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Review

# *Phyllanthus piscatorum*, ethnopharmacological studies on a women's medicinal plant of the Yanomamï Amerindians

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In memory of Helena VALERO (napëyoma) [1925–2002]

**Abstract**

The shrub *Phyllanthus piscatorum* Kunth (Euphorbiaceae) is cultivated by various ethnic groups of the Amazon because of its piscicidal properties. During ethnobotanical fieldwork among the Yanomamï Amerindians in Venezuela we observed that *Phyllanthus piscatorum* was exclusively cultivated and used by the women. Aerial parts of this herbaceous shrub are employed as fish poison and medicine to treat wounds and fungal infections. In addition, the leaves are used as tobacco substitute. Ethnobotanical data regarding the context of the use of this plant are presented. To validate ethnobotanical information related to its medicinal indications, antimicrobial, and antiprotozoal properties of water, methanol (MeOH) and dichloromethane (DCM) extracts were studied. No activity against Gram-positive bacterial strains but significant activity against the fungi *Aspergillus fumigatus*, *Aspergillus flavus* and the yeast *Candida albicans* were found. All extracts showed weak in vitro activity against *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense*. The extracts were further investigated for cytotoxic effects in an in vitro test system with leukemia Jurkat T, HeLa, and human peripheral mononuclear blood cells (PBMCs). During the first 48 h the extracts did not exhibit any cytotoxicity. After 72 h the DCM extract potently inhibited viability of HeLa cells. Although in several communities along the upper Orinoco the cultivation and use of *Phyllanthus piscatorum* is being lost because of the ongoing acculturation, the traditional medicinal use of *Phyllanthus piscatorum* might provide an effective and cheap remedy against dermatological diseases linked with *Candida albicans* infections.
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*Keywords:* Yanomamï; *Phyllanthus piscatorum*; Amazon; Medicinal plant; Antiyeast; Fish poison; Venezuela

## 1. Introduction

### *1.1. The ethnopharmacology of the genus Phyllanthus*

The genus *Phyllanthus* has been described to provide several piscicidal species, which are employed throughout the tropics, but predominantly in the Amazon basin (Acevedo-Rodríguez, 1990; Calixto et al., 1998; Heizer, 1953). In the literature, a number of species have been described as fish poisons that are used among different indigenous ethnic groups. *Phyllanthus acuminatus* M. Vahl has been reported as potent fish poison among the Tacana (Bourdy, 1999) and Mosetene Amerindians (Muñoz et al., 2000) of Bolivia. According to Muñoz et al. (2000), the Tacana also employ *Phyllanthus acuminatus* leaves in the treatment of dermatological infections. De Lucca and Zailles (1992) state that the species *Phyllanthus ichtyomethis* Rusby is employed as fish poison and pesticide in Bolivia. The species *Phyllanthus anisolobus* Müll. Arg. was reported as fish poison from Ecuador (Bachmann et al., 1993). In Guyana several ethnic groups cultivate *Phyllanthus brasiliensis* (Aubl.) Poir. for the same purpose (Van Andel, 2000). *Phyllanthus subglomeratus* Poir., which is closely related to *Phyllanthus brasiliensis* and *Phyllanthus piscatorum*, is known to be cultivated as fish poison and pesticide among the Creoles of Guyana since 1775, when this species was first reported by the French botanist Fusée Aublet (Grenand et al., 1987). The pantropical species *Phyllanthus niruri* is not only a well-known medicinal herb but also exhibits piscicidal properties (Quisumbing, 1947). The species *Phyllanthus urinaria* has been reported as a fish poison from India (Jhingran, 1975).

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The German botanist Carl Sigismund Kunth in Humboldt's and Bonplant's Nova Genera et Species Plantarum (1817) for the first time reported the use of *Phyllanthus piscatorum* as Amazonian fish poison, a circumstance that gave this plant its species name. Schultes and Raffauf (1990) state that *Phyllanthus piscatorum* is widely cultivated in the western Amazon as fish poison, and that it is also used as insect repellent among the Huitoto Amerindians. The first record of this fish poison (*barbasco*) in Venezuela is due to Henri Pittier (1926). The use of *Phyllanthus piscatorum* in poisoning fish has also been reported by others (Ramirez, 1943; Vellard, 1941; Fagundes, 1935). It is probable that *Phyllanthus piscatorum* belongs to an ancient group of culturally important plants, which have been cultivated for centuries by indigenous societies of the northern Amazon. This assumption is further confirmed by the fact that this shrub does not seem to occur in a wild form (Schultes and Raffauf, 1990). In the upper Orinoco, *Phyllanthus piscatorum* is cultivated and used by the Yanomamï women. The first accounts of the use of this plant among the Yanomamï go back to Knobloch (1967) and Lizot (1972). The latter for the first time collected a sample for botanical identification. Although several ethnographic studies among the Yanomamï report the use of *Phyllanthus piscatorum* as a fish poison, only Cocco (1972) mentions its use as medicinal plant. To date, a more detailed account of the overall importance of *Phyllanthus piscatorum* among the Yanomamï communities in Venezuela is lacking, and there are no pharmacological studies on this plant.

