME (98+2, v/m), ethanol, TBME, petroleum ether, and internal standard: α-tocopherol acetate (Sigma-Aldrich, Taufkirchen, Germany), α-, β-, γ-, and δ-tocopherols (Calbiochem, Darmstadt, Germany), and α-, β-, γ-, and δ-tocotrienols (Davos Life Science pte Ltd., Singapore). Tocopherol and tocotrienol stock solutions in ethanol containing approximately 1 mg/mL were diluted daily 1:100−1:5000 with *n*-hexane/TBME (98+2, v/m) to prepare the working solutions; the internal standard was diluted 1:100. The working solutions of tocopherols and tocotrienols were used for identification and quantification. A mixture of different forms of tocopherols and tocotrienols was also prepared to check the peak separation.

DNP reagent (a mix of 2,4-dinitrophenylhydrazine, thiocarbamide, and cupric sulfate), HPLC water, metaphosphoric acid, sulfuric acid, and trichloroacetic acid were used for vitamin C assessment. From 1 mg/mL ascorbic acid in trichloroacetic acid stock solution, the calibration

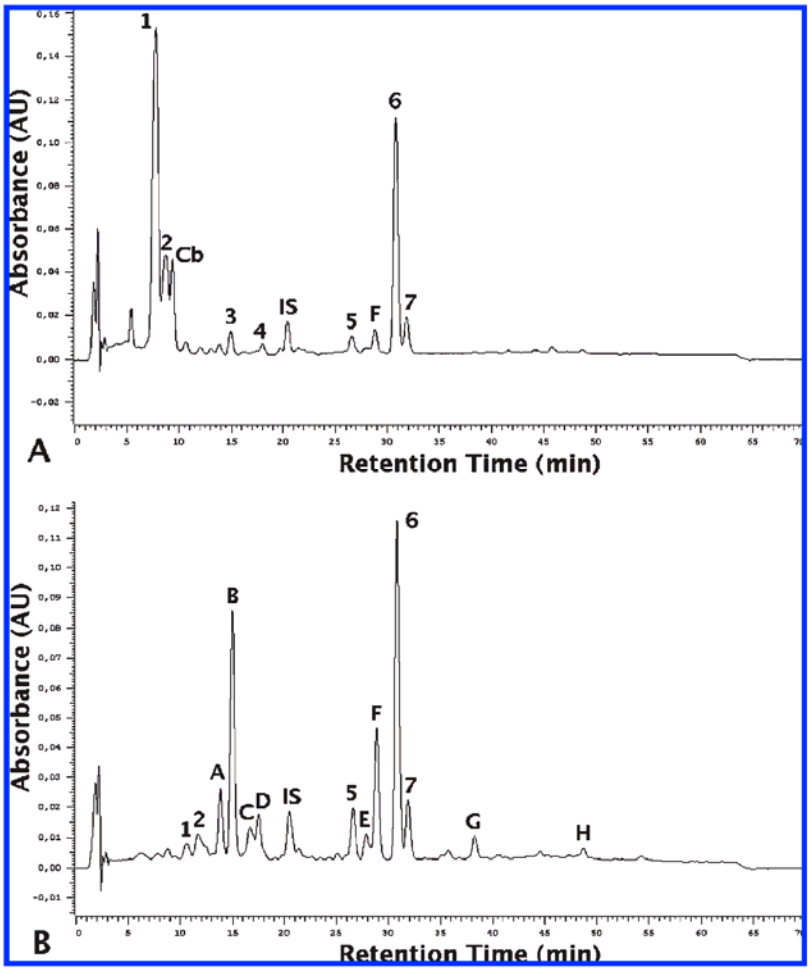

Figure 2. Chromatograms of the carotenoid extracts from *C. digitatum*: (**A**) raw material and (**B**) processed sample. IS and Cb denote internal standard and chlorophyll b, and A−H denote unknown peaks, which are still under investigation. Chromatographic conditions are described in the Materials and Methods, and peak identification (1−7) and characterization are given in **Table 1**.

solutions were done by diluting certain quantities of the stock solution in metaphosphoric acid.

Sample Preparation. Fresh leaves of *C. digitatum* were harvested from nature in August 2006 in the Baddan countryside in the southwestern highlands in central Yemen. This area was selected for sample gathering because it is away from any substantial human impact. A voucher sample was given to the Agricultural Research Centre in Taiz, Yemen. Part of the clean leaves was boiled for 30 min under pressure; then, the water was removed, and the leaf mass was mixed with a wooden spoon. The thick homogeneous stature baste was thinned into disks (8−12 cm in diameter) and dried in the sun in clean plates covered with tiny mesh, turning them upside down each day with 30 °C average temperature until complete dryness; this was called the "processed" sample. The other parts of the freshly cut leaves were dried in an oven at 40 °C for 90 min, and this sample was called "raw material". Both processed sample and raw material were used for the tests as dry materials. Both samples were packed in airtight dark containers and stored at ambient temperature (< 30 °C) for 3−5 months before use. Directly before preparation for estimation, both raw and processed samples were milled with a cyclotec mill (UDY Corp., Fort Collins, CO) (1 mm mesh).

Analysis of Vitamin C. According to the method of Roe and Oesterling (*20*), 1 g of each sample was dissolved in 5 mL of 4.5% *meta*-phosphoric acid, and the samples were shaken for 1 min and centrifuged (5000 rpm, 5 min). The aqueous phase was removed to a 20 mL flask. The extraction process was repeated twice, and the volume was completed to 20 mL by *meta*-phosphoric acid. Then, 1 mL of each sample was taken to an Eppendorf tube and centrifuged (12000 rpm, 5 min). Two hundred microliters from the supernatant of each sample, standard, and blank was taken, and 300 μL of trichloroacetic acid (5 g/100 mL) was added, mixed, and centrifuged again (12000 rpm, 5 min). Then, 300 μL of supernatant was taken, and 100 μL of DNP reagent was added. After they were shaken, all tubes were incubated in the thermal mixer at 60 ± 1 °C (800 rpm, 60 min). The tubes were removed to an ice bath for 5 min, 400 μL of sulfuric acid was added with shaking, and the tubes were left for 20 min in the dark before determining the absorbance at 520 nm. All analyses were done in triplicate.

Analysis of Vitamin E. An amount of 0.1 g of the sample was weighed into a centrifuge tube. Successively, 1 mL of distilled water, 40 μL of α-tocopherol acetat as an internal standard, 1 mL of ethanol, 1 mL of TBME, and 1 mL of petroleum ether were added. After each addition, the tubes were shaken for 30 s. Then, the samples were centrifuged (5000 rpm, 5 min), and the upper layer was transferred into a 50 mL pear-shaped flask. The extraction with 1 mL of TBME and 1 mL of petroleum ether was repeated until the solvent was colorless. The combined extracts were dried under reduced pressure at 30 ± 1 °C. The residue was dissolved in 2 mL of *n*-hexane/TBME (98 + 2, v/m) using an ultrasonic bath. Then, samples were centrifuged (14000 rpm, 5 min). The resulting solution was analyzed for vitamin E by using normal phase HPLC at 35 ± 1 °C with a Knauer Eurospher 100 DIOL-column (250 mm × 4.0 mm, 7 μm) and an appropriate guard column (5 mm × 4.0 mm, 7 μm) (Knauer, Berlin, Germany), with 1.5 mL/min of *n*-hexane/TBME (98 + 2, v/m) (**Figure 3**)

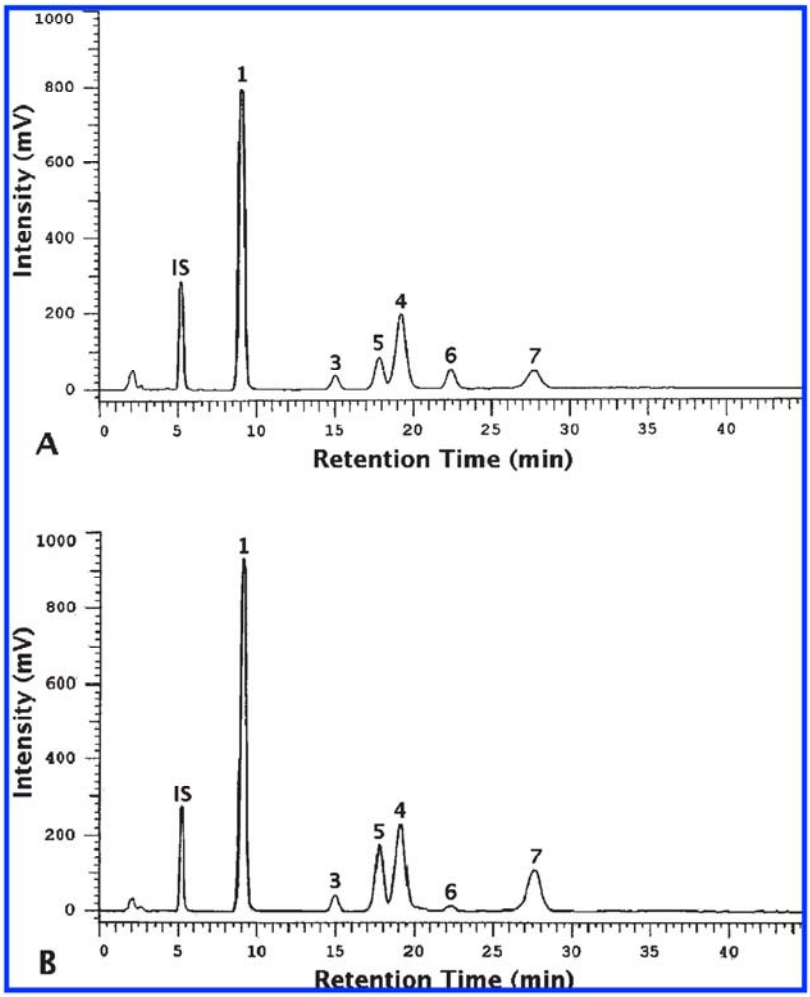

Figure 3. Chromatograms of vitamin E from *C. digitatum*: (**A**) raw material and (**B**) processed sample. Conditions are described in the Materials and Methods, and peak identification and characterization are given in **Table 2**.

with fluorescence detection (excitation, 292 nm; emission, 330 nm) (*19*). All analyses were done in triplicate.

Analysis of Carotenoids. The contents of carotenoids were analyzed by using C30-HPLC with diode array detection according to the method of Böhm (*18*). One milliliter of HPLC water was added to 1 g of the sample and mixed. After 5 min, 200 mg of magnesium hydroxide carbonate and sodium sulfate anhydrous, 200 μL of echinenone (i.e., internal standard), and 35 mL of MeOH/THF (1 + 1; v/v) containing 0.1% BHT were added. The mixture was homogenized on ice for 5 min using an Ultra Turrax (type T25, IKA-Werke, Staufen, Germany). After the residue was deposited, the solution was filtered through 390 paper (Filtrak, Niederschlag, Germany) on a Büchner funnel. The extraction was repeated at least three times until the solvent was colorless. The combined extracts were dried under reduced pressure at 30 ± 1 °C in a rotary evaporator.

The residue was redissolved in 10 mL of MeOH/THF (1 + 1, v/v) containing 0.1% BHT by using an ultrasonic bath. One milliliter of the solution was centrifuged (14000 rpm, 5 min) and then used for HPLC analysis, which was done with C30-HPLC column (250 mm × 4.6 mm, 5 μm) (Trentec, Gerlingen, Germany) and C30 guard column (10 mm × 4.6 mm, 5 μm) (Trentec) and 1.3 mL/min TBME/MeOH (gradient procedure) as the mobile phase at 17 ± 1 °C with diode array detection at 470 nm (**Figure 2**). All extractions were carried out under subdued light and were done three times for each sample.

Calculation of Vitamin A Activity. All calculations were performed on the dry mass of the raw material and the processed product; for details, see ref (*17*). Retention of each form of carotenoids, tocopherols, and tocotrienols after processing was calculated in % of that form in the raw material. The total carotenoids content was collected as mol/100 g for each form and converted to mg/100 g β-carotene equivalents for comparison purposes; also, total vitamin E was converted to mg/100 g α-tocopherol equivalents, since these two were the most abundant forms in both raw material and the processed sample (**Tables 1** and **2**). The vitamin A activities of the samples were calculated as mg retinol equivalents (RE) according to the FAO procedure (*21*). The activities in mg RE/100 g were calculated as one-sixth of β-carotene and 1/12 of other carotenoids with provitamin A activity

$$\text{mg RE}/100\text{ g} = \frac{\beta - \text{carotene}}{6} + \frac{\beta - \text{cryptoxanthin}}{12}$$

Statistical Analysis. Determinations were conducted in triplicate. Results were represented as means ± standard deviations (SDs). To ascertain differences between means, independent two sample *t* tests were done for each substance using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Methodological Considerations. Saponification was done in the beginning, but no improvement was found, which means that the samples contained no xanthophyll esters. This was anticipated since the searched material is GLV. Besides, some of the peaks were negatively affected, and small peaks became completely

Table 1. Carotenoids Composition and Vitamin A Value of Samples from *C. digitatum* before and after Processing[a]

		mg/100 g		
peak no.[b]	carotenoids	raw material[c]	processed sample[c]	retention in %
1	(*all-E*)-lutein*[f]	18.89 ± 0.73	0.19 ± 0.03	1.01
2	(*all-E*)-zeaxanthin*	9.46 ± 0.30	0.49 ± 0.01	5.18
3	(*all-E*)-canthaxanthin*	0.21 ± 0.01	ND	
4	(*all-E*)-β-cryptoxanthin*[d]	0.67 ± 0.03	ND	
5	(13*Z*)-β-carotene*[d]	1.07 ± 0.05	2.16 ± 0.18	201.87
6	(*all-E*)-β-carotene*[d]	11.38 ± 0.34	9.98 ± 0.31	87.70
7	(9*Z*)-β-carotene[d]	2.14 ± 0.07	2.24 ± 0.07	104.67
	total β-carotene[d]	14.60 ± 0.46	14.38 ± 0.46	98.56
	total carotenoids*[e]	42.20 ± 1.47	15.02 ± 0.60	35.60
	provitamin A (RE/100 g)	2.49	2.40	96.35

[a] The % of retention and provitamin A were calculated. ND, not detected. [b] Numbered according to **Figure 2**. [c] Mean ± SD ($n = 3$). [d] Denotes carotenoids with vitamin A activity. [e] Expressed as mg/100 g β-carotene equivalents; calculated from the sum of total carotenoids (mol/100 g). [f] Asterisks denote significant differences between raw and processed material ($P < 0.05$).

Table 2. Vitamin E and Vitamin C Content (mg/100 g) of *C. digitatum* before and after Processing; [a]

		mg/100 g		
peak no.[b]	vitamin	raw material[c]	processed sample[c]	retention in %
1	α-tocopherol*[e]	41.58 ± 0.41	49.57 ± 0.49	119.22
2	α-tocotrienol	ND	ND	
3	β-tocopherol*	3.00 ± 0.01	3.27 ± 0.06	109.03
4	β-tocotrienol*	22.93 ± 0.03	24.37 ± 0.37	106.31
5	γ-tocopherol*	6.16 + 0.06	12.33 + 0.18	200.23
6	γ-tocotrienol*	2.90 ± 0.02	0.68 ± 0.03	23.42
7	δ-tocopherol*	4.31 ± 0.11	8.36 ± 0.21	200.29
8	δ-tocotrienol	ND	ND	
	total vitamin E*[d]	82.74 ± 0.63	101.20 ± 1.38	122.31
	vitamin C[e]	49.50 ± 0.01	20.30 ± 0.02	41.01

[a] The % of retention was calculated. ND, not detected. [b] Numbered according to **Figure 3**. [c] Mean ± SD ($n = 3$). [d] Expressed as mg/100 g α-tocopherol equivalents; calculated from the sum of total vitamin E (mol/100 g). [e] Asterisks denote significant differences between raw and processed material ($P < 0.05$).

undetectable; hence, we decided to run the test without saponification. This is in accordance with previous research that emphasized that saponification can result in the destruction and/or structural transformation of some carotenoids (*22*). Therefore, HPLC methods that separate the various classes of carotenoids without saponification can be highly advantageous and provide valuable information on the identity and the levels of these compounds in their natural state in foods (*22*). All identified peaks (**Figures 2** and **3**) were determined by using standard reference materials.

In most cases, there is insufficient information on the history of the samples (i.e., cultivar, growing season, and location), the length of cooking, and the method of preparation of cooked foods (i.e., frying, steaming, boiling, baking, and microwave cooking) (*22*). Heat treatment was used to reduce the content of antinutritional substances and to diminish their effects (*23*). It is often difficult to compare analytical data about effects of cooking and processing on carotenoids levels in fruits and vegetables that have been reported in different studies. So, to determine changes in the phytonutrients content in *C. digitatum*, samples were analyzed in raw and cooked form. Estimations for *C. digitatum* are expressed in mg/100 g of dry mass, while for the rest (**Table 3**), values are shown in mg/100 g fresh matter. It is not

Table 3. Vitamin C, Vitamin E and Lutein Content of the Raw Material (rM) and the Processed Sample (pS) of *C. digitatum* as Compared with Other Well-Known Sources from German Food Composition Table (*24*), Quantified in mg/100 g

	vitamin C	vitamin E	lutein
C. digitatum (rM)[a]	49.50	82.74	18.9
C. digitatum (pS)[a]	20.30	101.20	0.19
asparagus	20.00	2.00	
blackberry	17.00	0.72	
blueberry	20.00	2.10	
broccoli	100.00	0.62	23.20
cabbage	50.00	1.70	
carrot	7.00	0.46	3.00
cauliflower	65.00	0.09	
corn salad	35.00	0.60	
garlic	14.00	0.01	
gooseberry	35.00	0.72	
grape	4.00	0.66	
green pepper	120.00	2.50	
kale	50.00	2.50	
leeks	25.00	0.53	
lettuce	13.00	0.60	11.30
onion	7.00	0.08	
pumpkin	12.00	1.10	9.20
raspberry	25.00	0.91	
red cabbage	55.00	1.70	
rhubarb	10.00	0.25	
rose hip	1250.00	4.20	
spinach	50.00	1.40	69.50
strawberry	65.00	0.12	
tomato	19.00	0.82	1.20

[a] For *C. digitatum*, estimations are expressed in mg/100 g of dry mass, while for all other substances, they are given in mg/100 g fresh matter (for an explanation, see the text).

meaningful to give results for *C. digitatum* per 100 g fresh matter, as it is used after redissolving in water in different concentrations according to the taste needs and person involved.

Vitamin C Content. The plant *C. digitatum* is a very promising and inexpensive source of vitamin C as raw material; hence, it could be used commercially for the extraction of ascorbic acid for culinary and pharmacological applications. We found 49.5 mg/100 g in the raw material and 20.3 mg/100 g in the processed material; this concentration could be compared to that in carrot, cauliflower, broccoli, corn salad, lettuce, leeks, Brussels sprouts, red cabbage, cabbage, rhubarb, asparagus, spinach, kale, onion, garlic, bumpkins, tomato, blackberry, strawberry, blueberry, raspberry, gooseberry, grape, kiwi, orange, lemon, and bananas (see **Table 3**) (*24*).

The vitamin C content of *C. digitatum* was decreased by the household processing; only 41% was retained after processing. However, this is rather high as compared to only 15% retention in all analyzed hot and sweet pepper cultivars, which are one of the important sources of vitamin C, in which the content in fresh fruits ranged from 101.2 to 167.5 mg/100 g of fresh weight (*13*). Because vitamin C is water-soluble, presumably reasonable amounts were discarded with the water during the processing of *C. digitatum* (see Sample Preparation).

Vitamin E Content. Many forms of vitamin E were found in both sample types of *C. digitatum*, but amounts were higher in the processed sample with remarkably very high extractability of especially γ-tocopherol and δ-tocopherol; only γ-tocotrienol was negatively affected by processing. In total, vitamin E was even enhanced by 22% through household processing (cf. **Table 2**). This high content of vitamin E in the processed sample could be attributed to two reasons: The processing may cause denaturation of proteins and a complete destruction of cell walls and cell

organelles and consequently result in liberation of vitamin E from the lipids, which then becomes more available for extraction. The other reason is that relatively thick and very dry discs were produced (as processed sample), which kept vitamin E and other functional food ingredients away from contact with oxygen and light, therefore, remaining intact, as compared with the thin pieces of dry leaves (as raw material). This is in accordance with the well-known stability of some forms of vitamin E like α-tocopherol, which significantly contribute to the stability of olive oil during potato frying (*25*).

C. digitatum proved to be an outstanding, very wealthy source of vitamin E: It contained 82.7 mg/100 g and 101.2 mg/100 g in the raw and the processed material, respectively; this concentration could be compared to green pepper, kale, blueberry, asparagus, cabbage, spinach, and many other fruits and vegetables (cf. **Table 3**) (*24*). Moreover, the vitamin E content of *C. digitatum* in both forms as a rich source of vitamin E could be compared with the well-known sources rich in vitamin E, as butter contains 2 mg/100 g, medium-fat margarine contains 6 mg/100 g, cocoa butter contains 1.1 mg/100 g, palm oil contains 9.5 mg/100 g, sesame oil contains 3.5 mg/100 g, and walnut oil contains 20.0 mg/100 g (*24*). Because the main sources of vitamin E are animal fat and plant oils, such extraordinary vitamin E-rich green leafy vegetables are important in food supplementation to avoid the high consumption of fats and oils. The high vitamin E dose of *C. digitatum* could be especially important as a food supplement for people with fats and oils malabsorption since they may suffer from vitamin E deficiency. Moreover, *C. digitatum* could be a proper resource for the extraction of pure vitamin E for medicinal applications and cosmetics and food industries.

C. digitatum also had a highly remarkable content of certain forms of vitamin E (cf. **Table 2**). For example, α-tocopherol reached 49.6 mg/100 g in the consumed form, which is rather high as compared to 61 mg/100 g in sunflower, recognized as one of the richest sources of this form and to 18 mg/100 g in canola oil and 12 mg/100 g in olive oil, respectively. According to the German Food Composition Table (*24*), *C. digitatum* seems to be the richest source in β- and γ-tocotrienol, which is very rare in nature as compared with the tocopherol counterpart class.

Carotenoids Content. The results showed that there is a high level of total carotenoids in *C. digitatum*: About 42 mg/100 g was determined in the raw material (dry sample), and 15 mg/100 g was still found in the processed form (dry sample) (see **Table 1**). These results are remarkable when compared to contents in vegetables, like 2.1 mg/100 g in beans, 8.2 mg/100 g in broccoli, 12.2 mg/100 g in chive, 2.2 mg/100 g in green bell pepper, 6.2 mg/100 g in lettuce (curly), 11.5 mg/100 g in lettuce, 23.2 mg/100 g in parsley, 3.2 mg/100 g in peas, and 15.6 mg/100 g in spinach, all as fresh samples (*26*). Our result is also in accordance with a survey study on selected leafy vegetables with medicinal value, but less commonly used for nutritional purpose, which generally contains higher levels of lutein than β-carotene (*27*). Moreover, the total carotenoids and provitamin A contents of *C. digitatum* were higher than those in *Brassica oleracea*, *Hydrocotyle asiatica*, and *Mentha spicata*, where total carotenoids were 10.32, 26.49, and 33.21 mg/100 g, respectively (*27*). *C. digitatum* also contained more provitamin A than *Allmania nodiflora*, *Beta vulgaris*, *Cucurbita maxima*, and *Murraya koenigii* (*27*).

Different classes of carotenoids differ in their stability toward heat treatment (*22*). The stability of carotenoids is also different among foods if the same processing and storage conditions are used. Thus, optimum conditions for carotenoids retention during preparation and processing differ from one food to another (*28*). Lutein and zeaxanthin are xanthophyll carotenoids found in a wide variety of plant foods, especially in GLVs. As in other GLVs, lutein is the most common vitamin A inactive carotenoid in *C. digitatum*. About 18.9 mg/100 g was determined in the raw material and 0.19 mg/100 g in the processed material. A very poor retention after processing of only 1% was observed. However, peaks A and B in the processed sample could be a derivative of lutein (the structural elucidation of these peaks is still in progress and will be reported separately) (**Figure 2**). Anyway, the lutein content in the raw material of *C. digitatum* can be compared to the lutein contents in carrot, broccoli, pumpkin, spinach, lettuce, and tomato, which contained 0.3, 2.3, 1.1, 6.9, 0.9, and 0.12 mg/100 g, respectively, expressed per 100 g edible portion (*24*).

Canthaxanthin and zeaxanthin are very rarely reported in GLVs, but zeaxanthin was found in both the processed sample and the raw material with less extent than lutein, while zeaxanthin had a better retention after processing than lutein. Lutein, zeaxanthin, canthaxanthin, and β-cryptoxanthin are very labile toward heat treatment and suffer tremendous losses (**Figure 2**). Only 1.0, 5.2, and 0%, respectively, were retained in the processed sample. Thus, to increase the dietary intake of carotenoids, including enhancement of bioavailability, it is recommended in general strategies to optimize cooking and processing conditions such that appreciable losses of carotenoids are prevented while the bioavailability is increased (*27*).

The high content of vitamin C in the matrix, which lowers the pH, together with high temperature and pressure during processing, resulted in a dramatic loss of lutein. This is in accordance with previous findings where the level of lutein in the extract of the autoclaved sample of spinach was substantially reduced, while in other green vegetables that contained weak organic acids, such as oxalic acid, lutein did not undergo dehydration, which finally led to degradation under these conditions (*22*).

The vitamin A active carotenoid β-carotene was unlike lutein under the household cooking conditions (see Sample Preparation); all isomers of β-carotene are quite heat resistant. β-Carotene levels in the processed sample as compared with raw material were not statistically different; 98.6% of β-carotene was retained in the processed sample (see **Table 1**). This high retention could be attributed to the inactivation of oxidative enzymes by the heat treatment, which prevents further and greater losses during storage (*27*, *29*). Moreover, the (13*Z*)-β-carotene isomer was significantly higher after processing, while (*all-E*)-β-carotene decreased in the same level, which implies that there is conversion of (*all-E*)-β-carotene to the (13*Z*)- and (9*Z*)-isomers (**Table 1**). This is obviously attributed mainly to the processing conditions but might be also a result of the UV light during the subsequent sun drying. The rise of β-carotene isomers was also reported in previous research, among them a research on green beans, broccoli, and spinach cooked under various conditions (*22*).

Provitamin A Content. It is well-known that VAD is a problem in less-developed areas of the world. Dark green leafy vegetables are the most common rich sources of provitamin A. Relatively easy to produce and available practically all year round, they are inexpensive and accessible sources of provitamin A for people in most of the developing world (*29*). In GLVs, β-carotene is essentially the sole contributor to vitamin A activity, while α-carotene and α- or β-cryptoxanthin are being reported only occasionally and at very low levels. However, the β-carotene content of leafy vegetables can vary markedly (*29*). Despite the importance of the provitamin A carotenoids in limiting eye illnesses, other carotenoids and vitamin E also play crucial role in that regard. In prospective observational data from large populations of female health professionals, a higher intake of the carotenoids lutein and zeaxanthin could reduce the risk of developing cataracts by about 18%, while a high intake of

vitamin E from food and supplements was associated with a 14% lower risk of cataract (*30*). Food based on *C. digitatum* appears to be a promising source in terms of provitamin A and also other phytonutrients like lutein, zeaxanthin, and vitamin E that sustain healthy eyes. The provitamin A content was found to be 2.49 and 2.40 mg RE/100 g in the raw material and the processed sample, respectively, corresponding to 96.4% retention after processing.

According to the central statistical organization in Yemen, there are 76000 blind people in Yemen, that is, 35 blind for each 10000 of the population, most of them living in rural areas (*31*). Another study was done in 18 districts of western Yemen on 338 children ages 1−5 years aimed to link the prevalence of xerophthalmia and night blindness with the extent of VAD (*15*). They found that about 2.2% of the children had active xerophthalmia; children ages 4−5 were more likely to have xerophthalmia than those less than 4 years, and boys were more likely ill than girls. Of the xerophthalmia cases, 77.8% had Bitot's spots, and 71% had spots in both eyes. The prevalence of Bitot's spots exceeded the minimum criteria for public health significance of xerophthalmia (1.72 vs 0.50%). The prevalence of night blindness stood at 0.45%. Children with xerophthalmia had much lower retinol levels than those without xerophthalmia (11.4 vs 18.8 μg/dL; $P < 0.001$). Likewise, children with night blindness had lower retinol levels than those without night blindness (10.9 vs 18.3 μg/dL; $P < 0.001$). The results of this study were very alarming; it came out with the conclusion that xerophthalmia and VAD are public health problems in western Yemen (*15*).

Anticipated Synergistic Effect. The beneficial effects of vitamin A, β-carotene, and lutein or zeaxanthin supplementation on human visual performance are well-known (*32*). Thus, data on carotenoid and provitamin A content of such a free and reasonably accessible source as *C. digitatum* may provide information to consumers and public health workers to support the dietary daily carotenoids intake in general and be helpful to create nutritional awareness among various target vulnerable age groups and to assess their relationship to health and disease. Other researches showed that both Alzheimer's and multi-infarct dementia patients had significantly lower levels of vitamin E and β-carotene than controls (*33*). Recently, use of vitamin E and vitamin C supplements in combination for Alzheimer's disease in elderly population was found to be associated with reduced prevalence by 78% and incidence by 64% (*34*).

With these high concentrations of carotenoids and vitamins, synergistic action is highly anticipated in vivo after a meal containing *C. digitatum*. This conclusion is strongly supported by previous findings, where the dose-dependent first evidence of a prooxidant in vitro effect of β-carotene under 100% oxygen pressure in a biological membrane model was illustrated, and the existence of cooperative interactions between β-carotene and α-tocopherol was shown (*35*). Meanwhile, it was pointed out that α-tocopherol should always function as an antioxidant, as long as the concentration of other coantioxidants, such as vitamin C, is high enough to convert α-tocopheroxyl radical back to α-tocopherol. Considering vitamin C's high concentration in human plasma, it is highly probable, in vivo, that vitamin E should act as an antioxidant regardless of the oxidative conditions (*36*).

Future Enhancement of the Product. *C. digitatum,* both raw material and processed sample, contained reasonably higher total phenolics contents and higher antioxidant capacities than asparagus and broccoli (rich sources for theses phytonutrients), although *C. digitatum* antioxidant capacities did not correlate with the total phenolics content (*17*). This antioxidant capacity was measured in hydrophilic matrix, resulting mainly from vitamin C and phenolic compounds. The high content of vitamin E and also the remarkable contents of carotenoids lead to the expectation that the antioxidant capacity of *C. digitatum* in the hydrophobic matrix might be even important.

Although traditional sun drying is the cheapest and most accessible means of food preservation, it is well-known that it causes considerable carotenoid destruction (*27*). So, drying in simple inexpensive solar dryers is highly recommended and can appreciably reduce losses in carotenoids. Modifications of the traditional cooking practices should be tried first (*29*). Various means of cooking (microwaving, boiling, and steaming) must be studied extensively to see the effect on the qualitative and quantitative distribution of carotenoids in *C. digitatum*. Optimization of processing time and temperature and of storage conditions is also recommended (*29*). Exclusion of oxygen may be assured by suitable packaging methods (e.g., through vacuum or hot filling, oxygen-impermeable packaging, or inert atmosphere). Low temperatures and protection from light can diminish carotenoid decomposition during storage, keeping storage time at a minimum (*27*).

The harvesting time of *C. digitatum* should also be studied to gain the best yield of this functional food ingredient. For example, in *B. oleracea*, carotenoid concentrations were significantly higher in the second year than in the first year (*37*). In lettuce and endive, the β-carotene content of the mature leaves was found to be three times greater than that of the young leaves taken from the same bunches of vegetables (*38*).

Many enzymes and secondary compounds of higher plants have been used in in vitro experiments to show protection against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (*7*). In that regard, some active phytochemicals were quantified, among them luteolin, in which bioactivity was tested in vitro (to be reported separately). More functional food ingredients and non-nutrient components must be determined in the fresh material to see the effect of drying and storage. It has become evident that several research questions need to be addressed soon; the high carotenoids contents suggest to progress in flavor and aroma constituents determination since many aroma compounds are generated from carotenoids by food cooking and processing (*3*); one of the main causes for using the processed sample is for its aroma.

Animal model experiments should be established soon with suitable strains vulnerable to vascular disorders and cancer. Serum and plasma response assessment will also be useful to study the bioavailability of these phytochemical micrometerutrients from *C. digitatum* after consumption, and the dosages that could promote health among the consuming public should be determined. Our findings proved that *C. digitatum* may become a new food source, being very rich in vitamin E, vitamin C, provitamin A, and other important carotenoids. Meanwhile, through this work on *C. digitatum*, we established research on Vitacea family as an important GLVs candidate for functional food ingredients.

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CHAPTER 4: Search for the Key Aroma Compounds in „*Cyphostemma digitatum*“ before and after processing.

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Mohammed Al-Duais*, Christine Bartzsch+

*) Institute of Ecology, University of Jena, Dornburger Straße 159, 07743 Jena, Germany

+) University of Applied Sciences Jena

Abstract:

Volatile components in *Cyphostemma digitatum* were extracted in two trails by solid phase microextraction. The extracts were then analyzed by gas chromatography. Twenty three compounds were characterised from *C. digitatum* samples, the raw material and the processed sample, both in dry form using the PDMS/DVB bi-polar fibre and the nonpolar PDMS. Among this volatiles Geranyl Acetone, Vitispirane, β-Cyclocitral, β-Myrcene, Safranal, Limonene, Furfural, Acetic acid and Formic acid, many of them are functionally important, meanwhile many reported in other sources. Processing enhances many of these compounds and some of them are only generated by processing.

Keywords: *Cyphostemma digitatum*; Aroma; Solid phase microextraction, Antioxidant; Carotenoids; Processing effect; Yemen

1. Introduction:

Among the very diverse volatile compounds in foods, only a few compounds can be defined as ‘aroma-active compounds’ responsible for the characteristic aroma of foods. (1) For that reason seeking a new aromatic plant with a special combination of aroma-active compounds was recognised as a new contribution in the food market. Nowadays capturing and application of volatile compounds becomes an important task in the standardisation of food products. Flavors and aroma therapy becomes an important branch of ~~the~~ alternative medicine. The determination of odor-active molecules present at trace levels (ppb or less) but with a high impact on the food quality can be performed with the development of different gas chromatography (GC) detectors~~,~~ such as GC mass spectrometric detector and GC flame ionization detector. However, the complex sample matrix causes some interference and makes analysis of trace compounds problematic. Solid phase microextraction (SPME) is a relatively new technique (2) that combines direct extraction and preconcentration without pretreatment of samples. It uses a polymer-coated silica fiber to extract the compounds from a sample in a single step. The fiber selectively extracts

compounds according to their polarity from the headspace of the sample container, and the extracted compounds can be thermally desorbed directly into the GC injector for analysis (3) ; (4), SPME is advantageous compared to the classical analytical methods used for GC analysis of aroma compounds (liquid liquid extraction and distillation) which have some drawbacks such as the possibility of contamination with solvents, artifact formation, the use of environmentally hazardous solvents, the length of time required, and insufficient selectivity.

The species *Cyphostemma digitatum* "halka" belongs to the vitaceae family and is a perennial, climbing, succulent undershrub species with compound fleshy leaves and tendrils. Its flowers in pedunculate axillary cymes, the fruits are one-seeded with red fleshy berries. The leaves are fleshy, petiolate, digitately 3-5 foliolate; leaflets are ovate and dentate. *Cyphostemma digitatum* usually occurs between 1400 m and 2500 m a.s.l. on the escarpment, often on cliffs and with a marked preference for shaded stony places such as gullies and terraced walls. It is usually associated with *Acacia* spp., *Agava* spp., *Senecio hadiensis*, *Clematis* spp. and *Euphorbia* spp. (5). The species is declining rapidly in its natural habitats.

Currently *C. digitatum* exported outside its natural area and sometimes outside Yemen; People started to cultivate *C. digitatum* by removing the plant completely from its original sites and replanting it in the gardens (6). There is an obvious decline of this species due to intensive commercial gathering. It is processed and sold as an ethnic medicine, flavouring food additive and as a main constituent to many traditional dishes in central Yemen (among them a very popular traditional soup known locally as marak). In addition it was traditionally recommended for gastroenteritis, fatigue, vomiting, general weakness, malaria, nausea and headache; the consumed parts are the leaves and fleshy young stem branches after processing (6). Therefore, *C. digitatum* disappeared completely from many regions in the southwestern highlands of Yemen. (6).

In the context of standardization of food products derived from the green leafy vegetable (GLV) *Cyphostemma digitatum*; phenolic contents of *C. digitatum* and the remarkably high antioxidant activity was quantified by four different methods and compared with the very well known vegetable source for antioxidant (7). Furthermore, it was proved that this plant is actually a new wealthy source of many functional food ingredients and micronutrients, among them vitamin E, vitamin C, provitamin A, and other important carotenoids (8). One of the main causes for using the processed sample of *C. digitatum* is for its aroma, since many aroma compounds are generated from carotenoids by cooking and processing (9). The high carotenoid contents proved

in a previous study (8); suggest a progression in flavour and aroma constituents' qualification for *C. digitatum* before and after processing which is the aim of this paper. The solid phase micro-extraction technique (SPME) combined with GC, provides a quick, simple, solvent less way of isolating flavour compounds (10).

2. Material and Methods

2.1. Samples preparation

Fresh leaves of *C. digitatum* were harvested from nature in August 2006 in the Ba'adan countryside in the south-western highlands in central Yemen. This area was selected for sample gathering because it is away from any substantial human impact. A voucher sample was given to the Agricultural Research Centre in Taiz, Yemen. Part of the clean leaves was boiled for 30 min under pressure, then the water was removed and the leave mass was mixed with a wooden spoon. The thick homogeneous stature baste was thinned into disks (8-12 cm in diameter) and dried in the sun in clean plates covered with a tiny mesh, changing upside down each day until complete dryness was achieved; this was called the "processed" sample. Other part of the freshly cut leaves were dried in an oven at 40 °C, and this sample was called "raw material". Both the processed sample and the raw material were used for the tests as dry materials. Heat treatment was used to reduce the content of antinutritional substances and to diminish their effects (11). Both samples were packed and stored at ambient temperature (< 30 °C) for 3-5 months before use. Directly before preparation for Aroma estimation both dry and processed samples were milled with a cyclotec mill (UDY Corp., Fort Collins, CO) (1 mm mesh).

Two trials with different equipment were involved in the direct SPME sampling and the gas chromatography identification; determinations were conducted in triplicates.

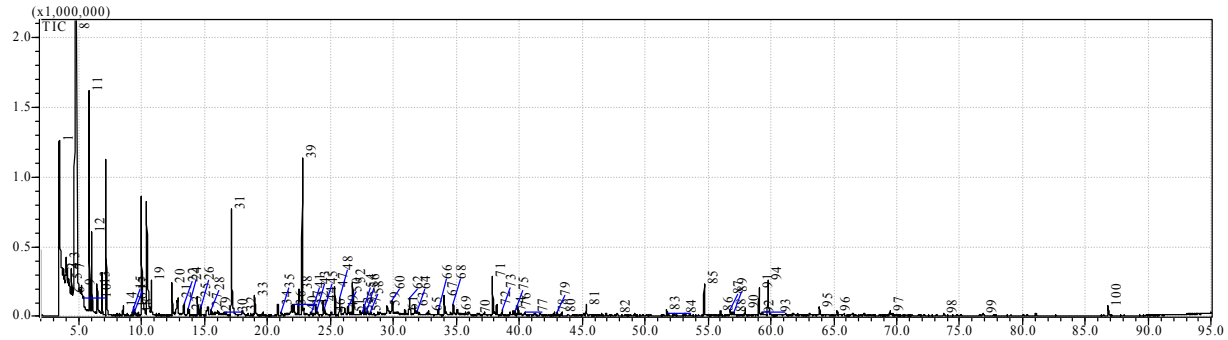
2.2. Trial 1: The sample was introduced into an auto sampler 1 mL glass vials, tightly closed with polypropylene caps with a Teflon liner and extracted for 120 minutes at 30 °C by a polydimethysiloxane-divinylbenzene (PDMS-DVB) 1 cm long 65 µm fibre. The fibre was placed in the headspace over the sample and thereafter the fibre containing the trapped volatile compounds were desorbed in the injection port of a gas chromatograph (GC17A, Shimadzu) equipped with a quadruple mass spectrometric detector (QP5000, Shimadzu) and an auto sampler AOC5000 was used, for 2 min and at 270 °C, for a quick thermal desorption of the analytes, Carrier gas was Helium of 99.999% purity , flowing at a rate of 1.6 ml/min. Volatiles were cold trapped by liquid nitrogen placed at the beginning of the GC column. The GC was equipped with a column (DB5MS, d = 0.25 mm, di = 0.25 µm, L = 60 m) obtained from J & W

Scientific. The best peak separation was obtained with the following temperature programme: 2 min at 40 °C, raised to 200 °C at 2 °C/min, then to 300 °C at 10 °C/min, 300 °C and held for 3 min. The set of substances, reaches from highly volatile substances up to n-hexacosan (Kovats-Index 2600). For identification of the substances the mass spectra libraries NIST (107886 spectra) and flavour & fragrances (1252 spectra) were used.

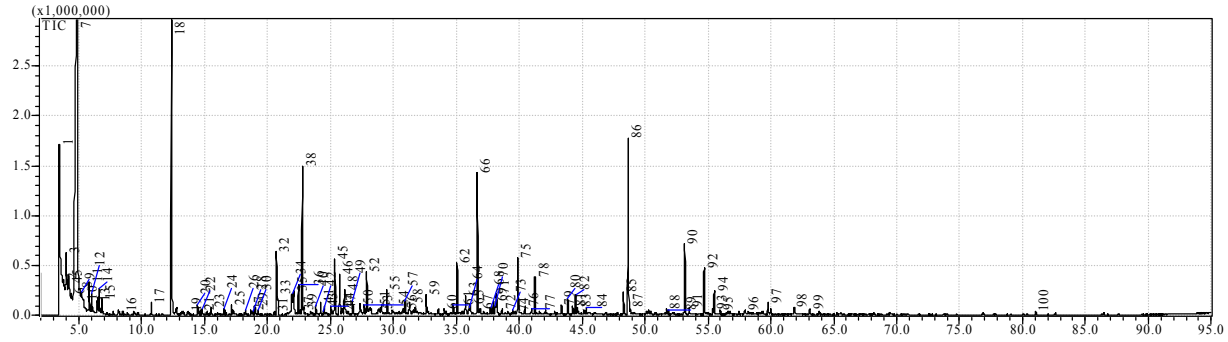
2.3. Trial 2: The sample was introduced into an auto sampler 1 mL glass vials, tightly closed with polypropylene caps with a Teflon liner and extracted for 60 minutes at 25 °C by Polydimethysiloxane (PDMS) 1 cm long 100 µm diameter fibres. The fiber was placed in the headspace over the sample and thereafter the fibre containing the trapped volatile compounds were inserted for 2 min into the injector port of the gas chromatograph (Agillnet Technologies) equipped with a quadruple mass spectrometric detector (5973, Agilent), where it was heated to 250°C for a quick thermal desorption of the analytes. The carrier gas was Helium at a flow of 1.5 ml/min. The GC was equipped with the column (DB5MS, d = 0.25 mm, film thickness = 0.25 µm, L = 30 m); from (J & W Scientific). The best peak separation was obtained with the following temperature programme: 2 min at 40 °C, raised to 200 °C at 5 °C/min, then to 300 °C at 10 °C/min, and held at 300 °C for 3 min. For identification of the substances the mass spectra libraries NIST and Wiley were used.

3. Results and Discussion

Solid phase micro-extraction analysis of *C. digitatum*, with two types of fibres, nonpolar (PDMS) and bi-polar (PDMS/DVB) were the volatile profile of the raw material and the processed sample as powder is shown in (Fig. 1). SPME was used to extract and concentrate *C. digitatum* volatiles because it was a solventless headspace technique that did not co-extract *C. digitatum* carotenoids. Diverse types of compounds were isolated from both samples, depending on the processing of each of the samples. The effect of the household processing reveals a positive generation of various aroma by-products isolated by the SPME technique; the total peaks area is clearly enhanced after the household processing (Fig. 1). This is in accordance with the previous findings with respect of both samples, where total carotenoids were dramatically reduced by the household processing (8). It is well known that carotenoids break down to produce aroma active compounds by such processing (9). This is not the case with respect to virgin olive oil where destruction of the existing volatiles in the oil has taken place by processing; the total peaks decreased from 120 in the initial virgin olive oil to 65–70 peaks after thermal oxidation because of its low content of carotenoids (12).



a)



b)

Figure 1: Chromatograms of *C. digitatum* using the PDMS/DVB bi-polar fibre **a)** raw material and **b)** the processed sample, gas chromatography (GC17A, Shimadzu) equipped with a quadruple mass spectrometric detector (QP5000, Shimadzu) see table 1 for details of some peaks.

(Table 1) lists the principal volatile compounds identified in the green leafy vegetable *C. digitatum* of the raw material (leaves after oven drying) and the processed sample, isolated by the SPME with two types of fibres, with the ratio of each compound as affected by processing. In particular, emphasis is given to the compounds, which had major quantitative changes in *C. digitatum* after processing; a special combinations of volatile compounds which gives the aroma of the processed *C. digitatum* have also other benefits as functional food ingredients; some of them with additional activity as natural food additives, analgesic or antioxidant which participate in the reasonably high antioxidant capacity of *C. digitatum* that were previously determined (7). Meanwhile, some of them are important aroma constituents in other food sources and have previously been widely reported in the literatures.

When using the SPME technique, two types of fibres, nonpolar (PDMS) and bi-polar (PDMS/DVB) were tested. Results were different, and were expected to be so because of the polarity compatibility between volatile compounds and the fibre material. As seen in (Table 1), the bi-polar PDMS/DVB material extracted and retained a broader class of compounds.

Table 1: Results of some volatile compounds isolated and identified from *C. digitatum*, by solid phase micro-extraction- Gas chromatograph (GC17A, Shimadzu) equipped with a quadruple mass spectrometric detector (QP5000, Shimadzu) using polydimethysiloxane-divinylbenzene (PDMS/DVB) and polydimethysiloxane (PDMS) fibers, according to their retention times (RT); Identified based on spectrum verification from the NIST and Wiley mass libraries or the literature retention indices.

Peak no.	Compounds	Ratio*	RT (min)	Fibre	Properities	Other source
3	**Formic acid**	1,300634	3,996	PDMS/DVB	Food additives	
8	**Acetic acid**	1,625316	4,836	PDMS/DVB	Food additives	Vinegar, sour
11	**Butanal, 3-methyl-**	0,178324	5,826	PDMS/DVB	Off flavour	
12	**Butanal, 2-methyl-**	0,275018	6,026	PDMS/DVB	Off flavour	
19	**Hexanal**	0,50764	10,744	PDMS/DVB	Off flavour	Many
20	**Furfural**	15,47981	12,399	PDMS/DVB		corncobs, oat and wheat bran
25	**2(3H)-Furanone, 5-methyl-**	0,791716	14,428	PDMS/DVB	Food additives	
39	**Beta-Myrcene**	1,295973	22,768	PDMS/DVB	Food additives, Analgesic, Antioxidant, Anticonvulsant, Antimutagenic, Bactericide	Rice, lemongrass, verbena, Caraway, hop and bay
47	**Benzene, 1-methyl-3-(1-methylethyl)-**	3,003791	25,351	PDMS/DVB		
59	**Methyl 2-furoate**	2,101201	29,499	PDMS/DVB		
76	**2-Furancarboxaldehyde, 5-(hydroxymethyl)-**	6,627724	39,891	PDMS/DVB		Grape & wine
48	**Limonene**	2,951393	25,725/ 11,64	PDMS and PDMS/DVB		Many Grapes
1	**5 Methyl Furfural**	New	9,71	PDMS	Food additives	
2	**6- Methyl -5-Hepten-2-One**	New	10,35	PDMS	Food additives	
3	**1,8-Cineole**	New	11,74	PDMS		
4	**Trans-Linalool Oxide**	New	13,48	PDMS		
5	**Unknown**	0,45	14,86	PDMS		
6	**Safranal**	1,97	16,74	PDMS		Saffron, Grapefruit Osmanthus, Mate, Black tea, Paprika, Grapefruit juice
8	**Beta-Cyclocitral**	New		PDMS	Minty, fruity, green	Roasted Mate, Tea, Peas, Rum, Melon, Paprika, Apricot, Brocolli, Tomato, Cantaloupe, orange juice
9	**Benzene, 1,3-bis(1,1-dimethylethyl)-**	New		PDMS		
10	**Vitispirane**	New	19	PDMS		Grape juice, wine, quince fruit and vanilla
11	**Geranyl Acetone**	0,95	23,44	PDMS		Grapes
12	**n-Hexadecane**	New	23,75	PDMS		

***** Peak area ratio after processing.

A total number of 12 and 14 compounds were isolated and identified by polar PDMS and the bi-polar PDMS/ DVB fibres respectively. Most of the compounds isolated by PDMS were newly formed after processing. A competition effect was expected to take place during extracting the active sites in the fibre, brought about by the limit in the size and the construction of the material

itself. Only those with the three identical reading at the same retention time and with more than 90% matching with the library were presented in Table 1 above.

The results showed that the majority of the flavour compounds isolated changed dramatically after processing. Off flavour volatile compounds such as hexanal were reduced after processing (Table 1). Because of the high demand of certain aroma combination and the scarcity of its plant source, various plant cell lines have been studied for the production of its natural flavour (13); (14). Recently due to its characteristic aroma, cell lines of the medicinal and culinary plant *Zanthoxylum piperitum* (prickly ash) of Rutaceae family were examined to produce its aroma combination commercially (15); interestingly that *Z. piperitum* shares with *C. digitatum* three (out of seven) aroma active compounds (limonene, geranyl acetate, and myrcene) as the major flavour compounds of this plant.

Some of the compounds have no commercial standards available like vitispirane (16). Vitispirane identified among the volatiles of grape juice or distilled white wines. It also occurs in quince fruit and vanilla aroma. Vitispirane belong to a class of norisoprenoid spiro ethers existing in nature in both the A and B forms (Fig. 2). Ever since their isolation, these compounds have been popular synthetic targets (17). Vitispirane was tentatively identified by GC mass spectrometry, comparing Kovats indices and mass spectra available in literature (Fig. 2).

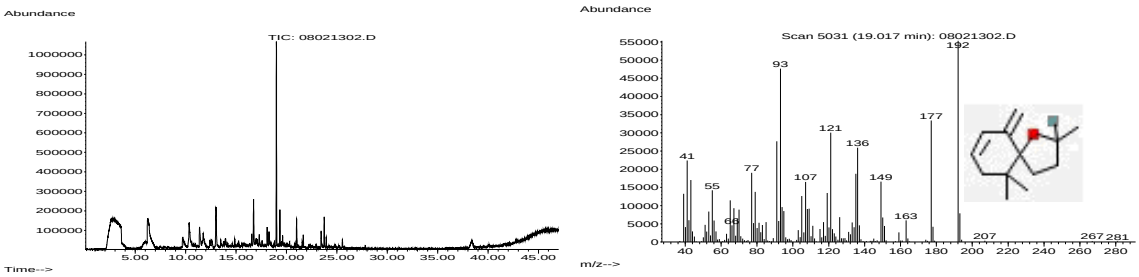


Figure 2 Mass spectra and interpretation of mass fragments of Vitispirane (C13 H20 O, 192.30) by gas chromatograph (Agillnet Technologies) equipped with a quadruple mass spectrometric detector (5973, Agilent)GC/MS.

4. Conclusion and Recommendations

A number of peaks were isolated by SPME (Fig. 1). This work introduces for the first time the volatile profile of *C. digitatum* in two forms, by showing definitively a special combination of aroma and functional food ingredients especially in the processed form. This report suggests moving toward quantification and examination of the enzymatic reactions in its biosynthesis as well as the possibility of utilizing this plant as a new natural source for a food-flavoring additive. There were notable quantitative enhancements in prominent compounds caused by the household

processing such as 2(3H)-Furanone, 5-methyl, 2-Furancarboxaldehyde, 5-(hydroxymethyl), Beta-Myrcene, Limonene, Safranal, Geranyl Acetone, Formic acid, Acetic acid, and Furfural which increased with processing, while another aroma active compound is formed completely by processing such as Beta-Cyclocitral, Vitispirane, 1,8-Cineole, Geranyl Acetone, 5 Methyl Furfural, Trans-Linalool Oxide, and 6- Methyl -5-Hepten-2-One. The off flavour compounds are off very trace concentrations and were reduced by processing.

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CHAPTER 5: Vegetation analysis of some communities harbouring the overexploited species *Cyphostemma digitatum* in Yemen

To be submitted to: Plant Ecology.

Mohammed Al-Duais, Gottfried Jetschke

Institute of Ecology, University of Jena,
Dornburger Strasse 159, 07743 Jena, Germany

Abstract

Cyphostemma digitatum (Vitaceae) is a perennial climbing succulent plant species with a broad ecological amplitude which inhabits the slopes in the southwestern highlands in Yemen. Due to overexploitation by local people the species seems is moving toward being endangered and denuded from many parts of its natural habitat. Therefore, the vegetation associated with *C. digitatum* was examined along an altitudinal gradient on the southwestern escarpment. Following a Braun-Blanquet approach 89 relevés with 467 species in total were analysed, followed by hierarchical polythetic agglomerative cluster analysis. Seven plant communities have been distinguished with evident differences in the main floristic composition. Each community type is characterized by particular indicators species easily seen in the field. Possible relationships of the community types to some environmental gradients were analyzed using non-metric multidimensional scaling (NMS). Good separation for the seven communities was obtained along the first three axes of an NMS plot, accounting for 78% of total variation in the floristic composition among stands. Altitude explained much of the distribution pattern obtained by NMS, mainly through an altitudinal gradient of temperature (especially frost) and precipitation. Also continentality exerts effects in this dissipation; matching the zonality conditions described for mountainous vegetation in Yemen. Local influence is superimposed by human impact and mesotopographic conditions (exposition, surface water, soil type and soil moisture).

Keywords

Yemen, *Cyphostemma digitatum*, phytosociology, centralized replicate sampling procedure, Braun-Blanquet, cluster analysis, indicator species analysis, non-metric multidimensional scaling, multi-response permutation procedure, coefficient of community, altitude, frost

1. Introduction

Yemen currently covers an area of 555,000 km^2 with 80% of it being arid or semiarid. However, situated at the south-western corner of the Arabian Peninsula, part of the area located in the southwestern highlands gets enough rainfall to allow dense vegetation and sustainable farming through a harmonious system of terraces with runoff water collection and other types of irrigation. Clear-cutting of trees, overexploitation and intensive agricultural use of the land led many species to nearly or complete extintion. However, recent projects in natural vegetation protection in Yemen with national and international aid have largely been concentrated in a handful of well-known high conservation status sites, among them the famous Socotra archipelago in the Arabian sea.

However, few data on the ecology and conservation of threatened and rare species have been compiled in Yemen and precise data on the status and number of rare and endangered plants are not available. Eight species (seven of these from the island of Socotra) are included in the IUCN Red Data Book as being endangered or rare, additional 19 species are considered to be endangered or rare at the national level in Yemen (UNDP/UNEP/GEF, 2001). Relatively reasonable efforts on protection were only done on the famous tree species *Dracaena cinnabari* (Attorre et al, 2007; Adolt & Pavlis, 2004).

There are many other endangered species in less spectacular sites waiting for a strategy of protection. Among them is *Cyphostemma digitatum*, a perennial climbing plant, where there is an obvious decline of this species resulting from intensive commercial gathering to be processed and sold as an ethnicmedicine, spice or as a food addidative. It is widely used as food flavouring and as a main constituent to many traditional dishes in central Yemen. It is also traditionally recommended for gastroenteritis, fatigue, general weakness, malaria, nausea and headaches. Due to overexploitation, the knowledge and use has even been partly lost. Recently it was proved that this plant is a wealthy source of many functional food ingredients and micronutrients with a variety of culinary and medicinal applications **(**Al-Duais et al 2009a, 2009b). This scientific confirmation is anticipated to increase demand on this species. Due to the declines of *C. digitatum* in nature, many inhabitants have begun to transplant *C. digitatum* from its original site to personal gardens.

Special highlighting on the Yemen vegetation as the most important site for Arabia and northeast Africa flora beside the special global important of Socotra were done in locations where *C. digitatum* does not exist in nature or was cleared through human activities. A brief description of *C. digitatum* was done by Wood (1997): *C. digitatum* (“halka”) belongs to Vitaceae family, is perennial, climbing, succulent undershrubs with compound fleshy leaves and tendrils. It flowers in pedunculate axillary cymes, has fruits which are one-seeded, and contains red fleshy berries. It is easily recognizable by its fleshy petiolate, digitately 3-5 foliolate leaves, and leaflets which are ovate and dentate. *C. digitatum* is spread between 1400 - 2500 m on the escarpment. Its preferred

habitat contains moderate moisture with a thin soil cover between stones. It also needs full sunlight and therefore almost never occurs in forested areas. It often occurs on cliffs with a marked preference for stony places such as gullies and traced walls with different slopes and aspects associated with *Euphorbia* spp, *Acacia* spp., *Agava* spp., *Senecio hadiensis* and *Clematis* spp. (Wood, 1997). To our observations *C. digitatum* seems to be inedible by indigenous herbivores. The used parts were the leaves and fleshy young branches **(**Al-Duais et al, 2009).

Despite the conservational value of *C. digitatum* there have been no published botanical studies which concern this species' autoecology, and no floristic surveys have been attempted to highlight the distribution of this species or its harbouring vegetation. Since this species been denuded from wide parts of the study area due to the intensive gathering, even if it could optimally sustain the species, studying the plant communities that harbor this species and the site conditions becomes a prerequisite to characterized the anticipated fundamental and realized niche for *C. digitatum*. Based on the community concept by Clements **(**1916) which emphasises that there is similar response of some plant species to environmental gradients which form the community as integrated distinctive unit, we felt the need to study the vegetation types that are associated with the occurrence of *C. digitatum* in the southwestern highlands of Yemen. Many researchers before stated that the vegetation situation in this region is not yet fully understood (Hall et al, 2008; Al-Hubaishi & Müller-Hohenstein, 1984; Wood, 1997). While our study is mainly concerned with this particular overexploited species and its harbouring communities in a region highly influenced by humans, it should also propose a methodology for developing a national mountain vegetation classification based on data from field surveys combined with up-to-date statistical techniques. Similar studies which traced individual species by characterizing its harbouring community were triggered before (Brullo et al, 2002; Abd El-Ghani & Marei, 2006).

Most of the existing vegetation classification in Yemen is based on physiognomy and mainly driven by experience of the authors, often concentrated to the five main natural vegetation regions of what was known prior to 1990 as North Yemen (Wood, 1997). Obviously, the vegetation zonation is highly influenced by topography, mainly altitude, through temperature and precipitation. Other site conditions like geology, soil, surface water availability and human influence still have reasonable influence on the mosaic shape of the vegetation in the study area (Al-Hubaishi & Müller-Hohenstein, 1984). We found some quantification based on physiognomy that was done by Wood (1997) for two particular places, one of them located in the vicinity of the study area named the southeastern mountains. Despite the somehow ambiguous picture about the flora in this region, tree species belonging to the genera *Acacia* and *Euphorbia* seem to play a key role in reflecting the zonation and patchiness impeding existing vegetation.

Our study was designed differently in a way that gathered the previously recognized knowledge together with the site conditions through application of modern statistical techniques and it is targeted differently, since an overexploited species with a well noted broad ecological amplitude

was traced in different plant communities spanning from 1000 to more than 2600 m in the southwestern mountains in central Yemen. This work was directed based on the history that was gathered from previous research, the local people and physiognomy. Additionaly, it was based on a well defined highly applied method, the Braun–Blanquet method, with its computational form by Van der marel in which all plant species were identified and their proportion was determined as percent coverage following the phyto-sociological methodology of Zürich-Montpellier (Braun-Blanquet, 1979; Mueller-Dombois & Ellenberg, 1974).

The present study was undertaken to analyse the vegetation associates with *C. digitatum*, in relation to the prevailing environmental gradients. It provides the baseline data on the vegetation structure of *C. digitatum*, and the communities in which the species occurs. We hope that the outcome may also provide, insights useful to establishing the most appropriate management and recovery measures for conserving these species. Multivariate statistical analysis techniques, like hierarchical cluster analysis, have long been applied to a wide variety of ecological scenarios (e.g. Williams et al. 1966; Stocker et al. 1977; Baeur 1989; Yom-Tov and Radmon 1998; Hupalo *et al.* 2000; Miserere *et al.* 2003), along with suitable ordination methods were applied to the data matrix to develop a comprehensive, floristically based classification of the vegetation that harbouring *C. digitatum* in the study area along an altitudinal gradient on the southwest highlands. It is used for the first time in the study of the Yemen vegetation.

This study was impeded by the main classical obstacle of searching the Yemen flora, namely the lack of climatic data and previous research. There is additionally the problem of fragmentation of habitat by new roads and new urban areas containing the highest population densities in the Arabian peninsula. Some times we encountered unaccessible places because of the rough landscape or suspected mine fields left from the civil war between the south and north regimes of Yemen before unification.

2. Material & Methods

The area of investigation is located in the southwestern part of the central highlands of Yemen around the cities of Taiz and Ibb, the rectangular study area extending between 13.25° – 14.16° N and 43,86°– 44,76° E with an altitude ranging from 1000 to 3500 m a.s.l. Since no *C. digitatum* was encountered at very high elevation, we limited the study sites between 1000 m and 2600 m (Fig. 1). These highlands contain a distinctive mosaic of vegetation (**33** plant communities according to Wood, (1997); This vegetation diversity is present to a lesser extent in other parts of Arabia. For this reason, it is the best place to develop a basic vegetation model which could be extended along the mountainous escarpment of Yemen and maybe throughout the whole of Arabia. Although the total study area seems to be rather small (150.000 km^2), it represents an essential part of the highly populated region in Yemen with enough rainfall to allow dense vegetation and agriculture. The southwestern highlands are remarkably dissected, resulting in many lowland corridors, valleys and steep slopes with terrace-based farming. Major

geomorphologic units can be distinguished including undulating plains of Tertiary volcanic material and Tertiary to Quaternary sediments, sharp granite outcrops like Jabal Sabir south of Taiz and Al-Manar Mountain north of Ibb, and smaller mountains consisting predominantly of Eocene basalt and partly of Palaeocene sandstone.

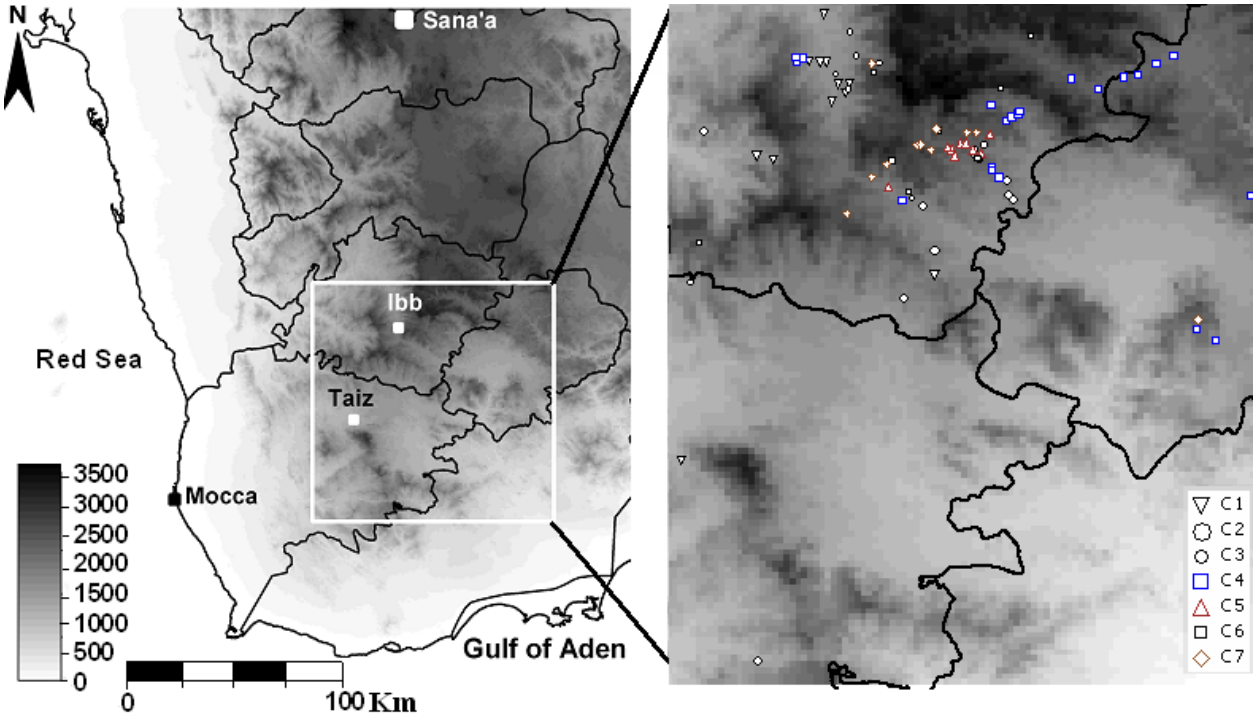

Figure 1: Distribution of the localities where relevés were made in the Southwestern Mountains in Yemen.

2.1 The climate

Meterological data are poorly available in Yemen. Detailed and systematic observation and analysis of precipitation in Yemen is scarce, data are manually and often inconsistency collected (Rappold, 2005). Standardization in the observational equipment and practices and quality of the data are low due to difficult access, limited funds and lack of maintenance and supervision (Bruggeman, 1997). A large proportion of the rain carried from the Gulf of Aden and the Red Sea is stopped by the mountains yielding high but extremely variable rainfall in the study area between 400 mm to more than 1000 mm per year (Al-Hubaishi & Müller-Hohenstein, 1984; Rappold, 2005). The seasonal precipitation pattern is characterized by equinoctial rainfall; the first peak occurs in April–May, the second one in August–October, with an intermediate three to six week dry period occurring in June–July. The latter rainy season has a considerably higher rainfall (Rappold, 2005; Al-Hubaishi & Müller-Hohenstein, 1984; Rappold, 2005). Interannual variation is high and potential evapotranspiration exceeds precipitation in every month in most of the study area (Rappold, 2005). The highest annual rainfall in the Arabian Peninsula with more than 1000 mm per year occurs around Ibb, averaging an impressive 1369 mm over five years in the 1970s while at Taiz, some 150 km south of Ibb, 563 mm was averaged over a much longer period (Rappold, 2005). Both are located in the core of the study area (see Fig. 1) where June to July dryness is very short or absent. Runoff in this mountainous region can mitigate the dry

period in many places and support a sort of vegetation patchiness, it also increases the damage of abandoned terraces and resulting soil erosion.

Mean annual temperature is highly dependent on elevation and, to a certain extent, on local annual rainfall. Average daily temperature ranges between 25 °C at the lower escarpment and 16 °C at the higher locations. Humidity changes with increasing elevation from high values in the coffee zone (1600 - 2200 m) to lower ones at high altitudes (Al-Hubaishi & Müller-Hohenstein, 1984). Yemen is situated at the fringe of the tropics. One possible definition to classify a region as tropical is the complete absence of frost (Herzog, 1998). This condition is fulfilled in the southwestern highlands mostly below 1800 m. At higher elevations, especially above 2000 m frost occurs more or less regularly in the winter, limiting the life conditions for all tropical lowland and most of the tropical submontane plant species (Al-Hubaishi & Müller-Hohenstein, 1984).