### 1.2. The Yanomamï Amerindians in Venezuela

The Yanomamï live in communities of 20–200 individuals in provisional shelters (*yahi*) or circular houses (*shapono or yano*) in southernmost Venezuela and northern Brazil (see Fig. 1). Each communal roundhouse has an open space in the middle, which offers an area where community affairs are discussed and feasts are held. The actual living area is thatched with palm leaves and is situated on the outside of the roundhouse with just one fence-like wall at the back. The *shapono* is divided up freely into sections, each representing a social nucleus, usually a family or a clan. Location and size of a Yanomamï community strongly depends on economic alliances with other communities. Probably more than half

Fig. 1. Area of fieldwork. The villages where the ethnobotanical study took place: (1) Aratha; (2) Ocamo; (3) Platanal (Mahekoto); (4) Hasupïwei; (5) Irokai; (6) Mokarita; (7) Hokotopïwei; (8) Shotemi; (9) Ashitowë. Hatched area shows where most of the data was recorded.

of the approximately 110 communities in Venezuela are still semi-nomadic and move their village every 3–7 years. This migration activity is an important custom in the sustainable use of biological resources (Gertsch et al., 2002). Generally between February and April or August and October the Yanomamï communities go on long treks (*wayumi*) and it is common to find that whole villages are abandoned for 2 or 3 months.

The Yanomamï are not only hunters and gatherers, but also shifting cultivators (Chagnon, 1968; Lizot, 1978). About 60% of their food originates from cultivated plants, mainly plantain varieties. Each settlement has one or more garden areas (*hikari*) outside the *shapono*. The garden is split up into sections, each area being kept by a different family or clan. Shifting cultivation is a key factor in subsistence activities, which are segmented into gender specific roles. In a typical garden, different magical and medicinal plants are cultivated, including *Phyllanthus piscatorum* (*yaraka kë henaki*). Among the most important crops we find *Musa paradisiaca* L. (*kurata* and *tate*), *Bactris gasipaes* Kunth (*rasha kë si*), *Nicotiana tabacum* L. (*pêê nahe*) and *Xanthosoma* spp. (*ohina*), *Manihot esculenta* Crantz (*nashi*), and *Zea mays* L. (*yono*). In addition to wild palm fruits, the Yanomamï collect other edible wild plant products, such as the fruits of *Clathrotropis* spp. (Fabaceae) (*wapu kohi*), *Bertholetia excelsa* Humb. & Bonpl. (Lecythidaceae) (*hawari kohi*), and *Micrandra* spp. (Euphorbiaceae) (*momo kehi*). During the treks the whole community may explore remote areas, which can be over 80 km away from their village. While the men hunt game and collect fruits, honey and small animals, the women collect a variety of wild plant and fungal products and also catch smaller animals, such as river crabs (*oko*), and fish (*yuri*). The traditional world of the central and southern Yanomamï is divided up into village (*yahi*), garden (*hikari*) and forests (*urihi*).

## 2. Materials and methods

### 2.1. Ethnobotany

The bulk of the information about *Phyllanthus piscatorum* was collected during three periods of fieldwork by the first author in July–October 1998, January–February 1999, and January–February 2002. During that time eight Yanomamï villages were visited: Ocamo, Aratha, Hokotopïwei, Shotemi, Mahekoto, Hasupïwei, Irokai, Ashitowë and Mokaritha in the upper Orinoco, Estado Amazonas of Venezuela (see Fig. 1). Field observations were also incorporated from other shorter visits by the first author to several other villages along the Río Mavaca and Río Siapa (Matacuni) in 1992 and Río Ocamo and Río Orinoco in 1998 and 1999. In Hasupïwei, Irokai and Mokaritha informal and open structured interviews were conducted in the communities. Plant material (twigs, leaves, inflorescence) was taken to the villages for interviewing. Ethnobotanical data is based on 34 use reports obtained in interviews with the women of the community.