Frost in winter nights is a crucial environmental factor determining plant distribution in Yemen. This fact was particularly noted in a study where it was used as a key variable for rating land qualities for forestation (Dent & Murtland, 1990). Frost free land was categorised as highly suitable, while land with severe frost hazard, i.e. below -3 °C for more than 20 days, was only partly suitable. Both the “Qat" plant, *Catha edulis* (a narcotic plant introduced from Africa where most of the adult males in Yemen are addicted), and coffee can serve as a good indicator for the frost free zone. In the Central Highlands of Yemen including the study area, an initial evaluation of land for forestry purpose highlighted drought and frost as the main constraints; hail is also a hazard to crops and young trees (Dent & Murtland, 1990).

2.2. Field methods

The study was based on a data matrix composed of 89 relevés and 467 taxa. Sampling was done in August and September (2005-2007) as it is the climax of the rainy season with a maximum species richness and easily identifiable grasses and herbs (although some bulbs of the spring flora could be lost (Wood, 1997)). Rodwell (1991) emphasized the need for the ‘ecological integrity’ of defined vegetation communities. Consequently, samples were chosen carefully to be intact and away from human impact as possible. Plot locations were subjectively chosen in areas of relatively homogeneous natural vegetation (see Fig. 1) using the centralized replicate sampling procedure (Mueller-Dombois & Ellenberg 1974). Sample area was predetermined to be 10 m by 10 m according to the minimal sampling area method. Estimates of vegetation cover were obtained by the standard Braun-Blanquet cover-abundance scale. The following site conditions were collected for each relevé: geographical coordinates and altitude (by GPS, ± 8 m precision), slope, aspect, geology, soil type, soil depth, surface water availability and human influence. Interviews with people including consumers, traditional healers and large scale collectors of *C. digitatum* provided additional information about the current situation and the few existing source regions of the species.

2.3. Data preparation and preliminary analysis

The ordinal Braun-Blanquet values were transformed into a 0 to 9 rank scale (not using 6) according to van der Maarel (1979). Syntaxonomic table sorting (Braun-Blanquet 1964; Mueller-Dombois & Ellenberg 1974; Westhoff & van der Maarel 1978) was applied to detect rough vegetation patterns in the data matrix. Rare species that were observed in only three plots in the study area were deleted from the dataset, leaving 337 species for further analysis. This procedure is known to reduce variance and noise in the dataset, while still permitting a robust assessment of community responses to environmental gradients (McCune & Grace 2002). A preliminary phytosociological table was constructed afterwards.

2.4. Statistical analysis

All subsequent multivariate analyses of the vegetation data were performed with PC-ORD v5 (MjM Software,Oregon). Species area curves were constructed based on repeated random subsampling to evaluate the adequacy of the overall number of relevés. Additional information on observed abundance was incorporated by calculating the average distance between the centroid of subsamples and that of the whole sample.

Methods of hierarchical polythetic agglomerative cluster analysis, among them complete linkage with Euclidean distance, were applied to partition the data into more manageable internally homogenous groups distinct from each other based on their floristic composition. Euclidean distance was adopted to compute the distance between every pair of samples in the resemblance matrix (McCune & Grace 2002).

Indicator Species Analysis (ISA) was used to identify species that consistently differed in their abundance between communities (Dufrêne and Legendre 1997) and to determine at what level the dendrogram resulting from the hierarchical clustering should be cut, i.e. ISA gives the optimal number of final communities by mathematically selecting the level that maximized the collective indicator values of the species. The indicator values were calculated from the relative abundance and frequency of species in each community (Dufrêne and Legendre 1997). ISA was run on the output from the hierarchical clustering cycles yielding 2-7 communities with 1000 randomisations used in the Monte Carlo tests. Number of significant indicators ($P < 0.05$) and average P value for each run were selected as criteria (McCune & Grace 2002).

Multi-response permutation procedure (MRPP; Biondini *et al.* 1988) was used to test whether the communities determined by the hierarchical clustering and ISA were significantly different in species composition and abundance calculated with Euclidean distances between sample plots. The MRPP method uses a repeated shuffling of the distance matrix to test the hypothesis of no difference and thus provides a non-parametric significance test of community differences (McCune & Grace 2002). In addition to a *P*-value, MRPP describes community tightness with the agreement statistics *A*, a value that compares the within-community heterogeneity to that

expected by chance (A = 1 when items are identical, A = 0 when heterogeneity within communities equals that expected by chance, and A < 0 when heterogeneity within communities is greater than that expected by chance) (McCune & Mefford 1999). MRPP avoids the normality requirements of parametric multivariate tests such as discriminate analysis (McCune & Grace 2002).

The coefficient of community (CC; floristic similarity) of Ellenberg (1956),

$$CC = \frac{Mc/2}{Ma + Mb + Mc/2} \cdot 100 \quad ,$$

was calculated for each pair of communities, where *Ma*, *Mb* and *Mc* denote the total cover of all species exclusive in the first community, exclusive in the second community and occurring in both of them, respectively (Ramírez *et al*., 1997).

Non-metric multidimensional scaling (NMS) was applied as an indirect ordination technique to illustrate the relationships between samples and species composition where sample sites were ordinated in species space (Kruskal 1964; Mather 1976). NMS is an iterative method that attempts to reduce differences between the ranked distances in the original multidimensional species space and ranked distances in the reduced dimensions of the ordination. These differences, termed stress, are measured as the degree of departure from monotonicity in the original space and the reduced space. This iterative ordination technique is particularly well suited for analysis of ecological community data as it works well with non-normal datasets, allows the application of many distance measures (McCune & Grace 2002). NMS works without assuming that a species responds in a linear or unimodal fashion to environmental gradients and, hence, is robust to large numbers of zero values (Kenkel & Orlóci 1986; Minchin 1987). Being based on ranked distances, NMS is less prone to distortion due to outliers. For ecological analysis, NMS has been recommended over the more widely used Detrended Correspondence Analysis (DCA) method, which has been seriously criticised by several authors, since it avoids many of the distortions of eigenvector-based ordination methods (e.g. Minchin 1987; Legendre and Legendre 1998; McCune & Grace 2002). Communities were superimposed on to the ordination diagram to compare classification and ordination results. Box plots of altitudinal ranges were constructed for each community with Excel and arranged along increasing mean altitude of the obtained plant communities.

2.5. Environmental data

Since climate data are scarce in Yemen, interpolated bioclimatic variables, including average monthly temperature and rainfall values, from WorldClim database (www.worldclim.org) with 30 arcseconds resolution were used as a surrogate. For each sample coordinate the values of the variables Bio1 to Bio19 (Hijmans et al, 2005) were obtained with free GIS software ILWIS. Highly correlated environmental variables were reduced step by step leaving only a few to be used as joint plot in the NMS ordination space. The used variables are Alt = Altitude, Bio 6 =

Minimum temperature of coldest month (or frost), Bio15 = Precipitation seasonality (Coefficient of Variation), Bio18 = Precipitation of warmest quarter and Bio19 = Precipitation of coldest quarter.

3. Results

Since above 80 plots the species-area curve nearly reached a plateau and distance of subplots approached low values rather fast, 89 plots were considered to be sufficient for further analysis (Fig. 2). The preliminary classification based on phytososciological table work ended up with a handful of rather clearly distinguished comm. unity types in accordance with field experience. The subsequent cluster analysis gave the best and most plausible results with Euclidean distance and complete linkage method. The resulting dendrogram is shown in (Fig. 3). Indicator species analysis provided evidence that the seven community stage of the cluster analysis was the most informative as this was the level with the maximum number of significant indicators and the lowest average *P* value (Fig. 4). Since ISA cannot be used when one of the clusters contains a single sample, further splitting to eight or more clusters was rejected due to the beginning of chaining in one of the clusters.

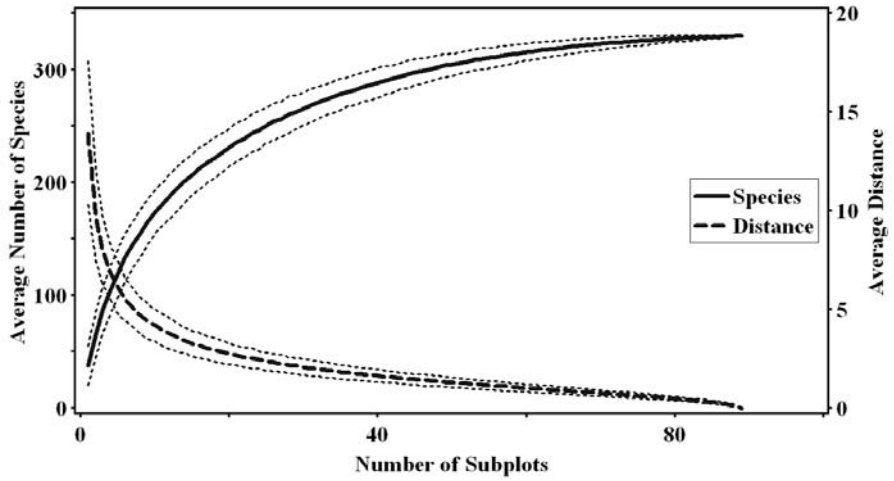

Figure 2: Species-area curve for all 89 relevés of 10 * 10 m each. The species area curve rises to a plateau, while the distance curve falls to zero. Confidence bands (plus or minus two standard deviations) were represented as dashed lines. Plateau of the cumulative number of plant species was reached within 80 relevés.

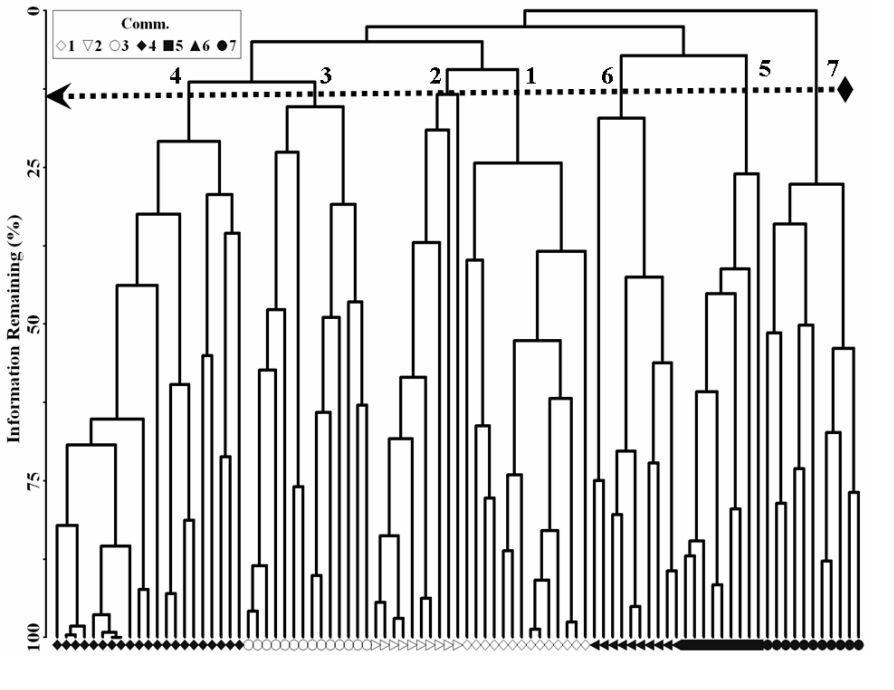

Figure 3: Floristic similitude dendrogram resulting from cluster analysis of the species composition in all 89 relevés. The broken line indicates where the dendrogram has been pruned based on the outcome of a previous indicator species analysis.

Multi-response permutation procedure indicated that there were very high significant differences between the seven communities produced by hierarchical clustering. The low *A* values proved that species composition differed reasonably among communities, while the very small *P* values underlined that it would be very unlikely to obtain this result just by chance (McCune & Grace, 2002).

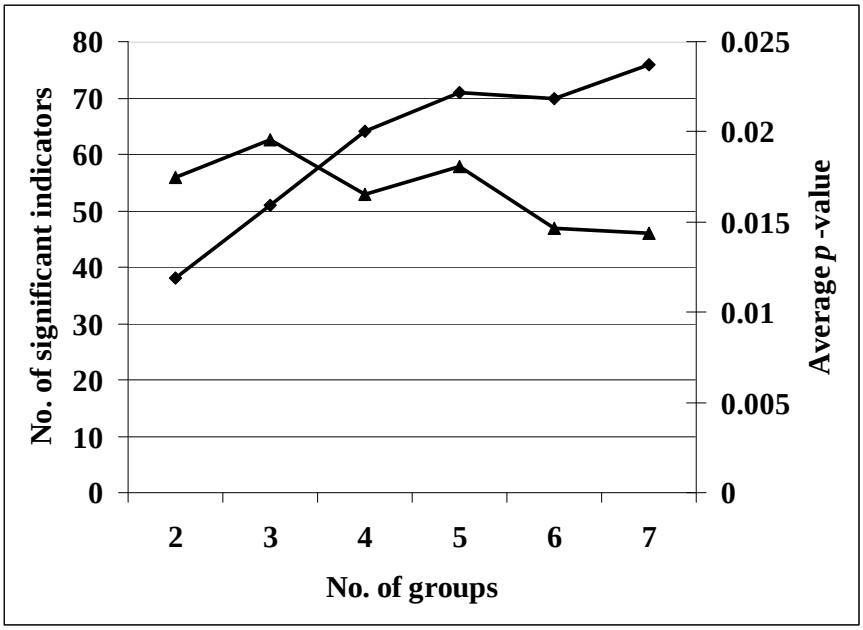

Figure 4: Variation in the number of significant indicators (diamonds) identified by ISA and the average *P* value of all species (triangles) at each step of hierarchical cluster analysis of the data matrix.

NMS was aimed to find a configuration of the samples in a predetermined *k*-dimensional ordination space, such that distances in ordination space correspond to dissimilarities; the statistical parameter "stress" has been used to measure the lack of fit between distances and these dissimilarities. Dimensionality was determined by running NMS on autopilot mode for 40 runs with real data and 50 runs with randomized data in each of six dimensions (McCune & Mefford 1999). Final value was chosen by selecting the highest number of dimensions that appreciably reduced stress and where the final stress for real data was significantly lower than that for randomized data. From the first run of NMS three-dimensional solutions were recommended. Then the 'slow and thorough' option in PC-ORD was used with Euclidean distance and varimax rotation. The use of this distance measure permitted ready comparison with the results of the hierarchical cluster analysis based on the same distance. Despite many zero values in the vegetation data set the ordination axes represented 78% of total variation in the dataset, (Axis 1 represented 14% of the variation in the species composition, Axis 2 accounted for 30% and Axis 3 for 35%). The successive decrease of the stress of the three NMS axes suggests a well-structured data set (Fig. 5). Stress on this solution was 17,3%, which indicates a good solution given the large size of the dataset (McCune & Grace 2002). The final instability criterion of 0.00003 for ordination with six environmental variables was achieved after 400 iterations or 50 continuous iterations within the criterion. Five of the seven communities (C1, C2, C4, C5, and C7) are very well separated in ordination space spanned by the first three axes. Axis 3, explaining the highest proportion of variance, is mainly an altitude and hence a temperature axis and gives an obvious separation between frost free and frost experiencing vegetation and has a

clear correlation with Alt and Bio 6. Because the extreme low and high altitiudes beyond the scope of this study, from now on it will refer to these groups as higher frost free vegetation (HFFV, at lower altitudes) and lower frost vegetation (LFV, higher altitudes), respectively.

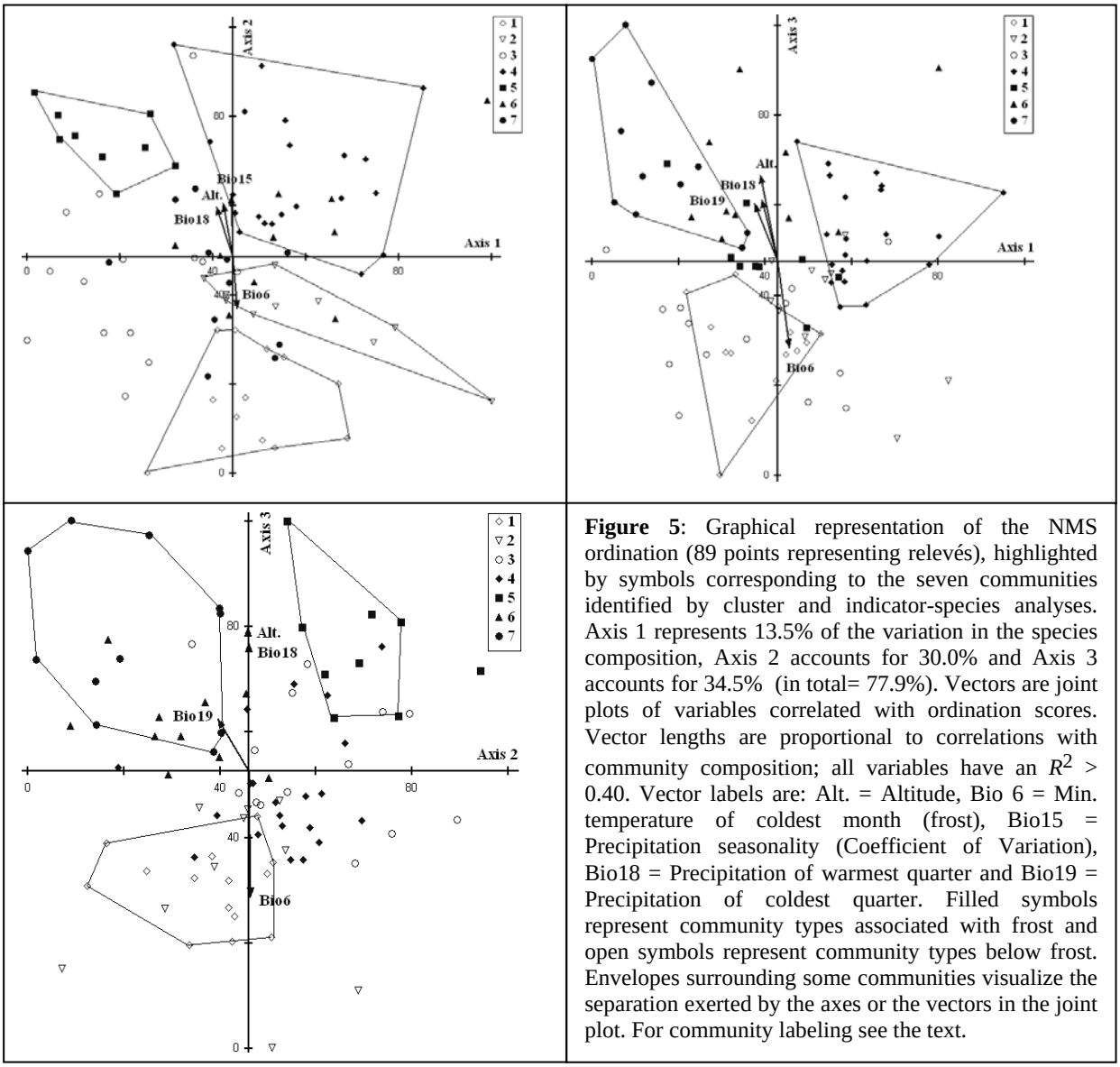

Figure 5: Graphical representation of the NMS ordination (89 points representing relevés), highlighted by symbols corresponding to the seven communities identified by cluster and indicator-species analyses. Axis 1 represents 13.5% of the variation in the species composition, Axis 2 accounts for 30.0% and Axis 3 accounts for 34.5% (in total= 77.9%). Vectors are joint plots of variables correlated with ordination scores. Vector lengths are proportional to correlations with community composition; all variables have an R^2 > 0.40. Vector labels are: Alt. = Altitude, Bio 6 = Min. temperature of coldest month (frost), Bio15 = Precipitation seasonality (Coefficient of Variation), Bio18 = Precipitation of warmest quarter and Bio19 = Precipitation of coldest quarter. Filled symbols represent community types associated with frost and open symbols represent community types below frost. Envelopes surrounding some communities visualize the separation exerted by the axes or the vectors in the joint plot. For community labeling see the text.

Only the variables altitude, Bio18 (= precipitation of warmest quarter) and Bio19 (= precipitation of coldest quarter) were found to be of explainable value in the joint plot in NMS ordination space (Fig. 5). Among them altitude was chosen as the most appropriate variable to ordinate samples, because it was highly correlated to all average temperature biovariables as an important biotic factor, especially to Bio 6 (= min. temperature of coldest month) expressing the possible occurrence frost. The communities were rather well separated in their altitudinal ranges as expressed by the corresponding boxplots and labelled C1 to C7 with increasing mean altitude (Fig. 6). Moreover, the coefficient of community expressed very well the pairwise dissimilarities between these communities and showed a clear inverse relation to the corresponding average elevation differences (Fig.7).

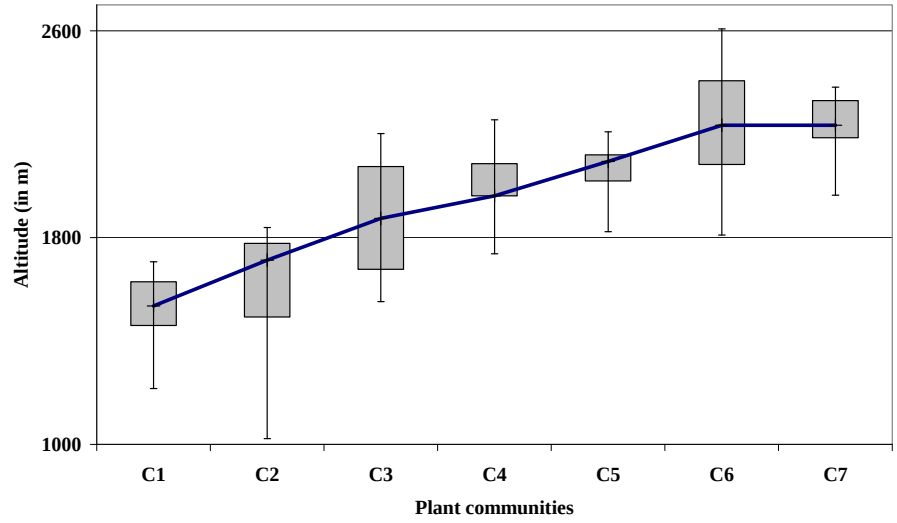

Figure 6: Box plots showing the altitudinal distribution of the seven plant communities and the estimated lower boundary (1800 m) between the higher frost free vegetation (HFFV, C1 – C2) and the lower frost vegetation (LFV, C4 –C7). Trend line connects the medians of the communities; numbers correspond to those of the seven tables.

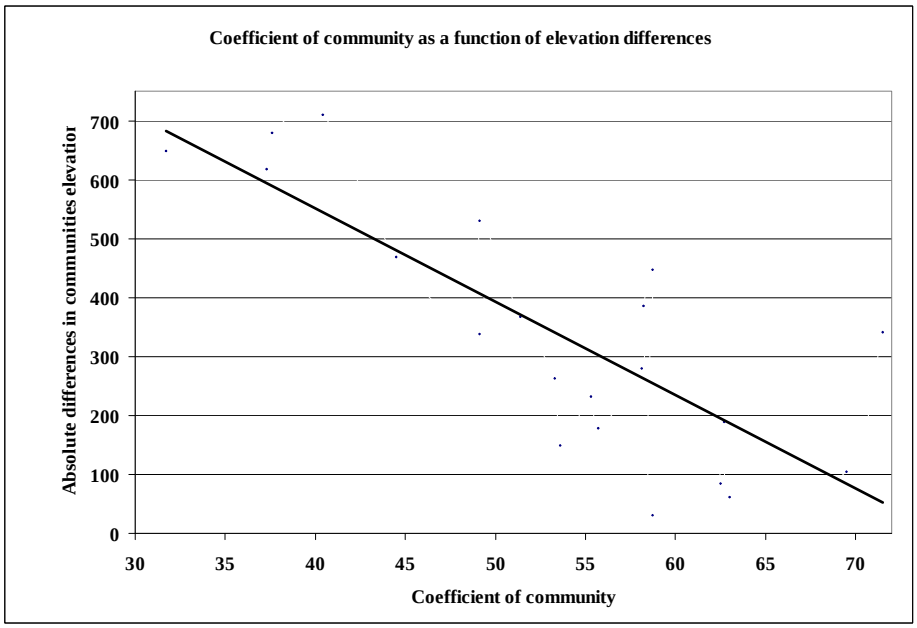

Figure 7: Inverse pairwise relation between the pairwise coefficient of community and the absolute differences in average elevation between communities.

Abbreviated floristic tables for these seven communities together with a summary of environmental data are presented in Tables 1-7. Each community was named after the tree or shrub species with the highest indicator values from ISA (not taking into account species which are constant and widely spread among several communities); they are listed together with their associational index with *C. digitatum* in Table 8. It should be emphasized that these results as they pertain to vegetation types are very much preliminary and names are simply intended to concisely distinguish the seven groups. A brief description of each community follows.

Acacia origena - Acanthus arboreus community

This vegetation type (C7, 11 sites; see Table 1) is the highest one along the altitudinal gradient that contains *C. digitatum* and was separated from the sample pool in the first clustering step (cf. Fig. 3). The community is located in a rather narrow altitudinal range of 2239 m ± 121 m s.d., where frost is very common in winter and precipitation is the highest among the seven

communities. Predominant soil type is heavy clay volcanic deep fertile soil with high pasture quality. The tree layer is dominated by *Acacia origena*.

Species	Association index	Species	Association index
Cyphostemma digitatum	100	*Pavetta longiflora*	38
Bidens pilosa	71	*Ocimum forskalei*	38
Hypoestes forskalei	69	*Commelina benghalensis*	35
Cyanotis nyctitropa	62	*Boerhavia diffusa*	34
Cynodon dactylon	58	*Digitaria sanguinalis*	30
Justicia flava	56	*Cyperus rubicundus*	25
Aristida adscensionis	53	*Reichardia tingitana*	24
Grewia erythraea	51	*Scilla hyacinthina*	24
Tagetes minuta	51	*Pancratium maximum*	24
Psiadia arabica	51	*Paspalum prostratum*	22
Hyparrhenia Hirta	48	*Commicarpus biossieri*	21
Leucas glabrata	46	*Leucas Spec.1*	20
Barleria trispinosa	44	*Agave sisalana*	19
Pupalia lappacea	40	*Tragia pungens*	19
Euphorbia inaequilatera	39		

Table 8: Association index for wide spread small shrubs and herbs in different habitat that coexist with *Cyphostemma digitatum* without coherence with any special community; for details see text.

This community is particularly well defined by ISA with a number of high indicator scores. The most characteristic species, *Acacia origena* (maximum domain score = 8) and *Acanthus arboreus,* are almost ever-present. Other frequent field layer species are *Arisaema flavum, Andropogon distachyos, Plectranthus asirensis* and *Vernonia bottae. Pennisetum villosum, Senecio hadiensis, Jasminum grandiflorum, Micromeria imbricate, Euphorbia inaequilatera, Plectranthus barbatu, Galium spurium* and *Rhynchosia minima* also show a relatively high association with this community. *Rosa abyssinica* which is the characteristic big shrub species of the higher mountains just starts to appear at this elevation.

Rumex nervosus - Euphorbia helioscopia community

This community (C6, 10 sites; see Table 2) has the highest overlap and similarity with the previous one, however it is more stretched with mean 2209 ± 265 m. s.d., also subjected to frost. Like above soils are heavy clay volcanic deep fertile soils with high pasture quality, but with less frequent tree layer. Interestingly, 50% of the sites ar abandoned terraces. The number of indicator species is low; among them are *Rumex nervosus, Commelina forskalei* and *Euphorbia helioscopia* the most frequent preferential species. Other frequent species are *Acanthus spira, Centaurea pseudosinaica, Malva parviflora* and *Ficus palmata,* while *Acanthus arboreus, Pennisetum villosum, Senecio hadiensis and Rhynchosia minima* are still frequent but markedly less than in the previous community. *Acacia origena* appears in 20% of the sites, while *Acacia gerrardii* starts to appear and is found in 40% of the sites. Interestingly, *Acacia yemenensis* which is very rare endemic species was encountered in this community two times out of three times in all the study area.

Table 1:- Abbreviated floristic table for the *Acacia origena – Acanthus arboreus* community.

Plant community No.	7	7	7	7	7	7	7	7	7	7	7		
Plant community variant	a	a	a	b	b	b	c	c	c	c	c		
Relevé No.	2	2	2	2	4	6	2	6	6	7	8		
	1	2	5	0	2	1	3	0	7	7	3		
Altitude (/ 10 m)	2	2	2	2	2	2	2	2	2	2	2		
	2	3	4	3	9	3	2	1	1	2	1		
	2	8	0	1	6	5	7	6	9	4	9		
Slope (x 10%)	2	1	2	2	1	4	2	1	3	3	5		
Vegetation Layer	2	4	3	3	2	3	4	4	3	3	3		
Soil depth	1	4	3	3	1	3	4	4	2	3	2		
Soil type	3	4	4	4	3	4	4	4	4	3	3		
Soil Fertility	2	4	4	4	3	4	4	4	3	4	3		
Pasture quality	2	3	3	3	2	3	3	3	3	3	2		
Stone cover	3	1	2	2	3	2	1	1	2	1	3		
Abandoned land	0	1	1	0	0	0	1	0	0	0	0		
Faithful and differential taxa:												**Max.**	**Freq.**
Acacia origena	7	8	5	4	0	2	7	7	7	7	5	**8**	**91**
Acanthus arboreus	3	0	3	4	0	3	3	2	2	2	2	**4**	**82**
Arisaema flavum	2	2	2	2	0	2	2	0	0	0	2	**2**	**64**
Andropogon distachyos	4	8	5	8	7	7	0	0	3	0	0	**8**	**64**
Vernonia bottae	0	3	5	0	0	0	0	0	0	2	0	**5**	**27**
Plectranthus asirensis	2	2	0	4	0	0	0	0	0	0	0	**4**	**27**
Senecio hadiensis	3	0	3	5	0	0	5	2	4	2	0	**5**	**64**
Pennisetum villosum	5	4	7	3	0	0	0	0	0	0	2	**7**	**45**
Digitaria abyssinica	4	4	5	0	2	0	2	2	2	0	0	**5**	**64**
Plectranthus barbatus	0	2	0	2	2	3	2	2	2	0	0	**3**	**64**
Jasminum grandiflorum	0	4	5	0	0	2	2	0	2	2	0	**5**	**55**
Rhynchosia minima	0	0	2	2	0	0	2	2	2	2	2	**2**	**64**
Micromeria imbricata	0	2	2	0	0	2	0	2	2	2	0	**2**	**55**
Galium spurium	2	0	0	0	0	2	2	0	0	0	0	**2**	**27**
Other taxa:													
Oxalis corniculata	2	2	2	0	0	0	0	2	2	1	0	**2**	**55**
Conyza pyrrhopappa	0	3	5	0	0	0	0	2	1	2	0	**5**	**45**
Selaginella yemensis	5	5	0	3	2	2	0	0	0	0	0	**5**	**45**
Rumex nervosus	5	4	3	0	0	2	0	0	0	0	0	**5**	**36**
Solanum nigrum	2	2	0	0	0	0	0	0	2	0	2	**2**	**36**
Hibiscus deflersii	0	0	0	0	0	0	0	2	2	2	2	**2**	**36**
Euphorbia ammak	0	0	0	0	0	5	5	7	0	5	0	**7**	**36**
Lantana rugosathunb	0	0	0	1	0	0	0	5	2	0	2	**5**	**36**
Commelina forskalei	0	0	0	2	0	2	2	0	2	0	0	**2**	**36**
Gomphocarpus fruticosus	0	0	0	0	0	2	0	0	2	1	2	**2**	**36**
Setaria flavidum	0	0	2	0	2	0	2	2	0	2	0	**2**	**45**
Moss Spec.	2	2	0	2	2	0	0	2	0	0	0	**2**	**45**
Opuntia ficus-indica	5	0	0	3	0	0	0	2	2	0	0	**5**	**36**
Acanthus spira	2	0	2	2	0	0	0	0	0	2	0	**2**	**36**
Cyperus rotundus	2	2	0	2	0	0	0	2	0	0	0	**2**	**36**
Kalanchoe glaucescens	0	2	0	0	2	0	2	0	2	0	0	**2**	**36**
Aloe vacillans	0	3	4	4	0	0	0	0	0	0	2	**4**	**36**
Cynoglossum lanceolatum	0	2	2	1	0	0	0	0	0	2	0	**2**	**36**

Additional taxa with three or less occurrences: *Euphorbia parciramulosa, Acacia hocki, Themeda triandra, Eragrostis papposa, Euphorbia Helioscopia, Snowdonia polystachya, Eragrostis pilosa, Kleinia odora, Cadia purpurea, Solanum incanum, Plectranthus hyemalis, Bothriochloa insculpta, Ruellia patula, Justicia odora, Carissa edulis, Withania somnifera, Indigofera arabica, Diplotaxis kohlaanensiss, Cotyledon barbeyi, Scadoxus multiflorus, Convolvulus arvensis, Pelargonium alchemilloides, Medicago critica, Torilis arvensis, Geranium ocellatum, Pupalia lappacea, Tragus racemosus, Euphorbia cactus, Maeroa triphylla, Tetrapogon villosus, Digitaria velutina, Panicum Maximum, Chenopodium schraderianum, Setaria viridis, Commicarpus helini, Becium serpyllifolium, Seddera arabica, Panicum acuminatum, Marrubium vulgare, Rhamnus staddo, Ipomoea eriocarpa, Orobanche cernua, Pulicaria arabica, pulicaria petiolaris, Ruellia praetermissa, Ipomoea nil, Pavonia kotschyi, Cistanche phelypoea, Verbascum decaisneanum, Bromus leptoclados, Anagallis arvensis, Cheilanthes coriacea, Cluytia myricoides, Justicia heterocarpa, Veronica opaca, Dorstenia foetida, Polygala tinctoria, Rosa abyssinica, Convolvulus siculus, Crassocephalum bojeri, Crinum yemense, Helichrysum foetidum, Huernia macrocarpa, kalanchoe yemensis, Onychium melanolepis, Pegolettia senegalensis, Vermifrux abyssinica, Campanula edulis, Convolvulus sagittatus, Craterostigma pumilum, Pentas lanceolata, Rhus retinorrhoea, Solanum villosum, Pavonia Spec.1, Cyclamen Spec.1, Lotus Spec.1, Reseda Spec.1, Ecbolium Spec.1, Orobanche Spec.1, Picris Spec.1, Rhynchosia Spec.1, Eulophia Spec.1, Silene Spec.1*

Euphorbia parciramulosa - Tragus racemosus community

This community (C5, 9 sites; see Table 3) was found within a narrow altitudinal gradient with mean 2061 ± 112 m. s.d.; There is a distinctive tree layer dominated by *Euphorbia parciramulosa* which is ubiquitous in this community and *Tragus racemosus;* both have high indicator value scores. Compared to C6 and C7, slopes start to be more variable and sites are dominated by fertile clay soils with different depth and high to moderate pasture quality. Other

frequent species are *Cenchrus ciliaris, Plectranthus hyemalis, Euphorbia schimperi* and *Acacia hocki,* while *Acacia gerrardii* appears occasionally.

Table 2: Abbreviated floristic table for the *Rumex nervosus – Euphorbia Helioscopia* community.

Plant community No.	6	6	6	6	6	6	6	6	6	6		
Plant community variant	a	a	b	b	b	b	c	c	c	c		
Relevé No.	0	0	2	2	9	9	0	1	1	2		
	3	4	4	6	2	3	7	3	4	7		
Altitude (/ 10 m)	1	1	2	2	2	2	2	2	2	2		
	8	8	3	3	6	4	1	0	0	4		
	1	2	2	6	1	2	5	8	9	4		
Slope (x 10%)	1	2	1	2	4	3	0	3	3	3		
Vegetation Layer	2	3	3	2	2	4	3	4	3	2		
Soil depth	4	3	4	4	2	3	4	2	3	1		
Soil type	3	3	4	4	4	4	4	4	4	3		
Soil Fertility	2	2	4	4	4	4	3	3	3	3		
Pasture quality	2	1	3	3	3	2	3	3	2	2		
Stone cover	3	3	1	1	2	2	3	2	3	3		
Abandoned land	1	1	1	1	0	0	1	0	0	0		
Faithful and differential taxa:											**Max.**	**Freq.**
Rumex nervosus	2	0	5	4	2	4	0	3	3	2	**5**	**80**
Euphorbia Helioscopia	0	0	2	2	2	0	0	2	2	2	**2**	**60**
Acanthus arboreus	3	0	3	5	2	2	0	0	3	0	**5**	**60**
Commelina forskalei	2	2	2	2	0	0	2	2	2	2	**2**	**80**
Acanthus spira	2	2	2	0	0	2	2	0	2	2	**2**	**70**
Ficus palmata	0	0	3	0	2	2	0	0	0	2	**3**	**40**
Malva parviflora	0	0	2	2	0	2	2	0	0	0	**2**	**40**
Acacia yemenensis	0	0	0	0	0	0	3	2	0	0	**3**	**20**
Centaurea pseudosinaica	0	0	0	0	2	0	0	2	2	0	**2**	**30**
Other taxa:												
Rhynchosia minima	0	0	0	2	0	0	1	4	2	2	**4**	**50**
Pennisetum villosum	0	0	7	4	0	2	0	2	0	0	**7**	**40**
Cyperus rotundus	2	2	0	0	0	0	2	2	2	2	**2**	**60**
Senecio hadiensis	4	0	0	0	0	0	4	3	4	8	**8**	**50**
Digitaria Spec.1	5	2	0	0	0	0	2	2	0	0	**5**	**40**
Acacia gerrardii	5	5	0	0	3	2	0	0	0	0	**5**	**40**

Additional taxa with three or less occurrences: *Euphorbia schimperi , Solanum nigrum, Conyza pyrrhopappa, Arisaema flavum, Portulaca oleracea , Solanum incanum, Carissa edulis, Euphorbia granulata, Plectranthus hyemalis, Andropogon distachyos, Micromeria imbricata, Cynoglossum lanceolatum, Orobanche cernua, Verbascum decaisneanum, Oxalis corniculata, Hibiscus vitifolius, Citrullus colocynthis, Euphorbia parciramulosa, Eragrostis pilosa, Opuntia ficus-indica, Acacia origena, Indigofera spinosa, Euphorbia ammak, Cadia purpurea, Digitaria abyssinica, Plectranthus barbatus, Jasminum grandiflorum, Blepharis ciliaris, Zizyphus spina-christi, Tribulus terrestris, Withania somnifera, Euphorbia cactus, Digitaria velutina, Amaranthus spinosus, Chenopodium schraderianum, Cotyledon orbiculata, Commelina communis, Scadoxus multiflorus, Ipomoea nil, pluchea dioscoridis, Nepeta deflersiana, Corchorus depressus, Arthraxon micans, Pennisetum setaceum, Veronica opaca, Helichrysum foetidum, Chenopodium ambrosioides, Cenchrus ciliaris, Selaginella yemensis, Cissus rotundifolia, Commicarpus plumbagineus, Tragus racemosus, Ruellia patula, Themeda triandra, Aloe vacillans, Setaria verticillata, Solanum sibicola, Cassia italica, Snowdonia polystachya, Dodonaea viscosa, Portulaca quadrifida, Xanthium strumarium, Abutilon fruticosum, Caralluma plicatiloba, Acokanthera schimperi, Pulicaria arabica, Convolvulus arvensis, Ficus sycomorus, Galium spurium, Cistanche phelypoea, Enicostemma verticillare, Actinopteris semiflabellata.*

Additional taxa with three or less occurrences continiue: *Anagallis arvensis, Cheilanthes coriacea, Cluytia myricoides, Medicago critica, Torilis arvensis, poa annua, Rhynchosia usambarensis, Rosa abyssinica, Ecbolium viride, Galinsoga parviflora, Tephrosia uniflora, Commicarpus fruticosus, Convolvulus siculus, Cordia africana, Geranium ocellatum, Launaea capitata, Onychium melanolepis, Ageratum conyzoides, Chenopodium murale, Achyranthes aspera, Lactuca serriola, Setaria flavidum, Rhus natalensis, Rhynchosia Spec.1, Silene Spec.1, Reichardia Spec.1, Solanum Spec.1, Solanum Spec.1, Geranium Spec.1*

Acacia gerrardii - Solanum incanum community

This vegetation type (C4, 21 sites; see Table 4) occurred most often with a very distinctive and constant vegetation composition in a narrow elevation zone of mean 1977 ± 134 m. s.d. (cf. Fig. 6). The tree layer is dominant in all sites with big canopies. We have mostly less clay alluvial soil with high variability in depth, fertility and pasture quality. This community is easily characterized by the tree *Acacia gerrardii*, which is almost ubiquitous, accompanied by the shrub *Solanum incanum* which has also a high indicator value and is widely spread in most of the samples. To a lower extent *Euphorbia inarticulata, Withania somnifera, Kleinia odora* and *Boerhavia diffusa* are markedly bound to this community. Frequent field layer species in this community are *Pergularia daemia, Seddera arabica, Marrubium vulgare, Fagonia indica* and *Pulicaria petiolaris*. *Cenchrus ciliaris* and *Digitaria abyssinica* are also rather common in this community. Worth to mention are some important, but less frequent species restricted to this

community, among them *Cometes abyssinica, Melhania philippine, Pulicaria jaubertii* and *Leucas inflata* which appeared in only three samples each; indicating that this community is a safeguard to other important species.

Table 3: Abbreviated floristic table for the *Euphorbia parciramulosa – Tragus racemosus* community

Plant community No.	5	5	5	5	5	5	5	5	5		
Plant community variant											
Relevé No.	2	3	3	3	3	3	3	3	5		
	8	0	1	2	3	4	6	8	0		
Altitude (/ 10 m)	2	2	2	2	2	2	1	2	1		
	0	1	1	1	1	1	9	2	8		
	3	0	2	1	5	2	9	1	2		
Slope (x 10%)	5	1	5	2	6	4	1	3	4		
Vegetation Layer	2	3	3	3	2	2	3	3	3		
Soil depth	2	3	3	2	3	2	3	4	4		
Soil type	4	4	4	4	3	3	3	4	3		
Soil Fertility	4	3	4	4	4	3	2	4	3		
Pasture quality	3	2	3	3	2	2	1	3	2		
Stone cover	2	3	2	2	2	2	2	1	1		
Abandoned land	0	0	0	0	0	0	1	1	0		
Faithful and differential taxa:										**Max.**	**Freq.**
Euphorbia parciramulosa	8	8	7	8	7	7	8	7	7	**8**	**100**
Tragus racemosus	2	0	0	2	2	2	2	2	2	**2**	**78**
Cenchrus ciliaris	2	2	2	2	0	2	2	2	2	**2**	**89**
Acacia hocki	0	5	3	0	0	0	0	3	3	**5**	**44**
Plectranthus hyemalis	3	2	2	0	0	7	0	2	3	**7**	**67**
Other taxa:											
Eragrostis pilosa	2	2	3	0	0	2	0	2	2	**3**	**67**
Hellotropium longiflorum	2	2	3	0	0	3	5	0	2	**5**	**67**
Euphorbia schimperi	0	5	0	0	0	5	4	2	7	**7**	**56**
Selaginella yemensis	4	3	0	0	2	2	0	3	0	**4**	**56**
Lantana rugosa thunb	0	1	2	5	0	2	0	2	0	**5**	**56**
Plectranthus barbatus	0	2	2	2	0	0	0	0	2	**2**	**44**
Blepharis ciliaris	0	0	0	0	0	2	2	4	2	**4**	**44**
Commicarpus plumbagineus	0	0	0	0	1	3	2	0	4	**4**	**44**
Tribulus terrestris	0	0	0	2	0	2	2	0	2	**2**	**44**
Ruttya fruticosa	2	2	1	4	0	0	0	0	0	**4**	**44**

Additional taxa with three or less occurrences: *Senecio hadiensis, Indigofera spinosa, Acanthus spira, Kleinia odora, Cadia purpurea, Solanum incanum, Cyperus rotundus, Jasminum grandiflorum, Kalanchoe glaucescens, Sansevieria forskaliana, Commiphora habessinica, Diplotaxis kohlaanensiss, Acacia gerrardii, Rhynchosia minima, Themeda triandra, Aloe vacillans, Indigofera arabica, Micromeria imbricata, Hibiscus vitifolius, Rhynchelytrum repens, Grewia tenax, Chenopodium schraderianum, Commicarpus helini, Gomphocarpus fruticosus, Commicarpus grandiflorus, Portulaca quadrifida, Portulaca oleracea, Priva cordifolia, Barleria proxima, Pavonia arabica, Ceropegia aristolochioides, Melhania incana, Indigofera costata, Crinum yemense, Ipomoea Cairica, Opuntia ficus-indica, Euphorbia ammak, Andropogon distachyos, Digitaria abyssinica, Acanthus arboreus, Rumex nervosus, Bothriochloa insculpta, Oxalis corniculata, Pennisetum villosum, Commelina forskalei, Justicia odora, Hibiscus deflersii, Bromus leptoclados, Conyza pyrrhopappa, Euphorbia cactus, Dactyloctenium aegyptium, Maytenus senegalensis, Tetrapogon villosus, Amaranthus spinosus, Eragrostis papposa, Setaria viridis, Seddera arabica, Becium filamentosum, Solanum sibicola, Euphorbia Helioscopia, Cynoglossum lanceolatum, pergularia daemia, Cotyledon orbiculata, Rhamnus staddo, Monotheca boxifolia, Xanthium strumarium, Ipomoea eriocarpa, Adenia venenata, Indigofera amorphoides, Melhania velutina, Cucumis prophetarum, Argemone mexicana, Launaea capitata, Rhynchosia buramenisis, Convolvulus Sagittatus, Phagnalon scalarum, Pavonia Spec.1,Cyclamen Spec.1, Ecbolium Spec.1, Cucumis Spec.1, Solanum Spec.1, Crotalaria Spec.1, Erodium Spec.1, Eulophia Spec.1*

Cadia purpurea – Selaginella yemensis community

This heterogeneous community (C3, 14 sites; see Table 5) is typical transitional vegetation with the highest species richness, but the lowest indication value among the seven groups. Unlike the previous one, this community has a wide elevation range of mean 1871 ± 234 m s.d. and a high variability in soil depth and soil type. Some of the sites are with low clay, hence low fertility and pasture quality. The vegetation is mostly shrubby and can be characterised by the relatively high prevalence of *Grewia erythraea, Selaginella yemensis, Cadia purpurea, Maytenus senegalensis* and *Kalanchoe glaucescens.* To a lower extent a good association of *Barleria trispinosa, Cyperus rotundus* and *Eragrostis pilosa* to this community was found. *Acokanthera schimperi* and *Heteropogon contortus* were found in five samples, four of them belong to this community.

Table 4: Abbreviated floristic table for the *Acacia gerrardii - Solanum incanum* community

Plant community No.	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4			
Plant community variant																							
Relevé No.	4	4	8	8	9	0	4	5	5	5	6	6	6	6	8	8	8	8	8	9	9		
	7	9	2	4	4	1	8	4	5	6	2	3	4	5	5	6	7	8	9	0	1		
Altitude (/ 10 m)	1	1	1	1	2	1	1	2	1	1	2	2	1	1	2	1	1	2	2	2	2		
	8	9	7	9	1	7	9	0	9	8	2	1	9	8	0	9	9	0	0	0	1		
	0	5	8	4	1	4	2	4	4	4	6	9	7	6	3	2	6	2	4	7	4		
Slope (x 10%)	8	7	2	2	2	3	3	1	5	2	4	1	0	6	0	5	0	1	3	2	2		
Vegetation Layer	4	4	4	4	4	3	4	4	3	3	4	3	4	3	4	3	3	3	3	4	4		
Soil depth	4	4	4	3	3	3	4	3	1	2	3	4	4	2	2	2	4	2	1	4	4		
Soil type	4	4	3	3	4	3	4	3	3	3	3	4	4	3	3	3	3	3	3	4	3		
Soil Fertility	4	4	4	3	4	2	4	4	2	3	4	4	4	3	2	3	4	1	2	3	1		
Pasture quality	3	3	3	3	3	2	3	3	2	2	3	3	3	2	1	2	2	1	2	3	1		
Stone cover	2	1	1	2	1	1	1	2	3	2	1	1	1	2	2	2	1	2	3	1	1		
Abandoned land	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1		
Faithful and differential taxa:																						**Max.**	**Freq.**
Acacia gerrardii	8	8	0	0	7	7	7	8	7	5	8	7	7	7	9	7	5	7	7	9	8	**9**	**90**
Solanum incanum	2	0	2	2	2	0	2	0	0	0	2	0	2	2	2	2	2	0	2	2	2	**2**	**67**
pergularia daemia	0	0	0	0	2	1	0	0	0	0	0	0	0	2	0	2	0	0	0	2	2	**2**	**29**
Euphorbia inarticulata	0	0	0	0	2	0	0	4	3	7	0	0	0	0	0	2	7	5	5	2	5	**7**	**48**
Kleinia odora	0	0	2	0	2	1	3	1	0	4	0	0	4	2	0	0	2	3	2	0	0	**4**	**52**
Digitaria abyssinica	2	3	0	2	0	0	2	0	0	2	3	2	2	0	2	0	0	0	0	0	0	**3**	**43**
Withania somnifera	2	0	2	2	0	1	0	0	0	0	0	2	2	0	0	0	0	0	1	2	2	**2**	**43**
Solanum sibicola	2	0	2	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	2	**2**	**29**
Marrubium vulgare	2	0	2	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	2	**2**	**29**
Fagonia indica	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0	0	0	0	**2**	**19**
pulicaria petiolaris	0	0	0	2	2	0	0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	**2**	**24**
Farsetia longisiliqua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	0	0	**2**	**19**
Cometes abyssinica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0	0	**2**	**14**
Melhania philippine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0	0	0	0	**2**	**14**
Pulicaria jaubertii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0	0	0	**2**	**14**
Leucas inflata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	0	0	0	**2**	**14**
Other taxa:																							
Cenchrus ciliaris	0	0	2	2	2	0	0	2	2	2	2	2	2	2	2	2	2	0	0	2	0	**2**	**67**
Hellotropium longiflorum	0	0	2	0	0	0	0	2	2	2	0	0	0	2	0	2	2	2	2	0	2	**2**	**48**
Eragrostis pilosa	0	2	0	0	0	0	0	2	2	2	2	2	2	2	0	0	0	0	2	0	0	**2**	**43**
Indigofera spinosa	0	0	0	0	0	3	0	2	0	2	0	0	2	2	0	0	2	2	2	2	0	**3**	**43**
Euphorbia schimperi	0	0	0	0	0	0	0	5	3	0	3	5	0	2	0	0	3	2	0	0	0	**5**	**33**
Acanthus spira	2	0	2	2	2	0	0	0	0	0	0	0	0	0	7	0	0	0	0	5	2	**7**	**33**
Plectranthus barbatus	2	2	0	0	0	1	2	0	0	0	2	2	2	0	0	0	0	0	0	0	0	**2**	**33**
Oxalis corniculata	2	2	0	2	2	0	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0	**2**	**33**
Hibiscus deflersii	0	0	0	0	0	1	0	0	1	1	0	0	2	2	0	0	2	0	2	0	0	**2**	**33**
Cadia purpurea	0	0	0	0	2	0	3	0	0	0	1	2	0	2	0	0	2	0	0	0	0	**3**	**29**
Lantana rugosa thunb	0	0	0	2	2	0	0	0	0	0	2	2	2	0	0	0	0	0	1	0	0	**2**	**29**
Commicarpus plumbagineus	2	2	0	2	0	0	0	3	0	0	0	0	0	0	2	2	0	0	0	0	0	**3**	**29**
Setaria verticillata	0	2	2	2	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	2	**2**	**29**
Themeda triandra	0	2	0	0	0	0	0	0	1	2	2	2	0	0	0	0	0	0	0	0	0	**2**	**24**
Hibiscus vitifolius	0	0	0	2	0	2	2	0	1	0	0	0	0	0	0	0	0	0	0	2	0	**2**	**24**
Tetrapogon villosus	0	0	0	0	0	0	0	2	0	2	0	2	0	2	0	0	0	0	2	0	0	**2**	**24**
Rhynchelytrum repens	0	0	1	0	2	0	0	2	2	0	0	2	0	0	0	0	0	0	0	0	0	**2**	**24**
Becium serpyllifolium	0	0	0	0	0	0	0	0	1	0	3	2	0	0	0	2	0	0	1	0	0	**3**	**24**
Diplotaxis kohlaanensiss	0	0	0	0	0	0	0	2	1	0	2	1	0	2	0	0	0	0	0	0	0	**2**	**24**
Cucumis Spec.1	2	0	0	0	0	1	0	0	0	0	0	0	4	2	0	0	0	0	0	0	2	**4**	**24**
Euphorbia parciramulosa	0	0	0	0	0	0	0	0	0	0	4	8	5	5	0	0	0	0	0	0	0	**8**	**19**
Opuntia ficus-indica	0	0	0	0	2	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	7	**7**	**19**
Kalanchoe glaucescens	0	3	0	0	0	0	2	0	0	0	2	2	0	0	0	0	0	0	0	0	0	**3**	**19**
Bothriochloa insculpta	0	0	2	0	0	0	0	0	0	0	3	0	2	2	0	0	0	0	0	0	0	**3**	**19**
Commelina forskalei	2	0	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**2**	**19**
Tragus racemosus	3	2	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	**3**	**19**
Pavonia Spec.1	2	0	2	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	**2**	**19**
Aloe vacillans	0	0	0	0	0	0	0	0	0	0	2	2	0	3	0	0	0	2	0	0	0	**3**	**19**

Maeroa triphylla	0	0	0	0	0	0	0	1	3	0	0	0	2	3	0	0	0	0	0	0	0	**3**	**19**
Eragrostis papposa	0	0	2	0	0	0	0	0	0	3	0	0	0	1	2	0	0	0	0	0	0	**3**	**19**
Aerva javanica	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	**2**	**19**
Pulicaria arabica	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0	0	**2**	**19**

Additional taxa with three or less occurrences: *Cyperus rotundus, Selaginella yemensis, Blepharis ciliaris, Zizyphus spina-christi, Plectranthus hyemalis, Rhynchosia minima, Justicia odora, Tribulus terrestris, Acalypha fruticosa, Solanum nigrum, Conyza pyrrhopappa, Maytenus senegalensis, Amaranthus spinosus, Panicum Maximum, Grewia tenax, Chenopodium schraderianum, Peristrophe paniculata, Cassia italica, Sageretia thea, Enicostemma axillare, Caralluma plicatiloba, Ipomoea eriocarpa, Andropogon distachyos, Jasminum grandiflorum, Acacia hocki, Acanthus arboreus, Rumex nervosus, Pennisetum villosum, Sansevieria forskaliana, Carissa edulis, Indigofera arabica, Micromeria imbricata, Dactyloctenium aegyptium, Setaria viridis,Commicarpus helini, Ormocarpum yemenense, Seddera arabica, Becium filamentosum, Gomphocarpus fruticosus, Cynoglossum lanceolatum, Alternanthera pungens, Rhamnus staddo, Commelina communis, Abutilon fruticosum, Lycium shawii, Priva cordifolia, Ipomoea nil, pluchea dioscoridis, Nepeta deflersiana, Cistanche phelypoea, Pelargonium alchemilloides, Celosia trigyna, Justicia heterocarpa, Commelina albescens, Dipcadi eurytherm, Ginidia somalensis, lavandula pubescens, Cleome schweinfurthii, Trichodesma Microcalyx, Senecio hadiensis, Acacia origena, Cissus rotundifolia, Ruellia patula, Acacia mellifera, Arisaema flavum, Flaveria trinervia, Panicum acuminatum, Blepharis maderaspatensis, Snowdonia polystachya, Arthraxon Prinodes, Dodonaea viscosa, Ruttya fruticosa, Vernonia bottae, Ficus palmata, Portulaca oleracea, Barleria bispinosa, Orobanche cernua, Cotyledon barbeyi, Ipomoea obscur, Malva parviflora, Scadoxus multiflorus, Euphorbia granulata, Oplismenus hirtellus, Orthosiphon pallidus, Convolvulus arvensis, Ficus salicifolia, Pavonia kotschyi, Adenia venenata, Amaranthus Graczance, Ehretia cymosa, Indigofera amorphoides, Indigofera articulata, Enicostemma verticillare, Cluytia myricoides, Dorstenia foetida, Melhania velutina, Phyllanthus tenellus, poa annua, Rhynchosia usambarensis, Striga gesneroioides, Ecbolium viride, Tephrosia uniflora, Argemone mexicana, Aspragus africanus, Commicarpus sinuatus, Crassocephalum bojeri, Heliotropium curassavicum, Bromus leptoclados, Setaria flavidum, Huernia macrocarpa, Pegolettia senegalensis, Vermifrux abyssinica, Achyranthes aspera, Campanula edulis, Capparis cartilaginea, Echinochloa colonum, Echinops squamulata, Lactuca serriola, Pavonia flavo-ferruginea, Phagnalon scalarum, Solanum villosum, Sonchus oleraceus, Moss Spec. 1, Reichardia Spec.1, Bouteloua Spec.1, Solanum Spec.1, Reichardia Spec.2, Phagnalon Spec.1, Digitaria Spec.1, Blepharis Spec.1, Solanum Spec.1, Indigofera Spec.4, Lantana Spec.1, Ecbolium Spec.2, Echinochloa Spec.1, Picris Spec.1, Lotus Spec.1*

Acacia mellifera - Cissus rotundifolia community

This group (C2, 10 sites; see Table 6) is the lowest in species richness. Despite the wide elevation range of mean 1591 ± 274 m. s.d. (with a minimum of 1022 m), we have a clear tree layer and moderate to low soil depth and clay content and mostly low pasture quality. This community is well distinguished. The two common tree species *Acacia mellifera* (with a reasonably high indicator value) and *Acacia asak* are restricted to this community, *Cissus rotundifolia* is almost ubiquitous. Generally shrubs and succulent climbers dominate the field layer. In the gaps left by *Cissus rotundifolia* small shrubs appear, especially *Indigofera spinosa, Hellotropium longiflorum* and *Justicia odora*, while grasses and herbs clearly becomes less common which could be attributed to the lower soil moisture and higher temperatures.

Acacia etbaica – Euphorbia inarticulata community

This well recognized vegetation type (C1, 14 sites; see Table 7) was found at the lower elevation boundary of *C. digitatum*. It appeared in our study within a narrow elevation zone with mean 1530 ± 135 m. s.d., being similar to the previous group C2. The tree layer is dominant, again we have moderate to low soil depth and clay and mostly a good pasture quality. *Acacia etbaica* is ubiquitous, as it is reported in the entire community. The most frequent big shrubs are *Euphorbia inarticulata* and *Acalypha fruticosa.* The field layer was distinctive with *Ruellia patula, Pupalia lappacea, Blepharis ciliaris, Sansevieria forskaliana, Tribulus terrestris* and *Gisekia pharnaceoides*. Interestingly the grass layer is characterised by the giant grass *Panicum maximum* and by *Dactyloctenium aegyptium.* The succulent climbers *Cissus quadringulata, Cissus rotundifolia* and the intruder herb *Alternanthera pungens* appeared less frequently.

Pairwise tightness coefficients and similarities between different communities are given in Table 9. Tightness coefficients vary between 0.03 and 0.10 (all *P* values being less than 0.0001) and indicate that there is much less heterogeneity within groups than just expected by chance.

Table 5: Abbreviated floristic table for the *Cadia purpurea – Selaginella yemensis* community

Plant community No.	3	3	3	3	3	3	3	3	3	3	3	3	3	3		
Plant community variant																
Relevé No.	1	3	3	5	5	5	9	2	4	5	6	6	7	7		
	2	5	7	2	3	9	8	9	3	1	8	9	3	5		
Altitude (/ 10 m)	1	2	2	2	2	1	1	2	1	2	2	1	1	1		
	5	0	0	2	0	7	6	0	5	1	0	7	7	6		
	5	0	6	0	6	4	7	8	6	2	8	2	5	2		
Slope (x 10%)	3	3	1	1	4	0	4	3	2	5	1	3	1	0		
Vegetation Layer	2	2	3	3	3	2	3	3	3	3	4	3	2	2		
Soil depth	1	3	3	1	1	4	1	2	2	3	3	2	4	3		
Soil type	1	3	4	1	1	4	1	4	3	4	4	3	3	3		
Soil Fertility	1	3	4	2	3	4	1	4	4	4	3	4	4	3		
Pasture quality	2	2	3	2	3	3	1	3	3	3	2	2	3	3		
Stone cover	3	2	1	3	2	1	3	1	3	2	2	2	1	1		
Abandoned land	0	0	1	0	0	1	0	0	0	0	0	0	1	1		
Faithful and differential taxa:															**Max.**	**Freq**
Cadia purpurea	0	4	3	0	4	4	2	2	0	3	0	3	2	0	**4**	**64**
Euphorbia schimperi	2	7	8	0	5	4	0	4	0	0	0	0	0	0	**8**	**43**
Selaginella yemensis	0	2	0	2	3	2	2	0	0	2	2	2	0	0	**3**	**57**
Acacia hocki	0	5	7	0	0	0	4	0	0	1	2	0	0	5	**7**	**43**
Maytenus senegalensis	1	0	0	0	4	0	0	0	0	2	2	3	0	3	**4**	**43**
Kalanchoe glaucescens	0	2	2	0	2	2	0	2	0	2	2	1	0	0	**2**	**57**
Eragrostis pilosa	0	2	0	2	5	2	2	2	2	2	0	2	2	0	**5**	**71**
Cyperus rotundus	0	0	2	0	2	2	0	2	0	2	2	2	2	0	**2**	**57**
Rhynchosia minima	0	2	2	0	0	0	0	2	2	2	2	0	0	2	**2**	**50**
Pavonia Spec.1	0	2	2	0	0	2	0	0	2	2	2	0	2	0	**2**	**50**
Heteropogon contortus	0	0	0	0	2	2	0	0	0	0	0	2	2	0	**2**	**29**
Opuntia ficus-indica	0	1	0	0	0	0	0	2	0	0	4	8	5	7	**8**	**43**
Acokanthera schimperi	2	0	0	0	0	0	0	3	0	1	0	0	2	0	**3**	**29**
Cyclamen Spec.1	0	0	0	0	0	2	0	2	0	2	2	0	0	0	**2**	**29**
Plectranthus Spec.2	0	0	0	0	0	0	2	2	0	0	0	0	0	2	**2**	**21**
Other taxa:																
Euphorbia ammak	0	0	0	0	0	0	0	7	7	8	8	0	0	0	**8**	**29**
Plectranthus hyemalis	0	0	0	0	0	2	0	3	2	2	0	2	0	0	**3**	**36**
Solanum nigrum	0	0	0	0	2	0	0	2	0	0	2	0	2	2	**2**	**36**
Jasminum grandiflorum	2	0	0	0	0	0	0	0	0	0	2	2	2	0	**2**	**29**
Hibiscus deflersii	1	0	0	0	0	0	0	1	0	0	2	0	0	2	**2**	**29**
Blepharis maderaspatensis	0	2	0	0	0	0	0	2	0	2	0	0	2	0	**2**	**29**
Hellotropium longiflorum	2	2	0	2	0	0	2	0	0	2	0	0	0	0	**2**	**36**
Euphorbia inarticulata	4	0	0	7	5	7	5	2	0	0	0	0	0	0	**7**	**43**
Justicia odora	0	0	0	2	2	2	0	0	2	0	0	0	0	0	**2**	**29**
Themeda triandra	0	2	3	2	2	0	0	0	0	0	0	0	0	0	**3**	**29**
Cenchrus ciliaris	2	2	2	2	0	0	0	0	0	2	0	2	2	0	**2**	**50**
Indigofera spinosa	2	3	0	0	0	2	2	0	2	0	0	2	0	2	**3**	**50**
Lantana rugosa thunb	0	2	3	0	0	2	2	0	2	1	0	2	0	0	**3**	**50**
Plectranthus barbatus	0	2	2	0	0	2	1	3	0	0	0	2	0	0	**3**	**43**
Blepharis ciliaris	2	0	2	0	0	0	2	0	2	0	0	2	2	0	**2**	**43**
Bothriochloa insculpta	0	2	0	2	0	3	2	0	0	2	0	0	2	0	**3**	**43**
Senecio hadiensis	0	3	0	0	0	0	1	3	0	0	5	2	0	0	**5**	**36**
Digitaria abyssinica	0	0	2	0	0	3	0	0	0	0	2	2	2	0	**3**	**36**
Tragus racemosus	0	0	0	0	3	0	2	0	2	0	2	0	2	0	**3**	**36**
Ruellia patula	2	0	2	0	0	2	0	0	2	0	0	0	2	0	**2**	**36**

Additional taxa with three or less occurrences: *Acacia gerrardii, Kleinia odora, Solanum incanum, Zizyphus spina-christi, Commicarpus plumbagineus, Oxalis corniculata, Commelina forskalei, Sansevieria forskaliana, Tribulus terrestris, Withania somnifera, Indigofera arabica, Arisaema flavum, Commiphora habessinica, Dactyloctenium aegyptium, Rhynchelytrum repens, Setaria verticillata, Ormocarpum yemenense, Flaveria trinervia, Dodonaea viscosa, Cassia occidentalis, Acanthus spira, Andropogon distachyos, Cissus rotundifolia, Acalypha fruticosa, Euphorbia cactus, Maeroa triphylla, Digitaria velutina, Amaranthus spinosus, Chenopodium schraderianum, Commicarpus helini, Becium serpyllifolium, Peristrophe paniculata, Seddera arabica, Becium filamentosum, Gomphocarpus fruticosus, Sageretia thea, pergularia daemia, Cotyledon orbiculata, Ruttya fruticosa, Xanthium strumarium, Lycium shawii, Priva cordifolia, Caralluma penicillata, Euphorbia hirta, Amaranthus Graczance, Actinopteris semiflabellata, Polygala tinctoria, Striga gesneroioides, Indigofera hochstetteri, Gladiolus dalenii, Acacia etbaica, Pennisetum villosum, Carissa edulis, Conyza pyrrhopappa, Hibiscus vitifolius, Tetrapogon villosus, Eragrostis papposa, Panicum Maximum, Setaria viridis, Diplotaxis kohlaanensiss, Panicum acuminatum, Cassia italica, Alternanthera pungens, Marrubium vulgare, Enicostemma axillare, Rhamnus staddo, Snowdonia polystachya, Cissus quadringulata, Setaria flavidum, Commelina communis, Monotheca boxifolia, Portulaca quadrifida, Sansevieria ehrenbergii, Bromus leptoclados, Caralluma plicatiloba, Adenium obesum, Barleria bispinosa, Ipomoea eriocarpa, Orobanche cernua, Ipomoea obscur, Ruellia praetermissa, Oplismenus hirtellus, Acacia yemenensis. Zingeria trichopoda, Celtis africana, Pavonia kotschyi, Andropogon Greenwayi, Cassia tora, Ceropegia aristolochioides*