### 2.2. Plant material and extraction

Plant material has been obtained in the garden of the village Hasupïwei in February 1999. Voucher specimens of *Phyllanthus piscatorum* (JG 287; JG 135) were deposited at VEN, STEY, and ETH Zurich. Voucher specimen of plant material for phytochemical and pharmacological investigations (JG 135), was identified at the National Herbarium in Caracas, Venezuela. The material extracted was collected and sun-dried in situ (semi-shade). The herbaceous plant material (twigs, leaves, inflorescence) was cut into small pieces and pulverized. Ten grams of plant powder was extracted exhaustively with either dichloromethane, methanol or deionized water (cartridge system, Kan, Switzerland). The solvents were removed in vacuo and the extracts were freeze-dried.

### 2.3. Antimicrobial tests

Bacteria (*Bacillus cereus*; ATCC 1070, *Escherichia coli*, ATCC 25922; *Staphylococcus aureus*, ATCC 25933; *Staphylococcus epidermidis*, ATCC 12228; *Pseudomonas aeruginosa*, ATCC 25922 and the yeast and fungi *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Candida albicans*, *Penicillium oxalicum* (clinical isolates obtained from the University of Zurich Hospital, Switzerland) were cultivated on nutrient agar plates (Sabouaud and malt extract agar, Oxoid, UK) and incubated in broth over night, diluted and spread over fresh nutrient agar plates. Few colonies from these cultures were inoculated in culture broth, which was then used for the assays (Rios et al., 1988). The MIC antiyeast and antifungal assay was performed according to the dilution method described previously (Frost, 1994) The DCM extract was dissolved in DMSO due to better solubility. Antimicrobial susceptibility testing was performed with the disk diffusion method (Lennette, 1985). Wells of 6 mm in diameter were made in Mueller-Hinton agar under aseptic conditions. Two hundred micrograms of plant extracts in solvent was added to the disks (Oxoid, UK) in triplicates for each concentration and dried. After 16 h incubation at 37 °C the plates were sprayed with methylthiazolyl-tetrazolium chloride (MTT) (Fluka, Switzerland). The diameters of the inhibition zones were measured for each plate. Miconazole-$NO_3$ (1 μg) (Signal Chemical Co., St. Louis, USA), chloramphenicol (Siegfried, Switzerland) (10 μg/ml)) and ampicillin (Fluka, Switzerland) (10 μg/ml) were used as positive reference. Solvent containing disks were used as negative controls.

### 2.4. Antiprotozoal assays

The in vitro antiprotozoal assays (*Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Plasmodium falciparum*)

were performed at the Swiss Tropical Institute, Basel, and carried out as described previously (Heilmann et al., 2000). The DCM extract was dissolved in DMSO. Antiplasmodial activity was determined using the *Plasmodium falciparum* strain NF54. Infected human red blood cells were exposed to serial drug dilutions in microtiter plates for 48 h. Viability was assessed by [$^3$H]hypoxanthine by liquid scintillation counting. Chloroquine (Fluka) was used as positive reference. Activity against *Trypanosoma cruzi* (Tulahuen strain C2C4 containing the galactosidase (Lac Z) gene) was assessed in rat skeletal myoblasts (L-6 cells) in microtiter plates after 3 days incubation at 37 °C. For determination of $IC_{50}$ values, the substrate CPRG/Nonidet was added to the wells and the color measured at 540 nm. The ratio trypomastigotes/cells was 1:1. Activity against *Trypanosoma brucei rhodesiense* (STIB 900) was determined using Minimal Essential Medium supplemented with 2-mercaptoethanol and 15% heat-inactivated horse serum for cell culture and Alamar Blue for detection. Alamar blue (10 μl) was added to each of the 96 wells an incubated for 2–4 h. The plate was read with a Millipore Cytofluor 2300 using an excitation wavelength of 530 nm and emission wavelength of 590 nm. Fluorescence development was expressed as percentage of the control and $IC_{50}$ values were determined by curve fitting. Melarsoprol (Arsobal) and Benznidazol (Roche) were used as positive controls.

### 2.5. Cell viability assay

The cytotoxicity of the extracts determined with HeLa cells (ATCC CCL 17) was carried out as described by Swanson and Pezzutto (1990). For the testing of cytotoxicity with soluble cells, $CD4^+$ Jurkat human leukemia and peripheral blood mononuclear cells were cultured in supplemented RPMI 640 medium. A cell suspension of $1.5 \times 10^5$ cells/well was incubated with six different concentrations of DCM, MeOH and water extracts in a 96-well plate for 1.5 and 19 h in a humidified atmosphere (37 °C, 5% $CO_2$). The DCM extract was previously dissolved in DMSO. The final volume was 150 μl/well. Ten microliters of WST-1 (4-[3-(4-iodophenyl-2-(4-nitrophenyl)-2*H*-5-tetrazolio)]-1,3-benzene disulfonate, Roche) was added and incubated for 1 h under the same conditions. The absorbance of the samples were measured at 405 nm (reference wavelength 650 nm) against a background control (culture medium with 10 μl WST-1), using a microplate reader (MRX, Dynex Technologies). For determination of the $IC_{50}$ the cell viability was determined as a percentage of the control response ($1.5 \times 10^5$ cells without test compounds plus 10 μl WST-1). Every test was performed at least in duplicates and all experiments have been repeated three times. Positive control experiments were performed with helenalin. Maximum observed standard deviation was 10% (absolute).