Additional taxa with three or less occurrences continiue: *Indigofera amorphoides, Pelargonium alchemilloides, Corchorus depressus, Aloe sabaea, Anagallis arvensis, celosia trigyna, Cheilanthes coriacea, Pennisetum setaceum, Citrullus colocynthis, Dipcadi eurytherm, Dorstenia foetida, Eleusine indica, Indigofera costata, Rhynchosia usambarensis, Cucumis melo, Cucumis prophetarum, Aspragus africanus, Indigofera oblongifolia, Acacia nilotica, Aloe inermis, Chenopodium murale, Pavonia flavo-ferruginea, Moss Spec. 1, Bouteloua Spec.1, Indigofera Spec.4, Orobanche Spec.1, Blepharis Spec.1, Micromeria Spec.1, Ecbolium Spec.2, Echinochloa Spec.1, Crotalaria Spec.1, Erodium Spec.1*

Table 6: Abbreviated floristic table for the *Acacia mellifera - Cissus rotundifolia* community

Plant community No.	2	2	2	2	2	2	2	2	2	2		
Plant community variant												
Relevé No.	0	1	3	5	5	7	7	8	9	9		
	6	9	9	7	8	8	9	1	5	9		
Altitude (/ 10 m)	1	1	1	1	1	1	1	1	1	1		
	7	4	0	8	7	7	7	6	2	7		
	9	5	2	4	8	0	2	1	3	6		
Slope (x 10%)	2	7	5	0	2	0	4	6	2	4		
Vegetation Layer	3	2	3	2	2	3	3	3	4	3		
Soil depth	2	2	1	1	2	3	1	1	3	1		
Soil type	3	1	1	3	3	3	1	2	3	1		
Soil Fertility	2	2	2	1	3	3	2	1	4	2		
Pasture quality	1	2	1	1	2	3	1	1	3	2		
Stone cover	2	2	3	3	2	2	3	3	2	3		
Abandoned land	0	0	0	0	0	0	0	0	0	0		
Faithful and differential taxa:											**Max.**	**Freq.**
Acacia mellifera	3	0	5	5	5	0	0	2	3	4	**5**	**70**
Cissus rotundifolia	5	5	4	4	3	4	3	2	3	0	**5**	**90**
Acacia asak	0	5	0	0	0	5	8	5	0	0	**8**	**40**
Indigofera spinosa	2	0	2	2	2	2	0	2	2	2	**2**	**80**
Hellotropium longiflorum	2	0	2	0	2	2	2	0	2	2	**2**	**70**
Justicia odora	0	0	2	2	2	2	0	0	0	2	**2**	**50**
Other taxa:												
Cenchrus ciliaris	0	0	0	2	2	2	0	1	0	2	**2**	**50**
Kleinia odora	2	2	0	0	3	0	4	0	0	2	**4**	**50**
Ruellia patula	2	0	2	0	0	0	1	0	2	2	**2**	**50**
Euphorbia inarticulata	0	0	0	3	4	7	0	0	2	0	**7**	**40**
Euphorbia schimperi	2	0	0	0	2	0	0	2	2	0	**2**	**40**
Solanum incanum	2	0	0	1	0	2	1	0	0	0	**2**	**40**
Blepharis ciliaris	0	2	0	2	0	0	0	0	2	2	**2**	**40**
Bothriochloa insculpta	0	0	0	0	2	0	2	2	2	0	**2**	**40**

Additional taxa with three or less occurrences: *Acacia etbaica, Eragrostis pilosa, Carissa edulis, Hibiscus deflersii, Acalypha fruticosa, Commiphora habessinica, Tetrapogon villosus, Ormocarpum yemenense, Becium filamentosum, Rhamnus staddo, Cyperus rotundus, Lantana rugosa thumb, Zizyphus spina-christi, Kalanchoe glaucescens, Commicarpus plumbagineus, Sansevieria forskaliana, Hibiscus vitifolius, Maeroa triphylla, Setaria viridis, Peristrophe paniculata, Seddera Arabica, Flaveria trinervia, Panicum acuminatum, Cassia italica, Enicostemma axillare, Arthraxon Prinodes, Commelina communis, Portulaca quadrifida, Sansevieria ehrenbergii, Caralluma plicatiloba, Aerva javanica, Adenium obesum, Caralluma penicillata, Orthosiphon pallidus, Euphorbia hirta, Andropogon Greenwayi, Crossandra wissmanii, Acacia gerrardii, Cadia purpurea, Digitaria abyssinica, Selaginella yemensis, Plectranthus hyemalis, Rumex nervosus, Pennisetum villosum, Commelina forskalei, Tribulus terrestris, Themeda triandra, Conyza pyrrhopappa, Indigofera Arabica, Micromeria imbricate, Dactyloctenium aegyptium, Digitaria velutina, Eragrostis papposa, Panicum Maximum, Rhynchelytrum repens, Commicarpus helini, Becium serpyllifolium, Diplotaxis kohlaanensiss, Solanum sibicola, Blepharis maderaspatensis, Sageretia thea, Alternanthera ,pungens, Dodonaea viscose, Cissus quadringulata, Setaria flavidum Commicarpus grandiflorus, Ficus palmate, Barleria bispinosa, Priva cordifolia Cassia occidentalis, Cotyledon barbeyi, Euphorbia granulate, Oplismenus hirtellus, pluchea dioscoridis, Lantana camara, Zingeria trichopoda, Commiphora myrrha, Pavonia Arabica, Salvadora persica, Adenia venenata, Amaranthus Graczance, Ceropegia aristolochioides, Cistanche phelypoea.*

Additional taxa with three or less occurrences continiue: *Ehretia cymosa, Indigofera articulate, Enicostemma verticillare, Actinopteris semiflabellata, Aloe sabaea, Arthraxon micans, Anisotes trisulcus, Commelina albescens, Dorstenia foetida, Jatropha variegate, Phyllanthus tenellus, Striga gesneroioides, Euphorbia articulate, Galinsoga parviflora, Cucumis prophetarum, Aloe revery, Commicarpus sinuatus, Indigofera oblongifolia, Ruellia oblongifolia, Tridax procumbens, Ageratum conyzoides, Echinops squamulata, Bouteloua Spec.1, Digitaria Spec.1, Ecbolium Spec.1, Indigofera Spec.4, Lantana Spec.1*

The pairwise similarities range from 32% to 72% with a clear trend such that communities are less similar if the difference in mean elevation is larger (Fig. 7). The highest pairwise similarities (72%, 70%, and 63%) were obtained between *Cadia purpurea - Selaginella yemensis* group (C3) and *Acacia etbaica - Euphorbia inarticulata* group (C1), *Acacia gerrardii - Solanum incanum* group (C4) and *Euphorbia parciramulosa - Tragus racemosus* group (C5), respectively. This is because of the nature of group C3 as transitional vegetation between the LFV and the HFFV. Lowest similarities were found between groups C1/C2 on hand side and C6/C7 on the other side, where we also have the highest differences in mean elevation.

The average total plant species richness in the collected samples is 39.4 species/sample with 0.97 level of evenness and 3.55 Shannon`s diversity index, which indicates a reasonably diverse vegetation. Species richness ranges from 35.2 in the *Acacia mellifera - Cissus rotundifolia*

community (C2) to 43,8 in the transitional *Cadia purpurea – Selaginella yemensis* community (C3) which is a mixture of frost tolerant and frost intolerant species (Table 5).

Table 7: Abbreviated floristic table for the *Acacia etbaica – Euphorbia inarticulata* community:-

Plant community No.	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
Plant community variant	a	a	a	a	b	b	b	b	b	b	b	b	b	b		
Relevé No.	1	1	7	7	4	4	4	6	7	7	7	8	9	9		
	5	7	2	4	4	5	6	6	0	1	6	0	6	7		
Altitude (/ 10 m)	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	6	4	6	6	4	5	6	5	2	6	4	7	4	4		
	2	6	5	9	8	0	3	7	2	0	0	1	3	7		
Slope (x 10%)	2	4	0	2	0	1	5	2	4	2	3	2	2	7		
Vegetation Layer	2	3	3	3	3	3	3	4	3	3	3	4	3	3		
Soil depth	2	3	4	3	4	4	1	1	1	4	2	3	3	1		
Soil type	2	3	3	3	3	3	1	3	1	3	1	3	1	1		
Soil Fertility	2	3	4	3	3	3	2	4	2	3	2	3	3	1		
Pasture quality	2	2	3	3	3	3	1	3	1	2	2	2	2	1		
Stone cover	3	1	2	1	1	2	3	2	3	1	2	2	2	3		
Abandoned land	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Faithful and differential taxa:															**Max.**	**Freq.**
Acacia etbaica	4	5	5	5	7	7	5	8	7	7	7	7	7	7	**8**	**100**
Euphorbia inarticulata	5	0	0	3	0	4	4	2	4	0	2	5	5	4	**5**	**71**
Ruellia patula	0	0	2	2	2	2	2	2	0	0	0	0	2	2	**2**	**57**
Alternanthera pungens	0	2	0	0	4	0	0	0	0	2	0	0	2	0	**4**	**29**
Blepharis ciliaris	0	2	2	0	0	2	2	2	2	2	0	0	0	2	**2**	**57**
Sansevieria forskaliana	0	2	2	1	0	0	0	7	0	2	0	0	0	0	**7**	**36**
Dactyloctenium aegyptium	2	0	2	2	3	2	0	0	0	2	0	0	0	0	**3**	**43**
Tribulus terrestris	2	0	0	2	0	2	0	2	0	2	0	0	2	0	**2**	**43**
Blepharis Spec.1	1	2	0	0	2	2	0	0	0	0	1	0	0	0	**2**	**36**
Gisekia pharnaceoides	0	0	0	2	2	2	0	0	0	0	0	0	0	0	**2**	**21**
Ruellia Spec.1	0	2	0	0	0	0	2	0	0	0	0	0	0	2	**2**	**21**
Acalypha fruticosa	0	0	0	0	2	2	0	2	2	0	4	0	2	2	**4**	**50**
Cissus quadringulata	0	0	0	0	0	5	0	0	0	0	2	0	2	0	**5**	**21**
Bouteloua Spec.1	0	0	0	2	2	2	0	2	2	0	2	0	2	0	**2**	**50**
Pancratium maximum	0	0	0	1	0	2	2	1	0	0	2	0	0	0	**2**	**36**
Other taxa:																
Opuntia ficus-indica	0	0	0	0	2	3	0	2	0	0	2	0	0	2	**3**	**36**
Indigofera spinosa	0	0	0	0	0	0	2	0	2	0	2	0	2	2	**2**	**36**
Lantana rugosa thunb	0	0	0	0	2	0	0	1	0	2	0	1	0	2	**2**	**36**
Acanthus spira	0	0	0	2	2	2	0	2	0	2	0	0	2	0	**2**	**43**
Solanum incanum	0	0	2	0	0	0	0	2	0	0	2	2	2	2	**2**	**43**
Cenchrus ciliaris	0	0	2	0	2	0	0	0	2	0	0	0	2	2	**2**	**36**
Zizyphus spina-christi	0	0	0	2	0	0	0	0	0	2	2	7	0	2	**7**	**36**
Cissus rotundifolia	0	0	0	0	0	0	0	0	0	0	3	2	3	2	**3**	**29**
Commicarpus plumbagineus	0	0	0	0	0	2	0	2	0	3	0	0	2	0	**3**	**29**
Peristrophe paniculata	0	0	0	0	3	2	0	2	0	2	0	0	0	0	**3**	**29**
Amaranthus spinosus	0	0	0	0	0	2	0	2	0	2	0	0	2	0	**2**	**29**
Justicia odora	0	0	2	2	0	2	2	2	0	0	0	0	2	0	**2**	**43**
Cyperus rotundus	0	0	2	0	0	0	0	2	2	2	0	0	0	0	**2**	**29**
Jasminum grandiflorum	0	0	2	2	0	0	0	2	0	0	0	0	2	0	**2**	**29**
Kalanchoe glaucescens	0	0	0	2	0	2	0	0	0	0	0	0	2	2	**2**	**29**
Bothriochloa insculpta	0	0	2	0	0	0	0	2	2	0	0	2	0	0	**2**	**29**
Digitaria velutina	0	2	0	2	0	0	0	2	0	4	0	0	0	0	**4**	**29**
Setaria viridis	2	0	2	0	0	0	0	2	0	0	2	0	0	0	**2**	**29**
Flaveria trinervia	0	0	2	0	2	2	0	0	0	1	0	0	0	0	**2**	**29**
Panicum acuminatum	3	0	2	2	0	0	0	0	0	0	2	0	0	0	**3**	**29**
Blepharis maderaspatensis	0	0	2	0	0	2	0	0	2	2	0	0	0	0	**2**	**29**
Cotyledon orbiculata	0	2	0	0	2	2	2	0	0	0	0	0	0	0	**2**	**29**

Additional taxa with three or less occurrences: *Kleinia odora, Cadia purpurea, Plectranthus barbatus, Oxalis corniculata, Tragus racemosus, Indigofera arabica, Hibiscus vitifolius, Eragrostis papposa, Becium serpyllifolium, Seddera Arabica, Becium filamentosum, Enicostemma axillare, Arthraxon Prinodes, Abutilon fruticosum, Barleria proxima , Ipomoea obscure, Ruellia praetermissa, Eragrostis pilosa, Commelina forskalei, Hibiscus deflersii, Solanum nigrum, Withania somnifera, Euphorbia cactus, Tetrapogon villosus, Setaria verticillata, Grewia tenax, Ormocarpum yemenense, Cassia italica, Snowdonia polystachya, Monotheca boxifolia, Xanthium strumarium, Lantana camara, Celtis Africana, Cassia tora, Eleusine indica, Euphorbia schimperi, Euphorbia ammak, Andropogon distachyos, Hellotropium longiflorum, Digitaria abyssinica, Selaginella yemensis, Plectranthus hyemalis, Rhynchosia minima, Carissa edulis, Conyza pyrrhopappa, Commiphora habessinica, Rhynchelytrum repens, Commicarpus helini, Diplotaxis kohlaanensiss, Solanum sibicola, Gomphocarpus fruticosus, Pergularia daemia, Marrubium vulgare, Rhamnus staddo, Commicarpus grandiflorus, Portulaca quadrifida, Portulaca oleracea , Aerva javanica, Acokanthera schimperi, Adenium obesum, Ipomoea eriocarpa, Cassia occidentalis, Caralluma penicillata, Cotyledon barbeyi, Fagonia indica, Heteropogon contortus, Oplismenus hirtellus, Orthosiphon pallidus, Zingeria trichopoda, Commiphora myrrha, Pavonia Arabica, Pavonia kotschyi, Andropogon Greenwayi, Ehretia cymosa, Indigofera amorphoides, Indigofera articulate, Setaria flavidum, Bromus leptoclados, Indigofera amorphoides, Indigofera articulate, Melhania incana, Anisotes trisulcus, Grewia villosa, Melhania velutina, Phyllanthus tenellus, Aloe revery, Aspragus africanus, Commicarpus fruticosus, Heliotropium curassavicum, kalanchoe yemensis, Rhynchosia buramenisis, Ruellia oblongifolia, Tridax procumbens, Rhus natalensis, Sonchus oleraceus, Pavonia Spec.1, Micromeria Spec.1, Becium Spec.1, Digitaria Spec.1, Cucumis Spec.1, Solanum Spec., Lantana Spec.1, Cucumis melo, Geranium Spec.1.*

4. Discussion

In general, *Cyphostemma digitatum* is associated with very common widespread small shrubs and herbs in the study area. This result is in accordance with information obtained from local people. Additional observations from ecophysiology and phenology substantiate that *C. digitatum* has a wide ecological amplitude and may have been found in different habitats before the regional overexploitation of the species. Collectively the associational index (Table 8) can be used as a good indicator for the habitat suitability and possibility of reintroduction of *C. digitatum* to such stands and may also give clues about the possible cultivation in the future for economical purposes. Meanwhile, many species from Table 8 show very low indicator values since most of them also have broad ecological tolerances including the invader species *Tagetes minuta.* Although a more detailed classification based on a larger sample data pool might be desirable, most of the community types suggested in this study can be easily recognized from far distances since each community is characterized by a few typical trees or big shrubs and a particular physiognomy.

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**
C1		0,04	0,05	0,07	0,10	0,07	0,09
C2	63,0		0,03	0,05	0,08	0,05	0,08
C3	71,5	58,1		0,04	0,05	0,04	0,05
C4	58,7	58,2	69,5		0,06	0,04	0,07
C5	49,1	44,5	62,7	62,5		0,07	0,08
C6	37,6	37,3	49,1	55,3	53,6		0,04
C7	40,4	31,7	51,4	53,3	55,7	58,7	

Table 9: Matrix of pairwise *A* values of multi-response permutation procedure (upper triangle) and pairwise coefficients of community *CC* (lower triangle) where *A* value is chance-corrected within-group agreement and *CC* measures floristic similarity (for details see text).

The differentiation of seven communities of vegetation may at first appear to be somewhat high compared with the relatively small study area, but there are several factors that may be of influence here. Firstly, we mention the geographical location and prehistoric connection of the study area to more than two different major vegetation units. Secondly, there is a relatively high range of environmental conditions, among them variations in the altitude of study sites from 1022 to 2607 m. Our result shows a wider zone for *C. digitatum* than the estimations made by Wood (1400 – 2500 m), moreover, he reported that *C. digitatum* almost never occured in forested areas while we encountered the plant under big canopies, which confirms a wide ecological niche. Thirdly, the study area is climatically divided into frost experiencing and frost free zones, enhancing the variability of habitats and species. Fourthly, the heterogeneity of local topography, edaphic factors and microhabitat conditions may lead to variations of the distributional behaviour of *C. digitatum* and its associates. This is in accordance with Parker (1991) where the spatial distribution of plant species and communities over a small geographic area was attributed primarily to a heterogeneous topography and landform pattern. Lastly, the common occurrence of non-native species with broad ecological tolerances, such as *Tagetes minuta, Eucaliptus spec.* and *Alternanthera pungens* may result in some blurring of native communities. For example, the huge *Eucaliptus* stands generally have a very poor field layer

vegetation, which could be attributed to the well known acidity of the *Eucaliptus* leaf litters; in our case *C. digitatum* was never present in *Eucaliptus* stands.
Lots of problems were encountered in afforestation projects performed in the northern borders of the study area, in which land evaluation to allocate specific species to suitable sites encountered many difficulties and needed lots of experimental work (Dent & Murtland, 1990). Forestry became a potentially attractive land use when site characteristics were unfavourable for arable farming. In this study, based on modern statistical methods, we were able to assign certain local tree species (at least the indicator species for each of the seven communities) as being suitable for afforestation in certain areas or zones. At least the high correlation of plant communities with altitude could be used as a guide for prediction of suitable species for further areas.

4.1. Classification

With respect to Rodwell's (1991) request for the 'ecological integrity' of defined vegetation communities, it is important to mention that the methodology presented here must be viewed as the first step in a two step execution process: firstly, accurately and objectively defining and describing the diversity of vegetation communities in the southwest highlands in Yemen ; and secondly, translating this information into a classification that is readily applicable in the field, Trimming the dendrogram from cluster analysis at seven communities (with only 13% of the information retained) seems to be a compromise to get a simple interpretable summary of ecological affinity among samples in each community.

Any classification of vegetation is a working hypothesis (McCune); to our knowledge this is the first attempt to use multivariate statistical analysis tools to classify part of Yemen flora. The presented classification might be used as a basis for the development of syntaxonomic vegetation units in the mountains escarpment of Yemen, in vegetation mapping, and in climatic change studies. However, for applied purposes, any evaluation based on statistical significance may need to be tempered by reference to ecological significance (Braun-Blanquet & Tüxen, 1952). A comparison of the communities defined by our preliminary analysis can hardly be made because of the lack of such research on Yemen flora. The best reference for this purpose is the effort of Wood (1997) to classify Yemen vegetation based on experience and physiognomic traits.

The *Acacia origena – Acanthus arboreus* community (C7) highly corresponds to the *Acacia origena* highland forest (2000 - 3000 m) described by Wood (1997). One of the famous terrains that contain this community are the Baddan Mountain southeast of Ibb (Wood, 1997) in which many sample of C7 type were located. There are still few patches with intact vegetation of this kind providing some indication of the nature of the prehistoric woodland (Wood, 1997). Recently it has been found among the typical progressing communities on abandoned terraces in this elevation zone.

Close resemblance was found between the *Rumex nervosus – Euphorbia Helioscopia* community (C6) and what Wood (1997) called “rough scrub on steep eroded slopes in the highlands (2000 – 2900m)”. Meanwhile 50% of the stands in this community are abandoned terraces with different levels of succession, which offer suitable niches for annuals, grasses and invader species. This latter group was slightly described by Wood as “abandoned fields between 1500 and 2000 m”. So this community seems to be a transitional one, while many characteristic species of the expected climax will appear later. Moreover, we strongly believe that most of the sites classified as C6 will be typical C7 in its climax if not disturbed by human landuse. This hypothesis was affirmed by the presence of the broad ecological tolerance invader species *Tagetes minuta* in all sites of this vegetation community. Besides, it is well known that *Rumex nervosus* and *Euphorbia helioscopia* are always among the first invaders of the abandoned lands.

The *Euphorbia parciramulosa* - *Tragus racemosus* community (C5) bears very close resemblance to the succulent euphorbia scrub in the southeast mountains between 1200 and 2000 m of Wood (1997), especially the subassociation dominated by *Euphorbia parciramulosa* which exists around the city Al Qaidah.

Precipitation is a key variable for the *Acacia gerrardii* - *Solanum incanum* community (C4). This distinctive community has the highest precipitation seasonality and the lowest precipitation of the driest quarter (December to February) among the seven communities. Consequently small shrubs start to dominate in the field layer while grasses and herbs decline compared to the C5 to C7 communities. There is a good agreement between our and Wood’s findings, that *Acacia gerrardii* exists north and east of Ibb, highly restricted to alluvial soil, but in contrast we found the species only on elevations above 1800 m . Interestingly the well defined community C4 is completely not described by Wood (1997). A sign of a good classification technique should be the ability to tease out more than is obvious and not simply reaffirm existing notions (Braun-Blanquet & Tüxen, 1952). This gives us a clear indication about the advantage and positive influence of the objectivity in this study by combining standard survey methods (centralized replicate sampling procedure and Braun-Blanquet in the field) with multivariate statistical analysis tools, which contribute to minimize the personal error and subjectivity even for the well-trained plant ecologist.

There are also no notes in Wood (1997) about the relatively heterogeneous *Cadia purpurea – Selaginella yemensis* community (C3). Two sub-communities could be even distinguished. which confirms that this species rich community with the lowest indication value is more a typical transitional stage. It appears as very defused community reflecting the relatively flexible upper border of the frost free zone which is influenced by other geographical factors than altitude, like location, exposure and continentality; it may also be more variable between years according to climatic conditions. More sampling in the transition vegetation is necessary for better understanding the frost tolerant and frost intolerant vegetation and whether there is vegetation in

such zone tolerating weak and unfrequent frost. Wood (1997) defined the dividing line between highland and lowland plants (what we describe as the LFV and the HFFV) to be around 1600 m to 1800 m; while Al-Hubaishi & Müller-Hohenstein (1984) mentioned that around 2000 m - 2200 m frost occurs more or less regularly. We found that 1600 m is obvoiusly in the frost free zone and most of the well known frost intolerant species, like *Acacia mellifera* and *Acacia asak* which mostly accompany each other (Al-Hubaishi & Müller-Hohenstein, 1984), were recorded eight times out of twelve in elevations more than 1600 m. One time *Acacia mellifera* was recorded at 1839 m, thus we are highly confident that the dividing belt will be around 1800 m to 2000 m, hence between the two previous estimations.

Finally, the *Acacia mellifera - Cissus rotundifolia* community (C2) highly matches much of the *Acacia-Commiphora* bushland (500 - 1600 m) described by Wood (1997), among them the most important and coexisting indicator *Acacia mellifera* and *Acacia asak.* However, we found this community in higher altitudes (up to 1839 m) than described by Wood. Müller-Hohenstein (1987) found this coexistence also in Haraz Mountains north of our study area. The *Acacia etbaica - Euphorbia inarticulata* community (C1) could be more or less compared to the "impoverished *Acacia-Commiphora* bushland (1600 - 2000 m)" described by Wood (1997). Obvious differences in the elevation between this study and Wood could be partly attributed to the high accuracy of GPS devices. In both C1 and C2 communities succulent climbers of the Vitaceae family shaped the physiognomy of the vegetation with gaps in between being occupied with small shrubs, while grass layer became very scarce compared with the frost experiencing communities. This could be explained by environmental variations (Aronson & Shmida, 1992) but also by edaphic factors where soils tend to be more calcareous than volcanic ones.

Axis 3, representing 35% of the variance of ordination, obviously reflects the elevation gradient which is highly correlated to all temperature indicators. Our interpretation is that temperature in the coldest month (Bio 6) is the crucial factor such that axis 3 well separates the LFV and the HFFV. Axis 1 clearly separates community *Acacia gerrardii - Solanum incanum* community (C4), this is difficult to interpret by any abiotic variable and could be related to disturbance or competition. This is in accordance with Jean & Bouchard (1993) who found that only half of the species variation could be related to abiotic variables. Axis 2 (represents 30% of the variation) clearly describes the degree of continentality, since it mainly isolates *Euphorbia parciramulosa - Tragus racemosus* community (C5) from *Acacia origena - Acanthus arboreus* community (C7) in combination with axis 3. Meanwhile axis 2 succeeds also to separate the two frost free communities from each other, e.g. *Acacia etbaica – Euphorbia inarticulata* (C1) from *Acacia mellifera - Cissus rotundifolia* (C2) despite the high CC between C1 and C2 (63 %) and separates both C1 and C2 from *Acacia* ger*rardii - Solanum incanum* (C4) when combined with axis 1. In the joint plot Bio 15, which expresses continentality, shows high correlation with the axis 2.

Despite the fact, the NMS does not escape the arch effect (Lepš & Šmilauer, 2003), the ordination analysis showed that a solid data structure was underlying. Visual inspection of the ordination plot showed a good separation between most of the communities, indicating a robust assessment of the real situation. The greatest degree of overlap occured between the *Cadia purpurea – Selaginella yemensis* community (C3) with the other communities in the LFV and the HFFV. This overlapping was anticipated since C3 is a transient vegetation spanning the frost boundary. This is also supported by the noticeably high species heterogeneity and richness in this community (Fig. 3) and the box plot (Fig. 6). *Rumex nervosus – Euphorbia helioscopia* community (C6) could be a result of deteriorations of many sensitive or indicator species, like *Acacia origena*, by the anticipated overwhelming human impact. Both *Rumex nervosus – Euphorbia helioscopia* and *Cadia purpurea – Selaginella yemensis* community reflect this situation with the high variability in the elevation gradient within samples in both communities. During the dry period lopping off trees and shrubs for fire and fodder purposes is ~~very~~ widespread in the study area (Müller-Hohenstein, 1987). More sampling between 1700 m and 2300 m will lead to a better assessment of the problem. High resemblance was noticable in the field between the two frost free communities *Acacia etbaica – Euphorbia inarticulata* and *Acacia mellifera - Cissus rotundifolia*, especially in small shrubs and herbs layers, which is supported by the high coefficient of community (63 %) from the analysis. Wood (1997) described this situation by the observation that the tree species *Acacia mellifera* and *Acacia asak* coexisting in C2 are completely replaced by *Acacia etbaica* in C1.

Indicator species analysis is a very valuable and leading tool in such a study area due to the high level of vegetation patchiness. This is in accordance with previous research in a case study at Haraz Mountains north of our study area in the western escarpments of Yemen, where microclimatic variables by natural topography or by indirect human influence exerted a tremendous influence to the patchiness of grass species in a very small area. As a consequence of aspect humid western escarpment facing the Red Sea and dry eastern slopes huge differences in plant species composition and distribution was reported (Müller-Hohenstein, 1987). Environmental variation often produces modifications in the pattern of vegetation (Aronson & Shmida, 1992). In semi-arid ecosystems, one of the main components of environmental change is water availability. Continentality in the study area exerts reasonable effect on the community composition and differentiation mainly through temperature and precipitation variables as shown through the joint plot of NMS ordination (Fig.5). While (annual and quarterly) temperatures are highly correlated with altitude ($r^2 > 0.87$), precipitation values are less correlated. Precipitation of coldest quarter (Bio19; $r^2 = 0.29$ with altitude) is highly favoured by the *Acacia origena - Acanthus arboreus* community (C7) and, to lower extent, by *Rumex nervosus - Euphorbia helioscopia* community (C6). Meanwhile precipitation of warmest quarter (Bio18; $r^2 = 0.79$ with altitude) is rather low for the lower altitude communities C1 - C3, but jumps to much higher values above 1900 m where communities C5 - C7 (and most of C4) are found. In accordance

with Westoby (1979) along such gradients of increasing precipitation, vegetation cover varies from shrubland to grassland as it was noticed in the study area from C1 to C7.

Floristically, our results show clearly the diverse vegetation nature of these mountains in Yemen which is in accordance with previous research (Al-Hubaishi & Müller-Hohenstein, 1984). A large variety of habitats and ecological niches along an altitudinal gradient is responsible for the rather rich flora and many different plant associations in the western escarpment (Müller-Hohenstein, 1987**).** However, huge areas of Yemen are still poorly known botanically, particularly in the southeast close to the former border with South Yemen (Wood, 1997) where most of our samples were located. It is, therefore, not surprising to find in a relatively small area at least seven plant communities containing taxa with varying distributions, which all harbour the target species *C. digitatum*. This is in accordance with another survey study on Yemen flora in a more remote area in Wadi Rijaf, the largest known relict valley forest in Yemen, where the valley forest canopy is composed of taxa with Yemeni endemic, Sudano-Zambezian, Somalia-Masai, Afromontane, widespread Afrotropical and riverine forest of northeast Africa origin (Hall et al, 2008). This diverse nature could be a common characteristic of the vegetation in the mountainous terrain in Yemen.

Until 30 to 50 years ago no special range management seemed to be necessary because of the population density was not very high as today (Müller-Hohenstein, 1987). Active management of the overused and disappearing forests in Yemen becomes the aim of a project targeting the protection of the natural environment through sustainable use, undertaken from 1988 to 1993 with FAO, GEF, UNESCO and the Swiss development cooperation; the civil war in spring 1994 stopped the activities of the project (Herzog, 1998). Since nobody knows about the carrying capacity of the rangelands and meadows in the study area, our study facilitates future range management and agroforestry projects that aimed to developed in the future since here the vegetation harbouring *C. digitatum* is focused as a “resource”. Meanwhile the resulted plant communities stressing the aspect of vegetation as an “ecosystem structure”, since the extended forests and woodlands in the study area have disappeared a long time ago. It is not that easy to reconstruct the original plant cover, but modeling the habitat of this target species and also the seven communities well lead to a better understanding of the previous forest structure.

4.2. Methodological considerations

The analysis reported here raises several important methodological considerations. For practical considerations, subjectively centralized replicate sampling procedure rather than randomly located plots have been used, as the latter approach would require much greater replication at each site. It is important that the limitations of sample data are acknowledged in interpreting the results (Braun-Blanquet & Tüxen, 1952). Jörg (2003) points out that subjective sampling may include a danger of simply reaffirming existing ideas, in which subjectivity tends to overemphasise what is regarded by the surveyors as typical vegetation, at the expense of less

well characterised transitional vegetation. As a result it is improper to draw conclusions about continuity or discreteness of vegetation communities from such datasets (McCune & Grace 2002). This problem of subjectivity in our study was minimized, since the initial selection of survey sites was mainly led by the search for the target species *C. digitatum*. This species, being overexploited in many areas, has obviously broad high ecological amplitude which led us to find it in a variety of communities. If there was any preconceived bias, it was ~~be~~ mitigated since we traced *C. digitatum* over a large altitude range and included only stands not visited yet by mass collectors of that species. Stands being too similar to recorded ones or stands with high human impact were avoided and recorded only as presence for another study. This is an important aspect concerning the highly fragmented nature of vegetation in the study area.

Hierarchical clustering was subjectively chosen over two other popular classification methods, namely Two Way Indicator Species Analysis (TWINSPAN) and K-means clustering. Serious weaknesses in the TWINSPAN method have previously been highlighted including its poor performance with heterogeneous datasets containing more than one important gradient and the loss of information from quantitative data inherent in the 'pseudospecies' concept (Belbin and McDonald 1993; Legendre and Legendre 1998). The lack of dimensionality in dendrograms resulting from hierarchical clustering is, conversely, an advantage when dealing with heterogeneous datasets (McCune & Grace 2002). A valid alternative would be K-means clustering, a non-hierarchical clustering technique, but it is rarely used for directly clustering ecological datasets (Legendre and Legendre 1998).

Complete linkage algorithm was used as this method is combinatorially compatible with Euclidean distance and most of the communities produced are explainable and highly matching the reality. This was reported for example by Sardinero (2000) and was noticed in parallel during the field work. Leps suggested the name "longhand method" for the complete linkage, because it tends to produce compact clusters of roughly equal size with low level of chaining, which can be better interpreted ecologically and compared to the existing knowledge of the study area (Lepš & Šmilauer, 2003). McCune also emphasized that this method is applicable when an intense community strategy is needed, which is almost always the case in such a mountainous subtropical area where elevation, continantality and frost seam to be of high influence in the floral distribution. The validity of the linkage method for this data set was verified by testing for community heterogeneity with MRPP and CC. Complete linkage in combination with Euclidean distance seems to especially be the method of choice to study vegetation communities along altitudinal gradients. It has been used successfully by Sardinero (2000) who got good reasons to assume that there is some real tendency in the data towards grouping which was also confirmed by other methods. Additional recommendations to apply the complete linkage when vegetation along elevation gradient investigated was given by Lepš & Šmilauer (2003) and Sneath (1966).

ISA offers a quantitative method for choosing the optimum number of end groups from a particular cluster analysis (Dufrene and Legendre 1997; McCune & Grace 2002). However, it does not always yield clear unimodal results. Since some degree of judgement is required to interpret ISA results, it cannot be regarded as purely objective. Since the plant communities in our dendrogram (Fig. 4) corresponded to those obtained from synoptic analysis, some real tendency in the data towards the grouping suggested by ISA was proven. At any given level of clustering, species are assigned to the community for which their indicator value is maximal; the significance of this assignment is tested using Monte Carlo randomisations. Dufrene and Legendre (1997) concluded that ISA is more sensitive at identifying indicator species than TWINSPAN. They also suggested that this method could be used as a stopping rule for clustering, as indicator values will be low when communities are too finely or too broadly defined, peaking at some intermediate and most informative level of clustering. Legendre and Legendre (1998) emphasized the importance of validating the results of hierarchical clustering; a problem in this respect is that it will always reveal communities even when the dataset is essentially unstructured (Pillar 1999). The strength of the results therefore needs a certain assessment. Two external executions, pairwise MRPP test and coefficient of community, were performed in this paper to validate and confirm the partition of the dataset into the seven communities suggested by ISA. The low *A* values of the pairwise MRPP test confirmed a reasonable degree of species composition heterogeneity among the seven communities. The very small *P* values emphasized that this heterogeneity is a reflection of reality and fairly unlikely to be obtained just by chance (McCune & Grace, 2002).

Ellenberg's community coefficients showed that there is a considerable amount of variation in floristic composition among the seven communities, which resulted from cluster analysis (Table 9). A similarity of less than 60% was obtained between most pairs. Some contribution to these similarities could be attributed to the fact that sites were (necessarily) selected on the presence of *C. digitatum.* The 89 studied sites showed, that *C. digitatum* is not part of a small coherent group of species which always occur together, but belong to seven vegetation communities. This floristic change is mainly driven by an elevation gradient, but partially modified by the presence of *C. digitatum* in different stages of man-made succession. The results confirm the hypothesis initially proposed in this study about the clear zonation in the Yemen escarpment (Al-Hubaishi & Müller-Hohenstein, 1984).

4.3. Recommendations:-

It should be emphasised again that the classification and ordination approach in this paper represents the first attempt to produce a national-scale vegetation classification system in Yemen, using a range of complementary methods and current best practices in statistical techniques. It will be, undoubtedly, refined in the future with an expanding dataset (hopefully to elevations higher than 3500 m which is not covered by this study) and accompanied by habitat modelling for predictive purposes. Success of this approach will guide the application of such methods to

other less studied regions in Yemen and in neighbouring countries with similar vegetation. In partucular, our observations showed that *C. digitatum* is a very tolerant species which is not connected to a particular plant community. Instead, it is highly associated with many other widespread species and could be found through a set of well distinguished plant communities covering the LFV and the higher HFFV zones in the southwest highlands in Yemen. This suggests that a reintroduction by seeds and old branch grafts could be done at many and different places to stop the rapid decline. Announcing *C. digitatum* as an endangered species could reduce illegal intensive commercial gathering and save time for a well-planned re-introduction into intact habitats. But the challenge to enforce such a label might still be beyond the ability of the weak central government in Yemen.

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CHAPTER 6: Ecological prediction modelling vigor as combination of species based and community based modelling: The overexploited species *Cyphostemma digitatum* in the western highlands in Yemen and its harboring communities as a case study.

To be submitted to: *Conservation Biology*

Al-Duais Mohammed & Jetschke Gottfried
Institute of Ecology, University of Jena, Dornburger Strasse 159, D-07743 Jena, Germany

Abstract
Habitat models for the overexploited species *Cyphostemma digitatum* and its harboring vegetation, namely the lower frost vegetation (LFV) and the higher frost free vegetation (HFFV) in the western highlands in Yemen, were obtained by the NonParametric Multiplicative Regression modeling approach (NMPR) using the statistical package HyperNiche. The first model for the species was of low prediction power because many pseudo-absence data due to human influence were recorded as real absence data. As an alternative, habitat models for the LFV and HFFV communities were constructed. They led during a second round of field work to record more presence data for the species. The relevès data were also increased by additional 25 relevès, providing more accurate presence and absence data. Based on these improved habitat models for *C. digitatum* and its harboring communities were constructed. An evaluation based on the resubstitution method classified the produced model as "very good", taking Cohen's Kappa and Area under curve (AUC) as indicators. Our approach showed that a community-based modelling can be a valuable substitute for species modelling if data for the latter are biased or incomplete.

Keywords*:* *Cyphostemma dagitatum*, habitat suitability, nonparametric multiplicative regression, community, altitude, lower frost vegetation, higher frost free vegetation, Yemen Mountains, prediction modeling.

1. Introduction

Modelling the distribution of the economic species in its natural habitat becomes a hot topic in conservation biology even for the species which are still in sustainable use (Tarkesh, 2008), This excusion becomes more necessary for a species like *Cyphostemma digitatum* in which no more sustainable use still exists. Many well known source areas for the species became cleared out from because of the intensive commercial gathering to be processed and sold as ethnic medicine, spice and food addidative (Al-Duais & Jetschke, 2009). Due to the decline of *C. digitatum* in nature it was cultivated by removing the plant completely from the original sites and replants it in gardens (Al-Duais & Jetschke, 2009). This plant is used mainly as food flavoring and as a main constituent of traditional Yemeni soup. Moreover, it was traditionally recommended for

gastroenteritis, fatigue, general weakness, nausea and headache. Further clearance of *C. digitatum* will be anticipated to accelerate after we recently proved that this plant is a really wealthy source containing many functional food ingredients and micronutrients with a variety of culinary and medicinal applications (Al-Duais et al., 2009a, 2009b).

Under pressure to make informed management decisions rapidly, conservation practitioners must increasingly rely on predictive models to provide them with information on species distributions (Ferrier, 2002; Loiselle et al., 2003). The problem of modelling the geographic distribution of species with conservation importance which may have extremely few georeferenced locality records is a critical problem in conservation biology. To save such threatened species, one first needs to know where the species prefers to live and what its requirements are for survival, i.e. its ecological niche (Hutchinson, 1957). Recent efforts to conserve biodiversity are moving beyond preserving only its pattern, such as particular species or populations, to include the many complex processes that produce and maintain biodiversity (Cowling and Pressey, 2001; Crandall et al., 2000).

The data available for this mission typically consist of a list of georeferenced occurrence localities, i.e. a set of geographic coordinates where the species has been observed and localities where the species is surely absent. In addition, there is data on a number of environmental variables, such as elevation, slope, aspect and some site condition, like soil type, soil depth vegetation structure, geology and runoff etc., which have been measured in the field. Other factors like many temperature and precipitation variables are only estimated across a geographic region of interest. The goal is to predict which areas within the region satisfy the requirements of the species' ecological niche and thus form part of the species' *potential distribution* (Anderson & Martínez-Meyer, 2004). The potential distribution describes where conditions are suitable for survival of the species, and is thus of great importance for conservation. It can also be used to estimate the species' *real distribution*, for example by removing areas where the species is definitely known to be absent because of deforestation or other habitat destruction.

Understanding the geographic distribution of a species or a community and their diversity and richness depends on how well their ecological niche is understood. It is widely accepted that measurement of environmental requirements to quantify the range size and patterns of species distribution and richness is an important step towards this understanding (Woodward, 1987). This generalization is true at a variety of spatial scales, suggesting the importance of measurements of environmental variables at different scales. For example, climate variables are of increasing importance as the scale increases from regional to continental to global scales. In most biogeographic theories, geographic distribution of species and their diversity or richness are conceived in terms of a multidimensional coordinate system, whose axes are various resource gradients (e.g. ecological and environmental variables). This coordinate system defines a hyperspace, and the range of the space that a given species occupies is its niche. The niche is an

abstract characterization of the intra-community position of the species that depends on time, space, and differences in resource gradients that cause the species evolution (Whittaker, 1972).

Most species, within the same assemblage, tend to have relatively small ranges that reflect how they share space (Brown et al., 1996; Gaston, 1998); this promotes the idea of modelling communities. Range size may depend on a variety of ecological and evolutionary processes and extrinsic factors of the physical environment such as soils, nutrients, water, and climate (Gentry, 1988; Hunter, 2003; Kreft et al., 2006; Smith et al., 2001). Capturing the interplay of these factors is fundamental to understanding the uneven distribution of diversity on regional and global scales.

From previous research on Yemen vegetation for conservation practice for a certain important species on a national and regional level like *C. digitatum* it is known, that studying the habitat and claiming for conservation of this habitat is an important requirement for the conservation of these plant species since there are only few areas of the country that are right now protected **(**Hall et al, 2008**).** However, many absence data can be caused by overexploitation of *C. digitatum*, leading to a wrong picture of natural distribution. Taking in consideration the advantage of the elaborated holistic concept of Clements (Clements, 1916), we aimed to additionally qualify the communities that harbor *C. digitatum* intensively. Seven communities spreading in two main vegetation units, the lower frost vegetation (LFV) and higher frost free vegetation (HFFV) were defined in this mountainous terrain (Al-Duais & Jetschke, 2009). Accordingly, an alternative way for modelling could be triggered by these communities in which absence data are extremely accurate. Meanwhile two approaches seams to be achievable: **(A)** Modelling and better understanding of some important plant communities in the Yemen mountainous massive are absolutely necessary, because many researchers stated that the vegetation situation in this region is not yet fully understood (Hall et al, 2008; Al-Hubaishi & Miller-Hohenstein, 1984; Wood, 1997). A proposed methodology for developing a national mountain vegetation classification based on data from field surveys combined with up-to-date statistical techniques was recently triggered by Al-Duais & Jetschke (2009). **(B)** We also need scientifically tracking and recording more presence date for *C. digitatum* in perhaps more intact places to possibly cover new combinations of environmental variables that can overcome any fitting problems during *C. digitatum* modeling until now.

This new execution that we suggest her seems to be extremely important for such cases and is supported by Whittaker opinion, that for a given resource gradient in a community, species evolve to use different parts of this gradient and competition between them is thereby reduced. Diversity of communities seem a result of non-extreme conditions, stable conditions, evolutionary and successional time, and the kind of community developed in that time (Whittaker, 1972).

Statistical models in general use empirical data to define relationships between current species distributions and environmental factors; when incorporated into a geographic information system (GIS), potential distributions can be mapped in this manner. Some common statistical approaches used at this field are generalized linear models (Guisan et al.1998), generalized additive models (Yee and Mitchell 1991), regression trees (Moore et al., 1991; Iverson & Prasad, 1998) and multivariate adaptive regression splines (Leathwick et al., 2005). Mechanistic models seem to yield superior results, particularly under climate change scenarios, but they are more difficult to build, often extremely time-consuming and rely on a greater knowledge of the biology of the target organism (Robertson et al., 2003). The prediction models are either strictly mathematical or based on certain ecological theories. The detailed discussion or review of these models and the ecological theories are beyond the scope of this paper (Elith et al., 2006; Graham & Hijmans, 2006).

Here, we are interested to model the distribution of *C. digitatum* and its harbouring vegetation over the Yemen mountainous massive. The strong gradients of climate variables due to the mountainous topology of the area of study and prediction made the potential use of environmental climatic variables for mapping distribution patterns more satisfactory and valid. We use a very novel statistical technique, the NonParametric Multiplicative Regression (NPMR; McCune, 2006a), which is an empirical smoothing technique of species or community response based on ecological niche assumptions. Based on this we integrated environmental variables and geographical point locality data to investigate the current distribution of *C. digitatum* and its harboring vegetation as a response to environmental factors.

The paper is organized into four sections: 1) description of the study area, the species, its harbouring vegetation, and the used climatic data, 2) description of the NPMR model and the environmental data, 3) assessment of potential range distributions, and 4) discussion on the contribution and significance of vegetation modelling for characterizing suitable areas of species habitat.

2. Material & Methods

2.1. The Study area

Yemen covers an area of 555.000 km², arid and semiarid land covers 80% of the total area. The study area has been chosen in the massive west highlands, the rectangular study area delimited by N 13° 04’ 19’’ - 16° 20’ 35’’, E 43° 14’ 55’’ - 44° 47’ 05’’ covered about 74000 km² (Fig. 1) with elevations ranging from 300 m to 3660 m. Inside this mountains there are many lowland corridors and valleys becomes very wide to form the high plateau mainly Qa’ Al-Hakel, Qa’ Gahran and Qa’ Sahman. The last one is located in the vicinity of Jabal Al-Nabi shu’ayb (the highest peak in Arabia, 3666 m) (Wood, 1997).

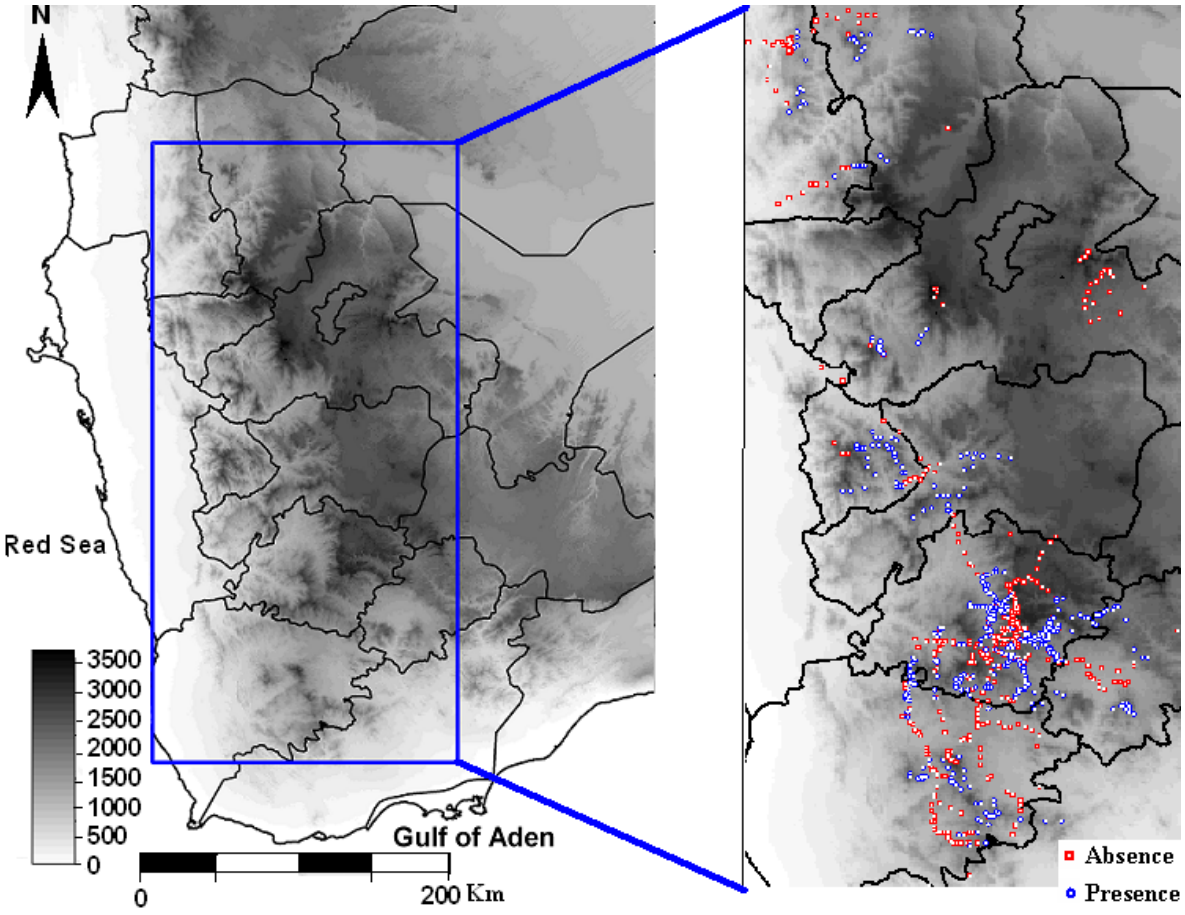

Figure 1: Distribution of the localities where presence/absence data for *C. digitatum* were recorded in the Yemen Mountains massive.

Thus major geomorphologic units can be distinguished including undulating plains of tertiary volcanic material and tertiary to quaternary sediments; a complex of basalts and metamorphosed rocks which give rise to very fertile soils (Wood, 1997). Limestone in the north around Mahwit and Khamir, sharp granite outcrops like Jabal Al-Nabey shuaib, Haraz west of sana'a, Jabal Sabir south of Taiz, Al-Manar Mountain north of Ibb and Hajah, Shuharah mountains and smaller mountains consisting predominantly of Eocene basalt and partly of Palaeocene sandstone, which are less common large deposits can be found around Tawilah and Al-Hujariah (Wood, 1997). According to Al-Duais & Jetschke (2009) Al-Hubaishi, A. & Müller-Hohenstein (1984) and Wood (1997) soil in the escarpment of Yemen is very mosaic-like and reflects the geology and topography of the region.

A large proportion of the rain carried from the gulf of Aden and the Red Sea is stopped by the mountains resulting in short heavy afternoon rainfall in the study area, being extremely unpredictable and variable. Going to higher elevations the precipitation (200 mm – 1000 mm) increase, while mean temperature decreases (22 °C to 16 °C) (Wood, 1997). Generally there are two rainy seasons which can be recognized, namely March - April and July – September. Through most of the years, still rains are anticipated out of the seasons and dryness also within in

the seasons (Al-Hubaishi & Miller-Hohenstein, 1984; Rappold, 2005). The highest annual rainfall in the Arabian Peninsula occurs in the west-facing slopes notably around Ibb, located within the stud area, which averaged an impressive 1369 mm over five years in the 1970s. In the south and west facing slopes frequent fog increases the water availability to plants. Runoff is rapid and in unterraced hills erosion can take place at a rapid rate (Wood, 1997). Mean annual temperature is also unequally distributed, highly subjected to elevation and to a certain extant to local annual rainfall (Al-Duais & Jetschke, 2009; Wood, 1997). Two main bioclimatic zones could be easily distinguished in the study area, which highly influence the plant distribution; these are the frost experiencing and the frost free zone, meanwhile aridity increases with continentality from the moist steppe mountains in the west to the dry desert in the east (Al-Duais & Jetschke, 2009).

2.2. Data collection

2.2.1. Species data

C. digitatum "halka" belongs to Vitaceae family; it is a perennial, climbing, succulent undershrub with compound fleshy leaves and tendrils; it flowers in pedunculate axillary cymes, fruita are 1- seeded red fleshy berries. The species is easily recognizable by its fleshy leaves, petiolate, digitately 3-5 foliolate with leaflets ovate and dentate **(**Wood, 1997). *C. digitatum* spreads between approx. 1000 m and 2600 m on the escarpment, it prefers habitats which are moderate moist with little soil cover between stones. It prefers full sunlight, but could also exist in forested areas. It occurs often on cliffs and with a marked preference for stony places such as gullies and terrace walls with different slope and aspects. It is usually associated with *Euphoria* spp, *Acacia* spp., *Agava* spp., *Senecio hadiensis* and *Clematis* spp., which implies a wide ecological niche (Al-Duais & Jetschke, 2009). *C. digitatum* is very toxic if eaten as fresh material and seems to be not edible by any herbivore (personal observation). The parts used by people are the leaves and fleshy young branches after household processing **(**Al-Duais et al, 2009a).

During the first and second field trips to the study area in 2005 and 2006 about 323 presence and 309 absence localities were recorded, guided by the questionnaires and interviews with local peoples and large scale collectors. Preliminary primary models and prediction maps were produced after these two years. During the third (final) field trip between 20 August and 30 September 2007 visit of presence or absence localities was guided by these primary produced prediction maps for LFV and HFFV communities that could harbor *C. digitatum,* which determined by Al-Duais & Jetschke (2009). Presences were determined by direct observation of the species for each circular plot while absence was defined as no observation within a random circular plot of at least 100 m radius. Finally, presence or absence for *C. digitatum* was determined on 1094 localities (with 596 presences, 498 absences). However, since this species became cleared from a wide part of the study area, several absences could be attributed to human activity, although the habitat properties could be suitable.

2.2.2. Community data

The sampling was done by implementing the Braun-Blanquet approach in the field, each year between 20 August and 30 September, because this is the climax of the rainy season. We made three field trips to the study area in 2005, 2006 and 2007, where 8, 56 and 25 relevés, 10 m * 10 m each, were recorded, respectively. All sites were selected to contain *C. digitatum*. The rectangular study area extended between 13.25° – 14.16° N and 43,86°– 44,76° E, it covered approx. 15.000 km² (Fig. 2). The resulting phytosociological table was subjected to hierarchical cluster analysis and ordination by NMS (for details see Al-Duais & Jetschke, 2009). It resulted in four communities in the lower frost vegetation (LFV) zone, *Acacia gerrardii - Solanum incanum, Euphorbia parciramulosa - Tragus racemosus, Rumex nervosus – Euphorbia helioscopia* and *Acacia origena – Acanthus arboreus* communities, and two communities in the higher frost free vegetation (HFFV) zone, *Acacia etbaica – Euphorbia inarticulate* and *Acacia mellifera - Cissus rotundifolia* communities. Both groups were included into LFV and HFFV modelling. The presence/absence localities for LFV and HFFV were taken from the matrix produced before by Al-Duais & Jetschke, (2009) .We did not include the transition community *Cadia purpurea – Selaginella yemensisin* in both LFV and HFFV modelling, meanwhile LFV and HFFV were each considered as absent points when modelling the other. Finally we had 24 absence and 51 presence records for the LFV and 51 absence and 24 presence records for HFFV. These were implemented into the construction of the model and the resulting prediction maps for both types of vegetation.

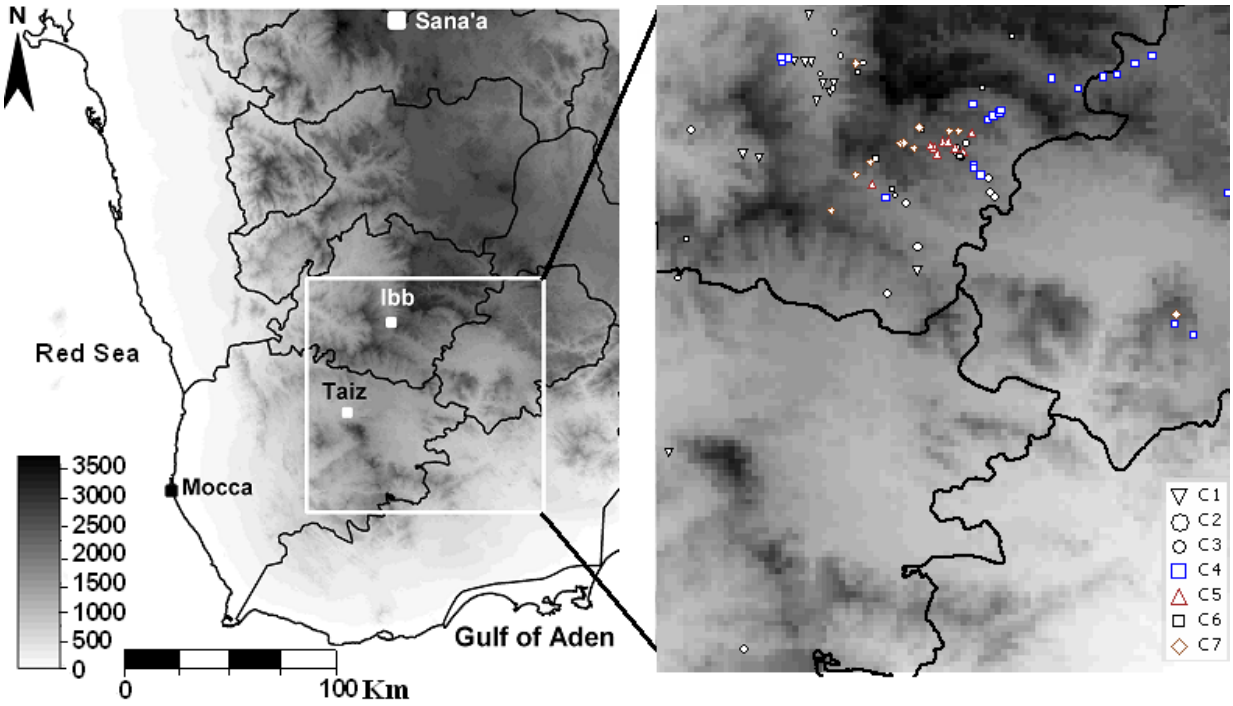

Figure 2: Distribution of the localities where Braun-Blanquet relevés were taken in the Southwestern Mountains in Yemen.

The geographical coordinates, altitude, slope and aspect for both species and community data were determined by GPS with ± 8m precision, slope is determined by special visual tool, vegetation structure, geology, topology, runoff, soil depth and type is also described. The climate data used in the analysis is an elaborated comprehensive set of bioclimatic variables, available from the data base of the climatic research organization "Worldclim" for the whole world in a grid format, in our case 30 arc seconds (http://www.worldclim.org; for explanation see **Table 1**).

After preliminary investigations using mainly the correlation matrixes, supported some times by the point-biserial correlation (r_{pb}) and the correlation ratio analysis (*Eta*), we selected elevation, slope and aspect which were measured in the field and used them in the analysis in all the three modelling process. With respect of modelling for of *C. digitatum* 12 bioclimatic variables which could control the distribution of *C. digitatum* were also chosen (Table 2). Monthly precipitation were removed because of its high correlation with the other chosen precipitation variables including Bio12, while the temperature variables Bio1 to bio3 and Bio5 to Bio11 were removed because of its high correlation with the altitude. With respect of the vegetation data Monthly precipitation were considered and most of the Bio variables because the high correlation wer declines because of the very low data set compare to the species data; Bio 1, Bio 3, Bio 5, Bio 6, Bio 8, Bio 10 and Bio 11 were ignored due to their high correlation with the altitude. We also used two nominal variables, soil (39 classes) and vegetation types (36 classes), in the three modelling process, because a Chi square test derived from contingency table showed a significant relation ($P < 0.001$) between these nominal variables and occurrence of the given species and vegetations.

Bio1	Annual mean temperature
Bio2	Mean diurnal range (mean of monthly (max temp. – min temp.))
Bio3	Isothermality (Bio2/Bio7)(*100)
Bio4	Temperature seasonality (Standard deviation*100)
Bio5	Max temperature of Warmest month
Bio6	Min temperature of coldest month
Bio7	Temperature annual range (Bio5 - Bio6)
Bio8	Mean temperature of wettest quarter
Bio9	Mean temperature of driest quarter
Bio10	Mean temperature of warmest quarter
Bio11	Mean temperature of coldest quarter
Bio12	Annual precipitation
Bio13	Precipitation of wettest month
Bio14	Precipitation of driest month
Bio15	Precipitation seasonality (coefficient of variation)
Bio16	Precipitation of wettest quarter
Bio17	Precipitation of driest quarter
Bio18	Precipitation of warmest quarter
Bio19	Precipitation of coldest quarter

Table1: Description of primary bioclimatic variables from Worldclim data set.

	HFFV			LFV			*C. digitatum*		
	***r*pb**	***Eta***	**Used***	***r*pb**	***Eta***	**Used***	***r*pb**	***Eta***	**Used***
Observ * Bio1	0.61	0.91	N	0.66	0.93	N	0.00	0.65	N
Observ * Bio2	0.52	0.88	Y	0.56	0.89	Y	0.03	0.55	N
Observ * Bio3	0.56	0.80	N	0.61	0.82	N	0.02	0.27	N
Observ * Bio4	0.44	0.95	Y	0.51	0.96	Y	0.01	0.69	Y
Observ * Bio5	0.56	0.93	N	0.63	0.94	N	0.00	0.64	N
Observ * Bio6	0.62	0.97	N	0.67	0.98	N	0.00	0.67	N
Observ * Bio7	0.64	0.95	Y	0.67	0.96	Y	0.02	0.56	N
Observ * Bio8	0.60	0.98	N	0.65	0.97	N	0.00	0.63	N
Observ * Bio9	0.59	0.93	Y	0.65	0.94	Y	0.00	0.67	N
Observ * Bio10	0.60	0.94	N	0.65	0.95	N	0.00	0.67	N
Observ * Bio11	0.62	0.93	N	0.67	0.93	N	0.00	0.65	N
Observ * Bio12	0.05	0.82	Y	0.08	0.82	Y	0.00	0.62	Y
Observ * Bio13	0.59	0.93	Y	0.62	0.94	Y	0.01	0.59	Y
Observ * Bio14	0.09	0.33	Y	0.12	0.36	Y	0.03	0.30	Y
Observ * Bio15	0.44	0.83	Y	0.50	0.84	Y	0.03	0.40	Y
Observ * Bio16	0.50	0.86	Y	0.54	0.86	Y	0.01	0.60	Y
Observ * Bio17	0.01	0.57	Y	0.04	0.57	Y	0.02	0.37	Y
Observ * Bio18	0.53	0.93	Y	0.59	0.93	Y	0.01	0.60	Y
Observ * Bio19	0.13	0.66	Y	0.20	0.70	Y	0.03	0.35	Y
Observ * Pr Jan	0.24	0.56	Y	0.34	0.62	Y	0.01	0.34	N
Observ * Pr Feb	0.31	0.67	Y	0.39	0.72	Y	0.01	0.38	N
Observ * Pr Mar	0.00	0.42	Y	0.00	0.38	Y	0.01	0.40	N
Observ * Pr May	0.28	0.70	Y	0.29	0.68	Y	0.01	0.41	N
Observ * Pr June	0.43	0.74	Y	0.46	0.76	Y	0.00	0.34	N
Observ * Pr July	0.53	0.83	Y	0.55	0.87	Y	0.02	0.54	N
Observ * Pr Aug	0.59	0.93	Y	0.62	0.94	Y	0.01	0.59	N
Observ * Pr Sep	0.47	0.80	Y	0.54	0.84	Y	0.00	0.33	N
Observ * Pr Oct	0.56	0.83	Y	0.60	0.84	Y	0.00	0.31	N
Observ * Pr Nov	0.00	0.35	Y	0.00	0.30	Y	0.01	0.36	N
Observ * Pr Dec	0.09	0.33	Y	0.12	0.36	Y	0.05	0.38	N
Observ * Slope	0.00	0.53	Y	0.00	0.51	Y	0.00	0.25	Y
Observ * Aspect	0.01	0.94	Y	0.02	0.95	Y	0.01	0.63	Y
Observ * Elevation	0.58	0.99	Y	0.64	0.99	Y	0.00	0.89	Y

Table 2: Pearson's correlation (= point-biserial) coefficient r_{pb} and correlation ratio *Eta* as measures of linear and nonlinear association between observations and predictor variables.

* donate the used variables

2.3.Habitat modelling and statistical analysis

With the rise of new powerful statistical techniques and Geographic Information System (GIS) tools, the development of predictive habitat models has rapidly increased in ecology (Guisan and Zimmermann, 2000). A novel highly objective statistical model, Non Parametric Multiplicative Regression (NPMR); which is a distribution-free method for fitting response surfaces to two or more predictors, where the predictors are combined with a multiplicative weighting function. NPMR use a "let the data talk" approach (Zurr, 2007) compare to parametric models, e.g. with a pre-specified model form to fit the data, have been used to predict *C. digitatum* and its harboring vegetation response and distribution maps in the study area, NPMR fitted relationships between

sites where the target species or community is known occurs and its environment which were relatively transparent to build a habitat model. This approach works by estimating species occurrence for new sites based upon the proportion of occurrences at known sites with similar environmental conditions. Model building is an iterative process in which NPMR searches through all possible multiplicative combinations of environmental variables to determine which predictors are the best ones for target species occurrence. In this context, we adopt NPMR models using software package HyperNiche version 1.30 (McCune, 2006a).

HyperNiche is gaining importance as a nonparametric multiplicative regression, non-linear alternative to parametric procedures. This technique is potentially more robust than parametric procedures to deviations from multivariate normality and equal covariation, and performed similarly, or better, than other techniques applied to presence-absence data (McCune, 2006b). HyperNiche have been used before to predict distributions and to map habitat suitability within GIS, with success (McCune, 2006b). We used a Gaussian weighting function and a local mean model (LM-NPMR) with a minimum average neighbourhood size of 5% of the number of sample points. A Gaussian kernel function assigns weights between 0 and 1 to all data points. Thus, for a given target point, not all sites contributed equally to the estimate. The form of the Gaussian weight function used for smoothing is based upon the standard deviation ("tolerance") of each environmental variable. Model quality was assessed with leave-one-out cross validation: (1) one data point was removed from the dataset (focal point); (2) the dataset minus the focal point was used to estimate the response for that point, using various combinations of environmental variables and tolerances; (3) this process was repeated for all points in the dataset and; (4) a Bayesian statistic, the log(*B*), was used to compare the accuracy (e.g. performance) of each model relative to a naïve model, where the probability of occurrence at a given site is constant and equals the overall frequency in the study area. (5) The response function of the best model was calculated and translated from ecological space to the geographical space. Ilwis GIS software was applied to allocate the points and to produce the prediction map for *C. digitatum* and its harboring vegetation types (LFV and HFFV) to identify locations suitable for restoration of the species. Potential restoration sites were initially identified on the basis of the capability of study area to support each of the evaluation habitats.

3. Model evaluation

We used resubstitution approach (Araujo et al., 2005) "Confusion matrix" is the classic way to evaluate the accuracy of an element distribution (Fielding & Bell, 1997; Guisan & Zimmermann, 2000; Pearce & Ferrier, 2000; Manel et al., 2001), which is based on a cross-table operation and records the number of true positive (*a*), false positive (*b*), false negative (*c*), and true negative (*d*) cases predicted by the model (Table 3 a). Each value from the matrix gives a synoptic view of overall model performance. For example, "true positive" (*a*) indicates how many observations are correctly classified as present in the model and real world. Another possibility is to derive some statistical indices from confusion matrix, such as correct classification rate, sensitivity,

specificity, differential positive rate (Ward, 1986) and Cohen's kappa (Table 3 b; Fielding, 1997). The last measure is a way to assess which models correctly predict occurrence better than chance expectation.

a)

	Observed	
Predicted	Presence	Absence
Presence	a	b
Absence	c	d

Table 3: Confusion matrix **(a)** and some indices derived from the confusion matrix **(b).**

b)

Measure	**Formula**
Correct classification rate	$(a+d)/n$
Sensitivity	$a/(a+c)$
Specificity	$d/(b+d)$
Differential positive rate	$\frac{a}{a+c}+\frac{b}{b+d}-1$
Cohen's Kappa	$\frac{(a+d)/n-\left[(a+b)(a+c)+(c+d)(b+d)\right]/n^2}{1-\left[(a+b)(a+c)+(c+d)(d+b)\right]/n^2}$

Another alternative for model evaluation is construction of receiver operating characteristic (ROC) plot methodology. ROC showing the predictive overall performance of a habitat model as a trade off between sensitivity and specificity (Schröder, 2004). A ROC plot is obtained by plotting the sensitivity of the model against the false positive fraction (1 minus specificity) (Table 3 b) over the whole of thresholds between 0 and 1. The area under the resulting curve (AUC) then indicates the probability that the model will distinguish correctly between the two observations. For a model with no discrimination capacity the area under the ROC curve will be 0.5, while for a model with perfect discrimination capacity the area will be 1. We calculated values of AUC of ROC plot and the derived indices from confusion matrix using ROC_AUC program (Schröder, 2004). This program calculates the the area under the ROC curve (AUC) as a threshold-independent measure of predictive performance with bootstrapped confidence intervals in addition to a variety of threshold-dependent performance criteria as described in Fielding & Bell (1997), e.g. Cohen's Kappa, correct classification rate, sensitivity and specificity and finally selects the best threshold based on optimisation of cut-off values regarding maximised Kappa, maximised correct classification rate and minimised difference between sensitivity and specificity. There are a number of rules-of-thumb available which help to interpret the measures of agreement between observed and projected events. For example, when using the kappa statistics, Landis & Koch (1977) suggested the following ranges of agreement: almost perfect agreement for kappa values between 1.00 and 0.80, substantial agreement 0.79-0.60, moderate agreement 0.59-0.40, fair agreement 0.39-0.20, poor agreement 0.19-0.00 and no agreement if less than zero. When using the ROC procedure, Swets (1988) recommended interpreting range values as: excellent for AUC > 0.9, useful 0.9-0.7 and poorly accurate if AUC is less than 0.7.

4. Results

4.1. Vegetation modeling

Previous primary models for both LFV and HFFV were produced based on 64 relevès. They were good enough to guide us during the final field work (in summer 2007) which led us far to the north to encounter both vegetation types and species *C. digitatum* in more intact situation, hence more species presence data were collected. Meanwhile the final modeling of the vegetation was also extended by 25 additional relevè. In the final runs of LFV and HFFV modeling many trials were done. Consequently the well defined *Acacia gerrardii* - *Solanum incanum* community (one constituent out of four of LFV) was omitted completely from the modelling process for the LFV because with including of this community as a part of the LFV presence data only low quality models were obtained. This is clearly attributed to the fact of the restriction of this community which made it appear in certain area with very specific variable combinations (see Al-Duais & Jetschke, 2009; Wood, 1997). So the final modelling process for LFV was based on 30 presence and 24 absence localities.

Among 4921 models (nested by increasing number of predictor variables) calculated by NPMR the best three models were chosen by the HyperNiche program for the LFV. Model number 2915 with three predictor variables indicated more precision (log(*B*) = 16.7), while model 3051 based on two variables was nearly as accurate. Among all temperature variables this model chosed as predictor the mean temperature of driest quarter (Bio9) which is also the coldest quarter and expressed well the frost in addition to altitude and the mean precipitation of March (the onset of the rainy season in the study area). A sensitivity analysis within NPMR showed that Bio9 turned out to be the most important predictor variable; it is also the most frequent predictor variable in all models (Table 4). The average response curves of the LFV with respect to the three variables are presented in Fig. 3. Occurrence of the LFV is highly probable at lower mean temperature of driest quarter (Bio9) not more than 17°C and higher precipitation in March, but mostly not exists lower than 1800m.

Model No.	No. Pred.	log(B)	Var1	Tol 1	Var2	Tol2	Var3	Tol3
193	1	16.1	Bio 9	5.2				
3051	2	16.6	Altitude	158.5	Bio 2	1.45		
2915	3	16.7	Altitude	237.8	Bio 9	10.4	Pr. March	1.65

Table 4: Characterization of the best single model(s) for the low frost vegetation (LFV) calculated by NPMR (No. pred. = number of predictor variables, Var. = name of variable, Tol. = tolerance of smoothing function in percent of range)

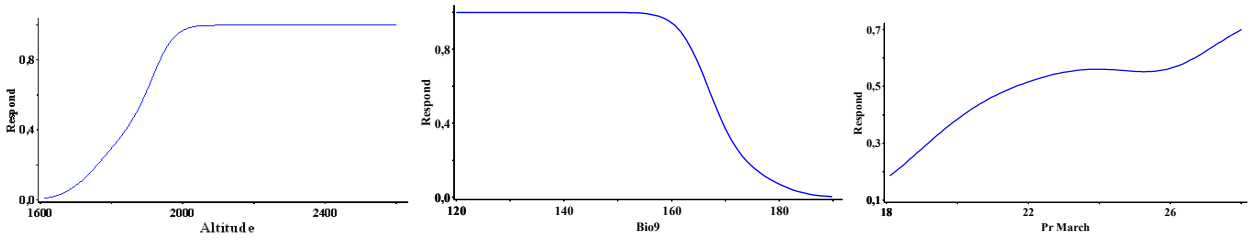

Figure 3: Response curves of the low frost vegetation (LFV) with respect to Altitude, Bio9 (= 10*Mean temperature of driest quarter) and precipitation of March.

Also, from 4694 models for increasing number of predictor variables calculated by NPMR the best three models were chosen to model the HFFV. Model number 1872 with three predictor variables indicated more precision (log (*B*) = 15.4), this model contained in addition to altitude the temperature variable Bio7 and also precipitation variable Bio15 (therefore both variables react differently when one moves through geographical space). A sensitivity analysis within NPMR showed that altitude turned out to be the most important predictor variable when smoothed with a (rather high!) tolerance of 79.3 percent of data range. It was also a very common predictor variable that had been selected in all subsets of best models (Table 4). The average response curves of the HFFV with respect to the three variables are presented in Fig. 4. Occurrence of the HFFV is highly probable in a zone below 1800 m, when the temperature annual range (Bio 7) is not more than 22.5 °C and precipitation seasonality (Bio15) is not more than 78%, but mostly not exists above this range. The bimodal structure of the response with respect to Bio 7 somehow reflects an inhomogeneity of the data structure of the input and could be smoothed a little more to a unimodal one for ecological reasons; more extended sampling will hopefully produce a smoother curve.

Model No.	No. Pred.	log(B)	Var1	Tol 1	Var2	Tol2	Var3	Tol3
17	1	15.1	Altitude	79.3				
715	2	15.2	Altitude	79.3	Bio 7	8.3		
1872	3	15.4	Altitude	79.3	Bio 7	8.3	Bio 15	5.7

Table 5: Characterization of the best single model(s) for the high frost free vegetation (HFFV) calculated by NPMR (No. pred. = number of predictor variables, Var. = name of variable, Tol. = tolerance of smoothing function in percent of range)

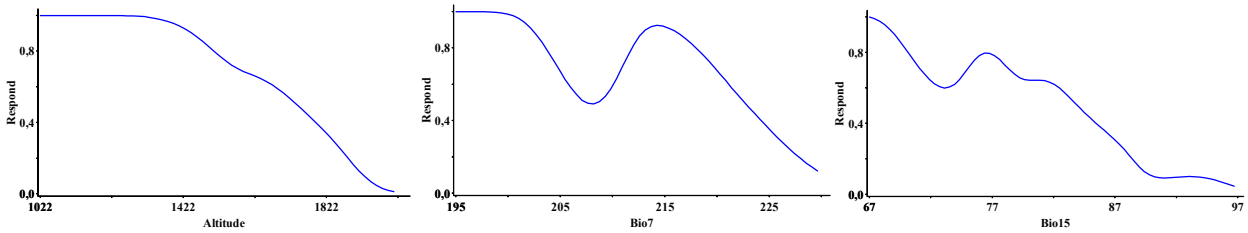

Figure 4: Response curves of the high frost free vegetation (HFFV) with respect to Altitude, Bio7 (= 10 * Temperature annual range) and Bio15 (= Precipitation seasonality: CV * 100).

4.2. Species modeling

Among a huge number (12165) of nested models calculated by NPMR ten were selected as best ones by HyperNiche. Model number 572 with three predictor variables was the most precise and accurate (log (*B*) = 141.33), slightly better values for log (*B*) could be obtained by other models but with an increasing the number of variables. Six of them are given below (Table 6). Due to parsimony and limited explanatory power of too high number of predictors, the three variable models were chosen as most appropriate. A sensitivity analysis within NPMR showed that altitude turned out to be the most important predictor variable when smoothed with a tolerance of

160 percent of data range. It was also the first predictor variable that had been selected by the program in all subsets of best models (Table 6) and is contained in all six models. In this model temperature is highly conveyable by altitude, meanwhile precipitation variables were expressed in the model by annual precipitation (Bio12) and precipitation seasonality (Bio15). The average response curves of *C. digitatum* with respect to the three variables are presented in Fig. 5.

Model No.	No. pred	Log (B)	Var 1	Tol 1	Var2	Tol 2	Var3	Tol 3	Var4	Tol 4	Var5	Tol5	Var6	Tol 6
1	1	108,8	Alt.	160,8										
283	2	127,7	Alt.	160,8	Bio15	3								
572	3	141,3	Alt.	160,8	Bio12	25	Bio15							
1377	4	149,7	Alt.	160,8	Bio12	25	Bio15	25	Bio17	1.5				
3633	5	152,7	Alt.	160,8	Bio4	42.2	Bio12	25	Bio15	3	Bio17	2,3		
5835	6	154,0	Alt.	160,8	Bio4	42.2	Bio14	1.2	Bio15	3	Bio16	13,6	Bio17	2,3

Table 6: Characterization of the best single model(s) for *C. digitatum* calculated by NPMR (No. pred. = number of predictor variables, Var. = name of variable, Tol. = tolerance of smoothing function in percent of range)

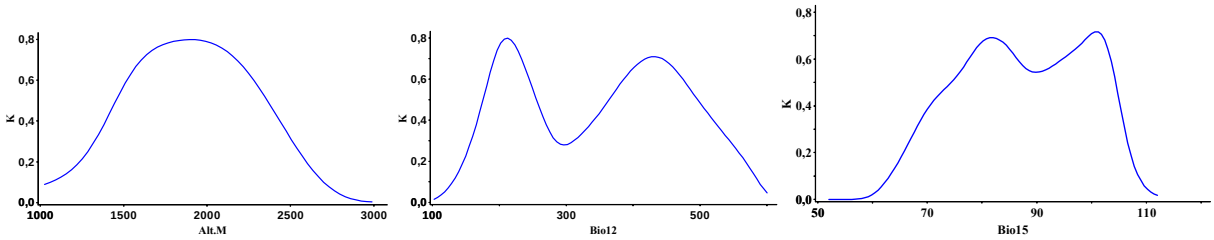

Figure 5: Response curves of *C. digitatum* with respect to Altitude, Bio12 (= Annual precipitation) and Bio15 (= Precipitation seasonality: CV * 100).

Presence of *C. digitatum* is highly probable between 1000 and 3000 m; typical normal distribution curves were obtained. Outside of this elevation zone the species seems to be not existing. Bimodal response of *C. digitatum* to the precipitation variables is anticipated since the species was proved to be of high ecological amplitude (Al-Duais & Jetschke, 2009), it can existed from semiarid to relatively high rainfall area. The three models were evaluated by the confusion matrix and its derived indices, calculated for the optimal threshold corresponding to the maximum kappa (see Table 7) and by AUC Fig. 6.

The maps of predicted current distribution (see Fig. 7, Fig. 8, Fig. 9), were produced after translation of the models from ecological space to geographical space using GIS. Predictions generated here have a natural probabilistic interpretation, giving a smooth gradation from most to least suitable conditions. Both the communities' and *C. digitatum* model were displayed in steps of 10% probability to indicate the chance of occurrence of the target in the study area. Table 8 shows additionally the potential occupied area by for each probability level.

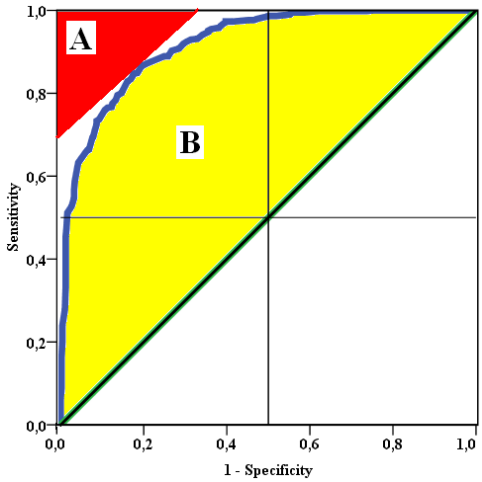

Figure 6: The area under the ROC curve (AUC) for *C. digitatum* chosen model. Area "**A**" resembles the true-negative responses and false-positive responses, and "**B**" resembles the number of true-positive responses and false-negative responses.

Evaluation approach	Model		
	HFFV	**LFV**	***C. digitatum***
max. κ	0.55	0.54	0.56
opt. th.	0.55	0.54	0.57
a	18	29	516
b	0	0	97
c	5	1	80
d	51	33	400
Ss	0.78	0.97	0.87
Sp	1.00	1.00	0.81
FPF	0	0	0.19
CCR	0.93	0.98	0.84
DPR	0.78	0.97	0.67
AUC	0.95	1.00	0.92

Table 7: Models evaluation based on area under curve (AUC) and confusion matrix and its derived indices, calculated for the optimal threshold (opt. th.) maximizing kappa (max. κ). The entries of the confusion matrix, false positives or commission error (*b*) false negatives or omission error (*c*), true negative (*d*), Sensitivity (*Ss*), Specificity (*Sp*), false positive fraction (*FPF*), correct classification rate (*CCR*) and differential positive rate (*DPR*) are given.

Classes of probability	Model		
	HFFV	**LFV**	***C. digitatum***
1-10 %	234097	197998	208866
11-20 %	1483	10840	3924
21-30 %	940	6883	2299
31-40 %	788	3606	2056
41-50 %	588	2548	2371
51-60 %	566	2115	2126
61-70 %	705	2287	1969
71-80 %	859	2631	2659
81-90 %	1194	4166	3909
91-100 %	10780	18926	16124
No data	0	0	5697

Table 8: Occupied area (in number of pixels of 1 km x 1 km) based on NPMR model, calculated by GIS, for each of the three models and for each probability class (of width 10%).

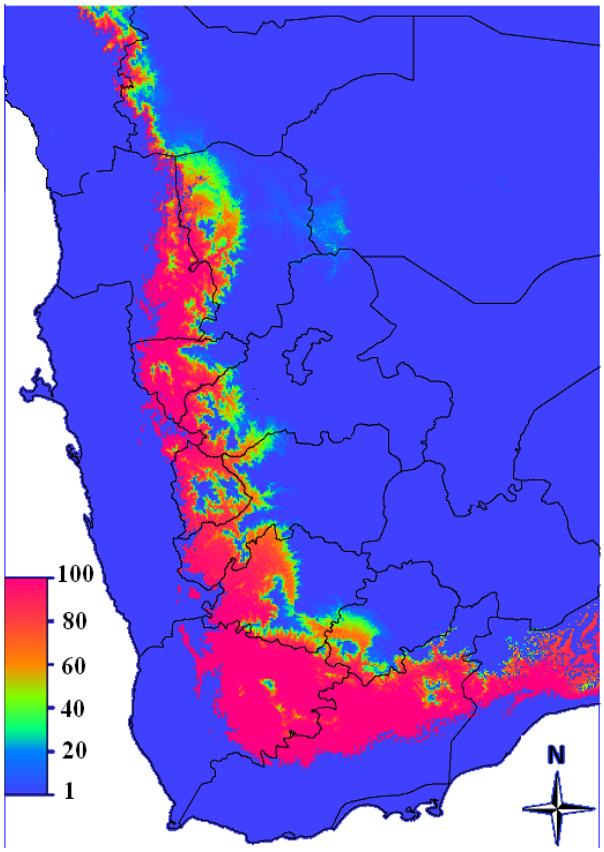

Figure 7: Map of predicted probabilities of occurrence (in percent) for the high frost free vegetation (HFFV) derived from NPMR.

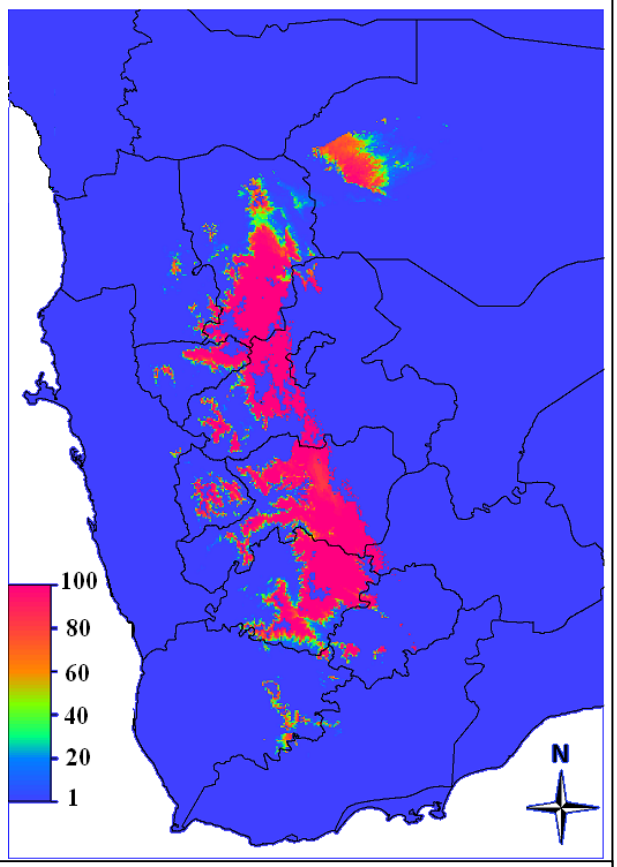

Figure 8: Map of predicted probabilities of occurrence (in percent) for the low frost vegetation (LFV) derived from NPMR

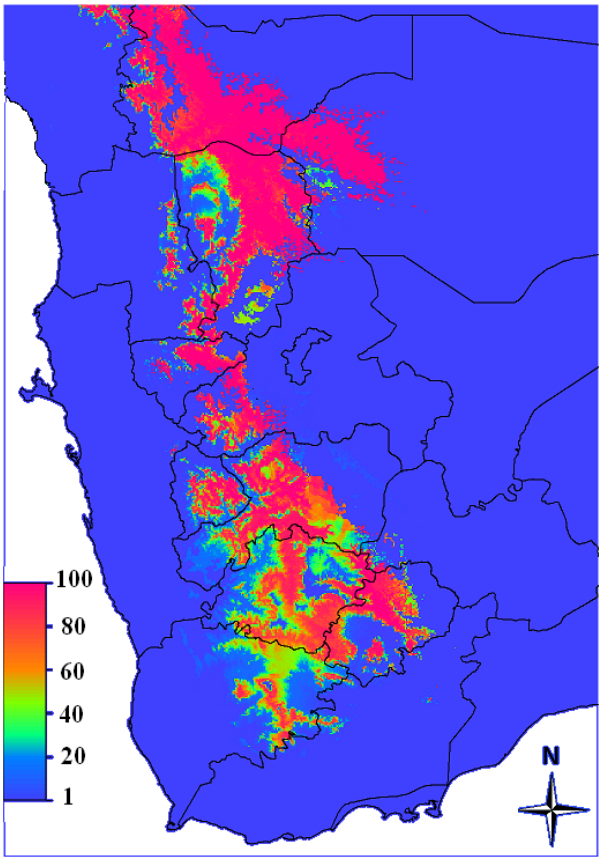

Figure 9: Map of predicted probabilities of occurrence (in percent) for the single species *C. digitatum* derived from NPMR.

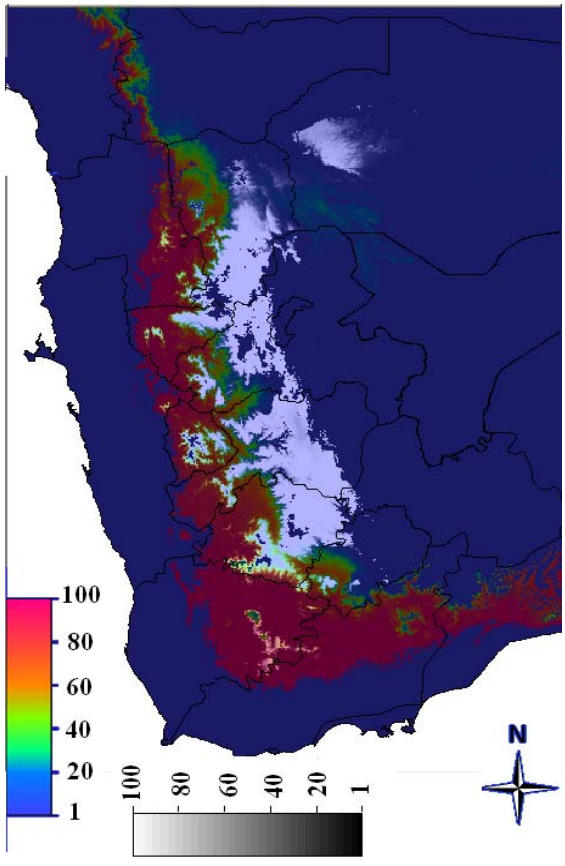

Figure 10: Transparent overlay of predicted probabilities of occurrence for LFV & HFFV (in percent) derived from NPMR.

5. Discussion

5.1. Models assumptions

Profile models usually created just a prediction map without any explanation or identification of variables being important in modelling process, so they need complementary analysis such as Jackknife statistical procedure to identify importance of variables (Levine et al, 2004) or the use of multivariate data analysis like PCA for the explanation of spatial distribution (backward insight). Quite contrary, NPMR with the software HyperNiche immediately visualisse the importance of the explanatory variables and is able to create one and two dimensional response functions with respect to selected environmental variables.

There are two main aims of predictive vegetation mapping, namely to know where a species or a specific community occurs (the "prediction") and to find the reasons for its distribution (the "explanation"). The three models obtained are based on certain equilibrium assumptions, namely that the HFFV, LFV or *C. digitatum* can occur in all environments where it is possible to survive, that it will not be found outside this range, and that it is in equilibrium with climate and biotic interactions (see Fig. 7, Fig. 8, Fig. 9). The opposite is probably true for many species due to several reasons including the anthropogenic influence (Loehle & LeBlanc 1996). The first and preliminary three models were produced with data collected during summer 2005/2006. Among them the species model was of low quality, which we explained by the fact of limited sampling area some places and especially the existence of a pseudo-absence data caused by the massive overexploitation of *C. digitatum.* Meanwhile the first HFFV and LFV models were of rather good predictive power, especially the LFV model. This suggested us to evaluate the model by additional field observations, consequently the predictions of the two community models moved us far to the north, where we indeed found the harbouring vegetation with the species being intact (because it was not recognised as culinary species by the people there). This opportunity was used for adding the newly collected data at the species level and community level to iteratively improve the modelling process, to obtain a higher prediction power and a better understanding of the species ecological requirements.

5.2. Variable selection

Three approaches can be to take decision to choose predictor variables which describe species distribution: (1) based on known biophysical processes, (2) based on ecophysiological knowledge, (3) based on correlations between species distribution and environmental variables (Austin 2007). Point-biserial correlation is one of the ways to investigate association between binary variables, in our case presence/absence, and continuous predictor variables (Kent and Cooker 1996). This index is actually equivalent to Pearson's correlation coefficient and measures only a linear (or at least monotonous) association between two variables. However, in niche theory, species response curves are assumed to be unimodal curves, often symmetric Gaussian shaped. Current evidence supports the occurrence of unimodal response curves with various skewed asymmetric or symmetric shape (Austin 2002). Thus, the point-biserial correlation

coefficient may not be a proper index, because the value for a unimodal response curve can be zero. To complement this, we also used the correlation ratio (Eta), based on ANOVA mean squares, to investigate a nonlinear relation between variables (Table 2). These indices, together with a correlation matrix among environmental variables, proved to be useful to find strong nonlinear relationships between the predictor variables and to reduce the number of variables initially entered in the predictive modelling process.

5.3. Models Explanation

There are many different approaches for the interpretation of such prediction models. Guisan and Zimmermann (2000) suggested that correlative models should draw the realised niche of organism. Austin (2002) suggested that statistical models may not represent the realised niche but rather an amalgam of realised niche and sink areas. Robertson (2003) suggested that the predictions produced by each model may offer different insights into the potential distribution and biology of the target organism. Although statistical models are based on correlations and do not necessarily reflect causal relations, their purpose is often prediction, such that a description of functional relationships can be achieved (Austin 2002).

Generally predictive vegetation modelling can tell us a lot about a species (or community) habitat and environmental niche and the reasons for its distribution in terms of its interactions with the environment around it. The probabilities displayed in the maps generated from NPMR model are interpreted as the probability of occurrence of the target community or species. Response curve is a plot of species or community presence data (e.g. proportion of presence or abundance, if available) in relation to changing environmental variables. In our case, three predictor variables, altitude, Bio12 and Bio15, were the most important ones for explanation of *C. digitatum* presence while others only slightly increased accuracy (expressed as Log(*B*)) while trailing parsimony if more than three variables used will be considered. Although all possible prediction maps were produced and evaluated for the six models, just the three variables one presented in (Table 6) was satisfactory to evaluation purpose and to our field observations Fig. 9.

Nevertheless, the predictive distribution maps derived from this study shall be used as a substantial tool in applied ecology with consequences in conservation, restoration, land use and rangeland management. Comparing the combinations of predictor variables altitude, Bio12 and Bio15 realised in the study area (see Fig. 9), very clearly the wide ecological amplitude for *C. digitatum* is confirmed. The LFV model reflects the previous continuous natural forest dominated by *Acacia origena* and *Euphorbia parciramulosa* which was mentioned by Wood, (1997) and Al-Duais & Jetschke (2009), while the HFFV model reflects the continuous natural lowland forest named *Acacia commiphora* and bushland which was also described by Wood (1997) More details of this vegetation were given by Al-Duais & Jetschke (2009), in which the indicator species *Acacia mellifera, Acacia asak, Cissus rotundifolia*, *Acacia etbaica* and *Euphorbia inarticulata* were determined for two clear communities. Both the LFV and HFFV

communities were later encountered during the field evaluation of the models in the final phase of field work in summer 2007.

Lots of problems were encountered in afforestation projects performed in the northern borders of the study area, in which land evaluation to allocate specific species to suitable sites encountered many difficulties and needed lots of experimental work (Dent & Murtland, 1990). Forestry became a potentially attractive land use when site characteristics were unfavourable for arable farming. In this study, our prediction approach were able to assign certain local tree species (at least the indicator species for LFV and HFFV) as being suitable for afforestation in the high probability areas in the produced prediction maps (Fig. 7 & 8). Austin (2007) suggested that data scale and resolution will determine the nature of the environmental niche models that can be fitted. In that sense our models are excellent for a resolution of 30 arc seconds (equal to approx. 1 km scale).

5.4. Models Prediction

Current methods for assessing predictive success are AUC and Kappa statistics (Fielding & Bell 1997; Allouche et al., 2006) using the scale of agreement proposed by Landis & Koch (1977) and Reineking (2004). While performance at maximum kappa threshold indicated fair agreement for LFV model and good agreement for both HFFV model and *C. digitatum* model (Table 7), the values of differential positive rate *DPR* were rather higher over a whole range of intermediate thresholds, also the correct classification rate is very high (Table 7). The values of AUC as an overall performance measure were outstanding, 0.95, 1.00 and 0.92 for HFFV, LFV and *C. digitatum* models respectively, with very small statistical errors. According to Swet´s scale of recommended interpreting range values for AUC, the models indicated excellent performances. AUC for the target species *C. digitatum* model Fig. 6, according to Jager, (2001) the highest discriminatory capability is obtained if sensitivity and specificity is almost equal. Also, the discriminatory ability is underestimated if sensitivity or specificity is low *C. digitatum* model reach very good performance since sensitivity and specificity is quite similar 0.87 and 0.81 respectively and both not low compare to the high data set (1094 P/A). This very optimistic result can be visualised by comparing the two areas in Fig. 6; "**A**" which resembles the true-negative responses and false-positive responses results, and "**B**" which resembles the true-positive responses and false-negative responses.

We also considered model performance based on objective intrinsic criteria (Anderson, 2003). Models with high omission error are definitively weak, models with high commission index produce an over prediction of presence area, while models with minimum commission and omission error (close to zero) often indicate an over fitting problem and could be weaker if applied to other areas. Thus, a good model should be located at low level omission error and moderate commission value (Anderson et al, 2003). However, in our case the situation may also interpreted in a different way: Commission error is acceptable, meaning that existence of *C.*

digitatum is predicted, but an extensive overexploitation stands behind this error, while omission error is lower and acceptable (the sensitivity of the model = 0.87), which could be also explained by the wide ecological amplitude of the species (Al-Duais & Jetschke, 2009) in the sense that still other places sustain *C. digitatum* which were not sampled in our survey. Both types of errors could be influenced by pseudo-absence data that could erroneously incorporated into modelling as real absence data. With respect of the vegetation models there is almost no commission error while the omission error is very low for FLV and moderate for the HFFV. However, the model results are very useful for our purpose since they have to be interpreted in the context of how the models will be applied. Some applications may be able to tolerate lower accuracy or precision, thus the indicator species of the modelled vegetation were frequently encountered in the field in the area of prediction along with the target species *C. digitatum* and also were recorded from previous studies like **(**Hall et al, 2008**).**

According to our knowledge, there are only few papers up to now that used NPMR for species distribution modelling (McCune 2006b). According to Cohen's kappa, AUC, ecological interpretation of response curves and visual inspection of produced maps, NPMR is a useful tool to reproduce the current spatial distribution of the two vegetation zones and the target species based on a few important predictor variables. Tarkesh (2008) demonstrated that NPMR is a powerful modelling tool with predictive capabilities comparable to other methods; likewise NPMR in our models identified a rather small subset of predictor variables (namely three) with excellent predictive capability performance (see Table 7). Another advantage of NPMR is its capability to immediately draw and inspect species response curves allowing a rather general form (see Fig. 2), compared with the rather restricted approach in generalized linear models (GLM) and generalized additive models (GAM) (Tarkesh, 2008).

The relationships quantified by NPMR between the binary presence/absence response of *C. digitatum* and the selected of predictor variables (Altitude, Bio12 and Bio15) are based on empirical observations and cannot be interpreted as causal ones. Unfortunately there is no previous botanical or ecological research published so far about *C. digitatum.* However, our model is in accordance with other concurrent studies by us (Al-Duais & Jetschke, 2009); besides our autecological observation from the field, the garden and the greenhouse accentuated the high ecological amplitude of the species. It seems to be clear, too, that precipitation during the early rainy season plays a key role for the biomass production of *C. digitatum*.

It is also important to mention that NPMR models do practically make no extrapolation of response functions due to the nonparametric character of the smoothing procedure. Therefore, application to areas with environmental conditions different from those observed in the study area is limited (Table 8). If the existing environmental combinations were completely out of the range observed in the study area, predictions will be reported as "No data" in such cases. Therefore, data for model building should cover as much as possible of the environmental range.

Despite the critical situation of the modelled species *C. digitatum,* this task was accomplished to a high degree in our case owing to the iterative process between vegetation modelling and species modelling. Further iteration between LFV and HFFV modelling and *C. digitatum* modelling will be desirable, such that it could also be extended to the southern mountains in Yemen and maybe even to the neighbouring countries. In a comparative view between Fig. 9 and Fig.10 we can find that LFV and HFFV models still predicted occupied areas as absence in *C. digitatum* model. Thus prediction and iteration between an overexploited species and its harbouring vegetation must be repeated to reach a level of high accuracy that enables conservation and restoration process of such endangered species to be applied in all parts of the ecological niche realisable under different conditions.

6. Conclusion

Vegetation modelling with NPMR showed a very good performance to our purpose; especially the iteration process led to record more presence sites for such an overexploited species. With relatively few predictorss the models generated by HyperNiche have a natural probabilistic interpretation, giving a smooth gradation from most to least suitable conditions. We have also shown that the models can be easily interpreted by human experts, a property of great practical importance. A model based on physiological mechanisms might produce even better results, thus *C. digitatum* model could be enhanced if data on the role of biotic interactions, seed dispersal, seed bank and germination were incorporated, but the necessary knowledge of autoecology and of historical changes is lacking. We propose comprehensive studies to include physiology and autoecology of the target species into the spatial modelling process to allow more details and finer grain size in future research.

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CHAPTER 7: General Discussion

Each of the previous five chapters (Ch. 2-6) on its own had already been discussed in its specific context. Therefore, this final chapter will highlight and summarize the major aspects of this research and provide general overview about the knowledge obtained from the literature, the field and lab work, the data analysis and modelling and some of the complementary laboratory work that was not included in the previous chapters. Beside that, some scientific ideas of the intended extension of this fruitful project in the future will be given. The project provided general answers to the questions that we addressed in the introduction (Chapter 1).

Authentication of the level of demand on culinary and medicinal *C. digitatum* products was an important motivation toward encompassing the critical situation of the plant and its culture of use. Beside the main product the discs (Ch. 1 figure 1) which could be used with different meals, in different regions we find different additional methods of preparation beside the large scale commercial preparation. Table 1 summarised the obtained information starting from the region of the highest demand (the lower latitudinal range of the plant) in the south in which *C. digitatum* produced commercially to the far and local markets moving to the north, the war arch in the far north was not visited for safety reasons.

To answer the first question (1), if there is any justification for this kind of high demand for a plant which is toxic before processing or if it was just a legendary heritage. We started by evaluating the capacity of functional food ingredients content in the plant. We tried to find a link between the high demand and the general medicinal and culinary benefits. Especially the antioxidant capacity and the total phenolics of the processed form and the raw material (both in dried form) in water and ethanol extracts were evaluated by using sophisticated micro plate reader techniques. The antioxidant capacity was elucidated by four methods, namely TEAC, DPPH, FRAP and ORAC while the total phenolics was done by Folin–Ciocalteu method (Chapter 2).

No or only very weak correlations were found between the remarkable antioxidant capacity and the reasonable total phenolics. The processed samples (the consumed form) showed a consistent positive pattern through all tests. Although there is a wide research in various plants, food application examples of antioxidants from less known plants, like ours, are very few. A more important scientific contribution of our research is that it is the first scientific work which visualised the importance of leaves from plants of the Vitaceae family as a possible rich source

of antioxidants and phenols, compared to the intensive research in the members of other families, like Lamiaceae, Asteraceae, Fabaceae, Geraniaceae and Rosaceae. Our approach anticipated to trigger more investigation in this plant family leaves. In this context experimental parts were already done by me in the same laboratory for the related species *Cissus rotundifolia*.

Table 1: Additional methods of preparation and use for *C. digitatum*.

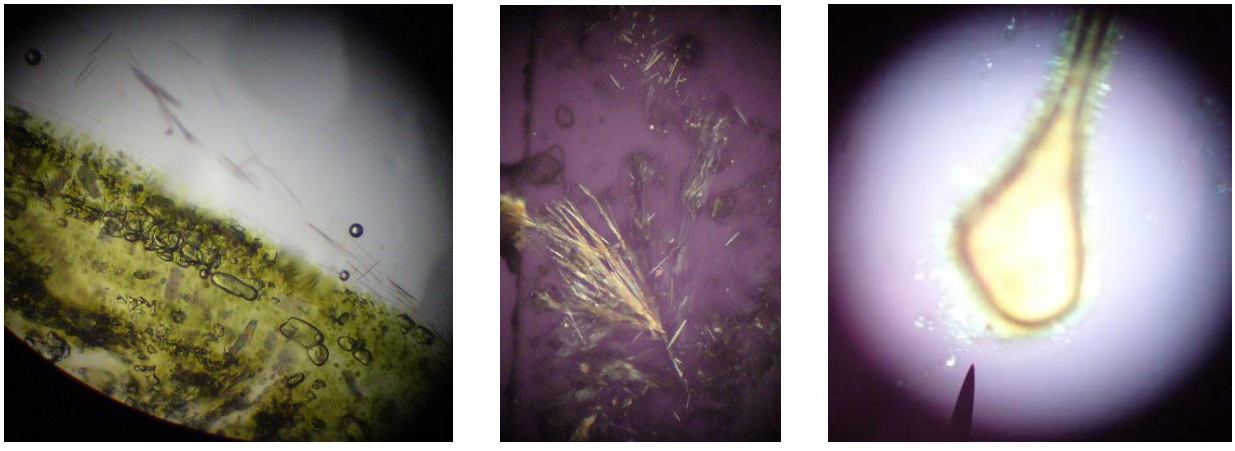
Governorate /Region	Preparations and use
Taiz /Saber Mountain and Blad Al-Hikey	Commercial large scale preparation by adding *Cissus rotundifolia* and *Romex Nervousus* then sale the produced discs as pure *C. digitatum* product.
Taiz / Al-Mesrak	For malaria by milling with garlic and black piper
Taiz / Shareb and Al-Audain	Cooked with spices, garlic, onion and special butter called Samn and directly eaten with bread or Asaid.
Taiz /Saber Mountain, Blad Al-Hikay and Dala'a /Jahaf Mountain.	Dressing for salad and other dishes.
Dala'a /Al-Aud	It is put directly on fire in traditional oven (called Tanour) then sorghum is baked, in which the bread becomes more toasted with special Aroma.
Ibb/Mothaikherah Al-Gawaleh	Boiling or overnight cooking in the Tanour with hot paper, garlic and thyme; thinning in discs and dried.
Ibb/Mothaikherah, Baddan, Shaer and Al-Gashen	Widely used for vomiting.
Ibb/Suhban	Cooked with potato, spices, garlic, onion and Samn and directly eaten with bread.
Damar/Al-Manar	Not eaten but overnight cooking in the Tanour then but it on the subcutaneous abscess for healing.
Raimeh/Al-Salafiah	Used for cleaning silver and antiques.
Hagah/Hsen azan	Edible after boiling with milk.

These results led the research toward the investigation for other functional food ingredients which stand behind the antioxidant capacity. Thus, the contents of vitamin C, vitamin E, and carotenoids and changes caused by the traditional household processing were investigated (Chapter 3). Carotenoids were determined by reversed phase C30-HPLC method with diode array detection at 470 nm, while tocopherols and tocotrienols component of vitamin E were analyzed by using normal phase HPLC with fluorescence detection (excitation, 292 nm; emission, 330 nm). Vitamin C was determined spectrophotometrically after reaction with DNP by measuring the absorbance at 520 nm. The carotenoids lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, and β-carotene (Provitamin A precursor) were determined; lutein, zeaxanthin and β-carotene are in high concentration, the first two were dramatically reduced by the household processing. Likewise, vitamin C (high content) was reduced to half by the processing; In contrast,

the outstanding high content of vitamin E (found in different forms, some of which were rare in other sources) was interestingly enhanced by the processing.

While still many carotenoids peaks are not identified in the consumed form (although arrangements were done to diagnose those compounds in the Max Planck Institute for Chemical Ecology, Jena, in July). These findings were of critical importance for the local people's health since culinary therapeutic synergistic effects of this combination of functional food ingredients from such free natural product can solve many health problems caused by malnutrition, especially many eye illnesses (Rosen et al, 1996; Ghaleb, 2007). Future enhancement of the product will most be triggered to escape the drawbacks of the main household processing that was performed to reduce the content of antinutritional substances and to diminish their effects (Ciganek et al, 2007).

Now we do no longer believe that the cause of toxicity of the fresh plant was caused by a chemical substance. We use the polarized light microscope to investigate the anisotropic character of the plant microscopic structures in *C. digitatum* and proved, surprisingly, that mechanical structures in the form of microscopic spiny crystals are the cause of the very cursive scorching effects of the fresh leaves on the mouth mucosa. Untreated homogenate of the fresh leaves can even burn the skin if applied on the soft skin parts like those between the fingers bases (see figure 1a & b). Surely this is the single cause that made the fresh plant completely unpalatable by all the herbivores as we observed in the field (Al-Duais et al, 2009), only humans can consume it but after processing (see figure 1 c).



a) b) c)

Figure 1: Photos taken for *C. digitatum* under the polarized light microscope visualising the spiny crystals **a)** at 40 X for the fresh leaves, **b)** at 100 X for the dry leaves, **c)** at 100 X for the consumed form.

When we asked people who consumed *C. digitatum* in daily bases, why they followed that demand, the replied "taste it and smell it". So this is the first attraction point in the processed form to the consumers. Beside there is a high content of carotenoids, but a dramatic reduction especially for lutein and zeaxanthin which is known to be broken down by processing to give the characteristic aroma during thermal food processing (Leffingwell, 2002).

Thus a quick descriptive analysis of the volatile components in *C. digitatum* is best obtained by using solid phase microextraction (using PDMS/DVB bi-polar fibre the nonpolar PDMS fibre) and a subsequent analysis by gas chromatography (Chapter 4). A remarkable collection of important volatiles with different aromatics, food additives and potential medicinal application were described according to their retention times (RT). The identification was based on spectrum verification from the NIST and Wiley mass libraries or the literature retention indices. Among them were the volatiles geranyl acetone, vitispirane, β-cyclocitral, β-myrcene, safranal, limonene, furfural, acetic acid and formic acid. This combination of volatiles obviously gives the characteristic aroma that attracts the high demand. Consequently moving towards quantification of this class of functional food products must be the next task in that line, because many of them are also of industrial importance.

Based in the promising results discussed in (Ch. 2-4) animal model experiments should be also established soon with suitable strains vulnerable to different disorders including vascular disorders and cancer. So the dosages that could promote health among the consuming public could be determined. The plant is highly promising in antibacterial and antifungal activity (see Figure 2), thus a chemical and biological screening of *C. digitatum* for more promising bioactive ingredients was already established in cooperation with Prof. Matthias Hamburger's group in the Department of Pharmaceutical Sciences, Basel University. However, the results obtained so far were beyond the scope and limit of this PhD thesis and will be published separately.

To answer the ecological requirements of *C. digitatum* we implement two contrasting concepts in plant ecology, namely the holistic concept of Clements (1916) and the individualistic concept of Gleason (1926). With the Clements concept we implemented a Braun-Blanquet approach guided by centralized replicate sampling procedure in the field. Multivariate statistical tools were applicable and finally ended up with seven well distinguished plant communities that harbour *C. digitatum.* Two of them represent frost intolerant vegetation; four of them contain frost tolerant vegetation, while one is just a transition community (Chapter 5).

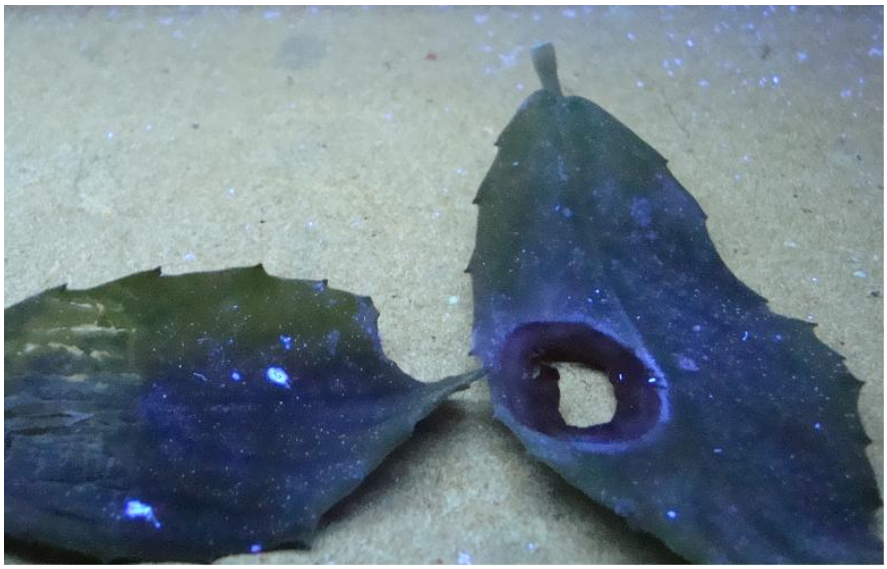


Figure 2: Illumination of *C. digitatum* leaves under the UV light. The intense illumination circle grasping a fungal infection suggests the production of bioactive chemicals which could be of application in natural plant protection.

The main explaining variable was altitude which was highly correlated with nearly all bioclimatic variables related to temperature. Among them, we selected Bio6, the minimum temperature of the coldest month, as the predictor with the most obvious ecological relevance. We strongly claim that our series of typical plant communities along an elevation gradient is shaped by winter temperatures. More observations in the field and greenhouse experiments have to follow, to even increase our insight to the real broad ecological amplitudes of the species. Moreover, we recorded the species in a wider elevation zone and in different habitat than that stated by Wood, (1997).

Floristically this is the first study that implements highly objective methods of sampling and statistics to characterise and classify the plant communities in the Western highlands of Yemen between 1000 m and 2600 m. Moreover we defined an important community as the lowest moderate frost tolerant community (*Acacia gerrardii* - *Solanum incanum* community) which is completely undescribed as community by many plant ecologists including Wood (1997). We also characterised and recorded some of the medicinally important and often endemic species which have such ambiguous situation for future further study and analysis. Based on the previously described objective methodology, that divided the relevés into frost intolerant vegetation and frost tolerant vegetation, we estimated the lower boundary of the frost sensitive zone to be around 1800 m. This contrast previous conflicting estimations which were produced by highly subjective methods to be 1600m, according to Wood (1997) or 2000 m by Al-Hubaishi & Müller-Hohenstein (1984).

With respect to the individualistic concept by Gleason the primary model produced by using the nonparametric multiplicative regression approach (NPMR) from the presence/ absence localities

data of the single species *C. digitatum* as response to the environmental gradients, we were very disappointed by the rather weak prediction capacity. We claimed that this situation must be mainly caused by the fact that *C. digitatum* is an overexploited species, which means that many pseudo-absence data were recorded but interpreted as real absence, leading to a bias in the modelling process for the species (Chapter 6). Thus we moved again towards Clements concept and dealt with the produced plant communities as basic entities according to Clements.

To keep the number of input data large enough for a sound model, we produced predictive models only for the two well defined large vegetation types simultaneously with the species (Chapter 6). In this respect we had mutual presence/absence data available for the two types of frost tolerant and frost free vegetation. Responses derived so far with respect to elevation and bioclimatic variables seem to be ecologically reasonable. Some bimodal shapes are attributed to the limiting amount of data or an insufficient smoothing and might be artefacts. Moreover, the predictor variables provided by the Worldclim data set are interpolated ones and may not properly fit the real climatic data (mostly missing throughout Yemen). In this respect it is satisfying to see the "very good" consistency of model predictions and observations.

The produced vegetation models led us to search for the species far away from the study sites, because predictions were made for a much larger part of the Yemen highlands. Surprisingly, we indeed found presence localities far to the north (more than 400 km away!) where the species was often recorded in more intact situation, sometimes with different combination of ecological conditions. This proved the high accuracy of our habitat models (for species and communities) and the very good prediction map for *C. digitatum* much more than any of the statistical indicators, as Cohen's Kappa or AUC. The iteration process between vegetation modelling and species modelling provided a very good understanding and characterisation of the broad ecological niche of *C. digitatum*. Hence area of high suitability can now be detected for the ultimate goal of restoration. Future large scale cultivation could also fulfil the rising demand which will be anticipated to expand especially after our tremendously important discoveries with respect to *C. digitatum* functional food ingredients content (Chapter 2, 3 & 4).

It should be emphasised again that the classification, ordination and modelling approach in this paper represents the first attempt to produce a national-scale vegetation classification and modelling system in Yemen, using a range of complementary methods and current best practices in statistical techniques. It will be, undoubtedly, refined and extended in the future with expanding the dataset horizontally and vertically to higher and lower elevations and cover more

endangered species and fragile communities in urgent needs for restoration. The immense productivity and success of this approach of methods combination will guide the application of such methods and techniques to other less studied regions in Yemen and in neighbouring countries with similar vegetation.

Currently, considerations of the exploitation and conservation of wild plant resources must be take ecological principles into account. The last four decades have witnessed a substantial change in the land use system in and around the southwest highlands of Yemen. Moreover, this has taken place with complete indifference to the fate of rare or endemic species causing destructive changes to plant life in such fragile habitats. It is necessary to involve conservationists and ecologists in the planning of these development scheme areas to ensure the conservation and regular monitoring of the flora and vegetation of *C. digitatum* habitats.

The species seems to have important indirect role in different food webs since it is among the few plants we encountered in the field with bad smell of flowers. Flowering season is relatively long and mostly continuous (a different degree of maturation probably exists in the same individual from flowering to the fully ripened berry). Thus all the time the plant attracts different insects pollinators (Figure 1, chapter 3), consequently Yemen chameleons (*Chamaeleo calyptratus,* becomes more and more house animal) noticed to have quite preference to stands that contains *C. digitatum.* Besides that, local peoples claimed that certain bird preferably consumed the fruits. Thus investigation of the role of *C. digitatum* in nature and the consequence of overexploitation of the species on the trophic levels will be a near future complementary task to our efforts. In the past only *C. digitatum* leaves and young branches were harvested at the end of September annually (end of the rainy season and climax of the growth of *C. digitatum* after that the plant remains dormant until the next season in March/April), prepared as discs and saved for consumption all the year, while the source plant flourished again in the next season. This sustainable use was only recently replaced by a marathon of intensive gathering and large scale commercial preparation, where the branches were removed completely leaving the plant severely injured.

Studying and understanding an economic species like *Cyphostemma digitatum* and claiming for its protection will hopefully give a response at the population and governmental levels and directly promote protection regulations and awareness. The restoration is very simple and needs no special high cost executions, while the species becomes more and more economic. This is not always the case in Yemen; for example, the important valley forest habitat on Jabal Bura received protected area status by the Environment Protection Authority. In the same time the core of the forest was irreversibly destroyed and the whole forest was made more accessible by a highway built by other Yemeni authority (Hall, 2008). Until now no real progress in restoration

and reestablishment of *Dracaena cinnabari* in Socotra despite the worldwide support and interest. Herzog (1998) was very annoyed from the attitudes in Yemen against forests ("forests don't make sense"), he concluded that forest management in Yemen has more to deal with people and their use of the forests (the destructive processes) than with trees themselves. Thus the previous sustainable of *Cyphostemma digitatum* and other species use could be recovered with: (1) Introducing the culture of cultivations by seeds which show a very high level of germination in the experiments, also by old branch grafts to be done at many and different places produced by our prediction map to stop the rapid decline, and (2) Announcing *C. digitatum* as an endangered species and prohibiting the mass transport in the highways between the regions of gathering and preparation places in the south in Saber Mountains and other places.

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Summary

1. The project was motivated by the problem of overexploitation of the species *Cyphostemma digitatum* (Vitaceae), being important as medicinal and (after processing) culinary source in Yemen. It is aimed to answer simple questions about the plant's situation, the phenomenon of the increasingly high demand on *C. digitatum* and for the ultimate goal of restoration of the plant in its natural habitat and the culture of it's sustainable use. We asked: (1) Was there any justification for the high demand? (2) Can we understand the ecological requirements of *C. digitatum*? (3) Could this ecological niche be generalised for possible reintroduction, cultivation or restoration of *C. digitatum?*

2. To answer the first question we started by searching the content and effect of functional food ingredients, namely the antioxidant capacity and the total phenolics of the processed form and the raw material (both in dried form) in water and ethanol extracts. We used 96-well micro plates with BMG FLUOstar Optima micro plate reader. The antioxidant capacity was elucidated by four methods: TEAC, DPPH, FRAP and ORAC. No or very weak correlations were found between the remarkable antioxidant capacity and the reasonable total phenolics. The ORAC assay proved to be more powerful than the other assays.

3. These results led the research toward the investigation for other functional food ingredients standing behind the antioxidant capacity. Thus the contents of vitamin C, vitamin E, and carotenoids as well as changes caused by common processing were investigated. Carotenoids were determined by reversed phase C30-high-performance liquid chromatography (HPLC) with diode array detection at 470 nm, while tocopherols and tocotrienols were analyzed by using normal phase HPLC with fluorescence detection (excitation, 292 nm; emission, 330 nm). Ascorbic acid was determined spectrophotometrically after reaction with DNP by measuring the absorbance at 520 nm. The carotenoids lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, and β-carotene (Provitamin A precursor) were determined; lutein, zeaxanthin and β-carotene were found in high concentrations. The first two were dramatically reduced by the household processing; likewise, vitamin C (high content) was reduced to half by processing. In contrast, the outstanding high content of vitamin E (found in different forms, some of which were rare in other sources) was enhanced by processing.

4. The high content of carotenoids and its dramatic redaction by processing implied that carotenoids were breaks down by the processing to gives the characteristic aroma of the consumed form. Thus a quick descriptive analysis of volatile components in *C. digitatum* is best obtained by using solid phase microextraction (using PDMS/DVB bi-polar fibre the nonpolar PDMS fibre) and analyzed by gas chromatography. Remarkable collections of important volatiles with different aromatic; food addidative and medicinal application were described according to their retention times (RT). The identification was based on spectrum verification

from the NIST and Wiley mass libraries or the literature retention indices. Among this volatiles Geranyl Acetone, Vitispirane, β-Cyclocitral, β-Myrcene, Safranal, Limonene, Furfural, Acetic acid and Formic acid were found.

5. To answer the ecological requirements of *C. digitatum* we implemented the two concepts in plant ecology, namely the holistic concept by Clements (1916) and the individualistic concept by Gleason (1926). With the first concept we implemented a Braun-Blanquet approach in the field based on 89 relevés. With multivariate statistical tools in the work place, especially hierarchical cluster analysis, indicator species analysis (ISA) and nonmetric multidimensional scaling (NMS), adding experience from the field, we ended with seven well distinguished plant communities, that harbour *C. digitatum.* Two of them are frost tolerant vegetation and four of them are frost free vegetation while one is just a transition community. All were characterised by indicator species; a complete synoptic table was constructed. Thus we got an insight to the real broad ecological amplitude of the species. The efficiency of Braun-Blanquet approach combined with multivariate statistics in a subtropical area was proved by us for the firt time.

6. The individualistic concept seemed to be suitable with respect of studying the plant species' response to the environmental gradients. Hence, a predictive habitat model for was constructed by a nonparametric multiplicative regression approach (NPMR), taking altitude and bioclimatic variables as predictors and approx. 1000 presence/absence observations as response. However, this approach was not as effective as expected due to the situation that is an overexploited species. This implies that many pseudo-absence data due to human influence were recorded as real absence data. Therefore, we moved towards habitat modelling for the communities harbouring *C. digitatum* resulting from our classification approach, however compacted to only frost tolerant vegetation and frost free vegetation. Hence, presence/absence data were taken from the recorded relevès and the appropriate models were constructed. Evaluation based on resubstitution method classified the model as "rather good", based on Cohen's Kappa and Area under curve (AUC).

7. The predictive vegetation models above led to search for the species at places in the north far away from the main source and overexploited areas. Many new presence localities of the species were found based on predictions, often recorded in more intact situation, sometimes with different combination of ecological conditions. Consequently a model and a prediction map of *C. digitatum* with much higher accuracy was obtained, because of the iteration process between species and vegetation modelling, implementing the conflicting concepts of Clements and Gleason. Hence, areas of high suitability can now be detected and used for the ultimate goal of restoration. In the future large scale cultivation could fulfil the rising demand, which is anticipated to expand after our important functional food ingredients discoveries.

Zusammenfassung

1. Im Jemen wird die zur Familie der Vitaceae gehörende Art *Cyphostemma digitatum* in zunehmendem Maße sowohl medizinisch als auch kulinarisch (nach entsprechender Zubereitung) verwendet und deshalb lokal stark übernutzt. Vor diesem Hintergrund bestand die Motivation zu diesem Projekt darin, zum einen die Inhaltsstoffe der Pflanze und zum anderen die Möglichkeiten ihrer nachhaltigen Nutzung im natürlichen Lebensraum zu untersuchen. Im Mittelpunkt der Dissertation stehen (1) die chemische Zusammensetzung als Grundlage für eine verstärkte Nachfrage, (2) das Verständnis der ökologischen Anforderungen unter Freilandbedingungen, und (3) die Nutzung dieser Kenntnisse bei einer möglichen natürlichen Wiedereinführung oder Kultivierung von *C. digitatum*.

2. Im ersten Komplex geht es um die Bestimmung der Inhaltsstoffe von *C. digitatum* als funktionelle Nahrungsbestandteile. Dazu wurden die antioxidative Kapazität und der Gesamt-Phenolgehalt des jeweils getrockneten rohen bzw. traditionell zubereiteten Pflanzenmaterials bestimmt. Dabei sind Wasser- und Ethanolextrakte auf 96-well micro plates mit einem BMG FLUOstar Optima micro plate reader analysiert worden. Die antioxidative Kapazität wurde mit vier Methoden (TEAC, DPPH, FRAP and ORAC) bestimmt. Antioxidatives Potential und Gesamtphenolgehalt korrelierten nicht oder nur sehr schwach miteinander. Dabei erwies sich die ORAC-Methode als besser geeignet als alle anderen Methoden.

3. Als weitere funktionelle Nahrungsbestandteile sind die Gehalte an Vitamin C und E sowie an Carotenoiden und deren Veränderungen durch die traditionelle Zubereitung bestimmt worden. Die Carotenoide wurden mit der Reversed-Phase-C30-High-Performance-Liquid-Chromatography (HPLC) mit Diodenarray-Detektion bei 470 nm bestimmt, Tocopherole und Tocotrienole mit der Normal-Phase-HPLC mit Fluoreszenzdetektion (Anregung bei 292 nm; Emission bei 330 nm) analysiert. Ascorbinsäure wurde spektralphotometrisch nach DNP-Reaktion und Absorption bei 520 nm analysiert. Die Carotinoide Lutein, Zeaxanthin, Canthaxanthin, β-Cryptoxanthin und β-Carotin (Provitamin A) wurden quantifiziert. Von den im pflanzlichen Rohmaterial in hohen Konzentrationen auftretenden Substanzen Lutein, Zeaxanthin und β-Carotin kam es bei den beiden erstgenannten durch die Zubereitung im Haushalt zu einer sehr starken Abnahme der Gehalte. In ähnlicher Weise halbierte sich bei Zubereitung auch der (hohe) Gehalt an Vitamin C. Im Gegensatz dazu blieb der außergewöhnlich hohe Gehalt an Vitamin E (in verschiedenen, teils selten auftretenden Formen) durch die Zubereitung erhalten.

4. Der ursprünglich hohe Gehalt an Carotinoiden und die dramatische Verringerung durch die Zubereitung führte zur Vermutung, daß deren Abbau das charakteristische Aroma der Speise entstehen läßt. Dazu wurden die flüchtigen Substanzen von *C. digitatum* mit der Festphasen-Mikroextraktion (Verwendung der bipolaren PDMS/DVB-Faser bzw. der unpolaren PDMS-Faser) bestimmt und diese Stoffe dann mittels Gaschromatographie analysiert. Dabei wurde eine

breite Palette an wichtigen flüchtigen Substanzen mit verschiedenen Aromen gefunden. Aufgrund unterschiedlicher Retentionszeiten (RT) konnten weitere nahrungsergänzende und medizinisch wirksame Stoffe beschrieben werden. Die Identifizierung beruhte auf einem Vergleich der Spekten mit den umfangreichen NIST und Wiley Bibliotheken bzw. auf Retentionsindizes aus der Literatur. Darunter waren Geranylaceton, Vitispiran, β-Cyclocitral, β-Myrcen, Safranal, Limonen, Furfural, Essig- und Ameisensäure.

5. Im zweiten Komplex geht es um die ökologischen Anforderungen von *C. digitatum* unter Freilandbedingungen. Zur Untersuchung der ökologischen Nische der Art im jemenitischen Verbreitungsgebiet wurden das holistische Konzept von Clements (1916) und das individualistische von Gleason (1926) herangezogen. Basierend auf dem ersten wurden in drei Feldaktionen insgesamt 89 pflanzensoziologische Aufnahmen sensu Braun-Blanquet erhoben. Mit Hilfe multivariater statistischer Auswertungen, insbesondere hierarchische Clusteranalyse, Indikatorart-Analyse (ISA) und Ordination durch nichtmetrische multidimensionale Skalierung (NMS), kombiniert mit empirischen Erfahrungen, konnten gut 7 unterschiedene Pflanzengesellschaften herausgearbeitet werden, in denen *C. digitatum* vorkommt. Zwei davon enthalten frostintolerante Arten, vier sind frosttolerant, während eine Übergangscharakter hat. Dies belegt die breite ökologische Amplitude von *C. digitatum* und die typische Abfolge von Pflanzengemeinschaften entlang eines Höhengradienten. Die Anwendbarkeit eines solchen pflanzensoziologischen Zugangs, kombiniert mit multivariater Statistik, in einem bisher kaum erfaßten Gebiet konnte erstmals nachgewiesen werden.

6. Das individualistische Konzept scheint geeignet, um die Reaktion einer Art auf Umweltgradienten zu untersuchen. Deshalb wurde mittels nichtparametrischer multidimensionaler Regression (NPMR) ein prädiktives Habitatmodell konstruiert, das Höhe und bioklimatische Variable als Prädiktoren und etwa 1000 Präsenz/Absenz-Daten als Response enthält. Dieser Zugang war allerdings nicht so wirksam, da die Spezies sehr übernutzt ist. Als Folge wurden viele Pseudo-Absenz-Daten als Folge menschlicher Nutzung als tatsächliche Absenz gewertet. Deshalb wurden alternativ Habitatmodelle für die Pflanzengesellschaften, die *C. digitatum* enthalten, basierend auf der gefundenen Klasifikation, konstruiert, allerdings reduziert auf die beiden Klassen frosttoleranter und frostfreier Vegetation. Die entsprechenden wechselseitigen Präsenz/Absenz-Daten wurden den 89 Relevés entnommen. Eine Bewertung der Modelle mittels Cohens Kappa-Wert bzw. dem AUC-Wert ergab, daß sie die Beobachtungen „ziemlich gut“ wiedergeben.

7. Unsere prädiktiven Vegetationsmodelle führten uns zur Suche nach der Art in Gebieten, die weit entfernt vom Kerngebiet mit der hohen Übernutzung liegen (weit im Norden) Viele neue Präsenz-Orte wurden dann registriert, oft in gutem Erhaltungszustand, mitunter in neuen Kombinationen der Umweltparameter. Nachfolgend wurden dadurch ein Modell und eine potentielle Verbreitungskarte von deutlich höherer Genauigkait erstellt, speziell durch den

wechselseitigen Iterationsprozeß von von Art- und Vegetationsmodell. ein hochauflösendes Modell. Nunmehr können Gebiete mit einer guten Eignung für die natürliche Wieder- einführung der Art vorhergesagt und genutzt werden. In Zukunft könnte eine großräumige Kultivierung der Art den steigenden Bedarf decken, der aufgrund der von uns entdeckten wichtigen funktionellen Nahrungsbestandteile zu erwarten ist.

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Declaration Statement

Herewith I state that the work presented here as a PhD thesis is my own. I have conducted the field work, analysed the data with the statistical methods and geographical information system described, performed the laboratory extractions and evaluation. I wrote all the manuscripts in all published, accepted or in future to be submitted manuscripts presented here. I am the first and corresponding author in all. Collaborations with other people are mentioned below.

Volker Böhm gave laboratory space to achieve the biochemical characterization and discussed with me the general approach. Lars Müller, Juliane Hohbein and Susanne Werner gave training in the applied methods and techniques installed in the laboratory; they also helped in revising the manuscripts before submission for publication (Chapters 2 & 3).

Christine Bartzsch and Michael Reichelt gave laboratory space and training in the techniques installed in the laboratory for aroma determination (Chapter 4).

Gottfried Jetschke provided training in field methods, introduced me to basic methods in modelling and plant ecology (Chapters 5 & 6), facilitated my working in different laboratories, gave continuous intellectual input and support in English language and supervised my PhD work (Chapters 1 to 7).

Winfried Voigt contributed in skilfulness in multivariate statistical analysis methods (Chapter 5).

Mostafa Tarkesh Esfahani contributed in skilfulness in GIS and modelling methods (Chapter 6).

Herewith I state that this thesis has not been nor will be submitted as a PhD thesis at any other university or research institute.

June 26, 2009, Jena, Germany

Mohammed Al-Duais

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Curriculum Vitae

Name: **Mohammed A. M. Al-Duais** Date / Place of birth: **12. Jan. 1974**, Ibb (Yemen)

Education

[2005 - Present] Ph.D. student at University of Jena, Germany, Institute of Ecology, Plant Ecology and Modelling Group, Supervisor: PD Dr. Gottfried Jetschke

[1999 - 2003] MSc. In Biology, Al al-Bayt University, Mafrak, Jordan; Dissertation topic: "A Survey Study of the Bioaccumulation of Some Heavy Metals in Exposed Human Groups"

[2001] Euro-Mediterranean Master Class in "Technologies, services and strategies for the development of a more sustainable and competitive food packaging sector"; Napoli, Italy.

[1990 – 1996] Degree in Applied Biology, with double minor in Applied Microbiology and Genetics & Molecular Biology, Jordan University of Science and Technology, Irbid, Jordan.

[1989] Secondary School, Scientific Sector, Yemen.

Scholarships:

[2005 - Now] Ph.D. scholarship from DAAD; Germany
[1999 - 2003] Master scholarship from Ministry of Higher Education, Yemen.
[1990 – 1996] Undergraduate scholarship from Ministry of Higher Education, Yemen.

Research, Experience and Training:

[2005 - Now] Ph.D. project Vegetation Ecology and Ethnobotany of *Cyphostemma digitatum* in the Western Highlands in Yemen.

[2003 - Now] Teaching and Research Assistant, Department of Biology, Ibb University, Yemen. Still have the position while I have scientific vacation to do my Ph.D.

[2002 - 2003] Ecological consultant; Via-nova organization; Local communities' consultant, Amman, Jordan.

[2002 - 2003] Water analysis at the Jordanian Water Authority, Central Laboratory in Amman, September 2002 to January 2003.

[2001 - 2002] Teaching Assistant; Biochemistry Practicals, Department of Biology, Al al-Bayt University.

[1997 - 2000] Research assistant; Part time Researcher in the Jordanian Royal Society for Conservation of Nature.

[Aug., 1994]Plant Tissue Culture; King Hossain`s Agriculture Research Station; Jordan from 16 July to 18 August 1994.

[1989 - 1990] Teacher (Sciences); Organised, planned and coordinated class work and activities on a day to day basis for a multilevel class in primary education; Yemen.

Professional Affiliations:

[2006 -] International Carotenoid Society
[2006 -] American Association of Yemeni Scientists and Professionals
[2004 -] German Academic Exchange Service
[2002 -] The Yemeni Society for Biological Sciences
[1998 -] Royal Society for Conservation of Nature; Jordan