## 3. Results and discussion

### 3.1. Ethnobotany

In the Yanomamï communities visited (see Fig. 1) the cultivated shrub *Phyllanthus piscatorum* was commonly called *yaraka kë henakï* (*yaraka* fish leaves). This name has previously been reported by other investigators (Knobloch, 1967; Cocco, 1972, and Lizot, 1972). *Henakï* is the plural of *hena*, which is the Yanomamï term for leaf. *Yaraka* fish stand for a group of small, edible fish (*Tetragonopterus* spp.) (Knobloch, 1967, Finkers, 1983) that are among the first ones affected by the poison. We conclude that this might be the reason why the Yanomamï name *yaraka*-fish leaves is used.

The use of *Phyllanthus piscatorum* as fish poison was found to be similar in all communities: the leaves and twigs are harvested by the women and taken to a stream or creek. If the stream does not already have pond-like areas it might be channeled and blocked up. The twigs and leaves are put into special baskets with holes (*yorehi* and *warama*) and crushed. Otherwise, the twigs are smashed first in a hole made in the ground. The basket containing the pieces and powder of the yaraka (*Phyllanthus piscatorum*) shrub is then submerged. With a wooden stalk the plant material is squeezed and compressed until a whitish broth (*moshi*) is released to the surrounding water. The pool finally turns slightly turbid. After 30–40 min, the women and girls return to the pond to collect the little fish that are floating on the surface of the water. The effect of *Phyllanthus piscatorum* is believed to be transitory and not lethal to the fauna (Anduze, 1982), as it is the case with the group of wild lianas called *ayari thotho* (*Lonchocarpus* spp. and *Derris* spp.), which are rotenoid-containing fish poisons only employed by the men. Although the men may help in the collection and preparation of the *yaraka kë henakï* poison, the actual fishing process is exclusively carried out by the women. The men and boys may sit on the riverbank and watch. While the women cultivate *Phyllanthus piscatorum* the men mainly use wild plants for fish poisons, such as the bark of *Annona* spp., *Lonchocarpus* spp., *Derris* spp., and the fruits of *Caryocar* spp.

Interestingly, fishing with *Phyllanthus piscatorum* is associated with certain taboos, which resemble the ones involved in the preparation of curare (*mamokori*) as described previously by Lizot (1972). According to the women of Hasupïwei, it is prohibited to urinate into the water because this would render the poison inactive. Likewise, the men preparing curare are not allowed to urinate during the process. Furthermore, sexual relations of the women the day before the fishing with *Phyllanthus piscatorum* are prohibited just as it is the case with the men preparing curare.

In the communities Hasupïwei and Mokaritha, the women believe that the water poisoned with *yaraka* leaves is an efficient medicine to treat itching skin infections, especially of the vagina. When the women enter the water to gather the intoxicated fish their skin gets into contact with the extract of

*Phyllanthus piscatorum*. Also, according to our female informants in Hasupïwei, it was said that, in this area, women often suffer from skin infections, such as *si rurupï* and *pare*. It was also reported that *si rurupï* occurs mainly in elderly people and persons suffering from malaria. Though it was not possible to determine exactly what were the pathogens involved in *si rurupï*, we suspect *si rurupï* to be a candidiasis infection of the skin, or a fungal one. *Pare* is generally associated with an abscess caused by insect bites, and might possibly be ringworm (*Trichophytum* spp.).

Mainly dermatological conditions of the vagina (mycotic vulvovaginitis), most likely to be related to *Candida albicans* infections, are healed with the *yaraka* (*Phyllanthus piscatorum*) leaves. In order to treat their dermatological diseases the women wait in the turbid water for up to half an hour until the first fish appear floating on the surface. According to the women in Hasupïwei, the fishing procedure is repeated in another pond if the infection and itching does not get better after a few days. Also head injuries from fights between communities or conflicts within the group are occasionally treated with *yaraka* leaves. For that purpose, the collected leaves are soaked in water and wrapped up into a big pisha leaf (*Calathea altissima*) (Maranthaceae) to form a package with an open end. This package is then heated up over a coal fire and squeezed until the sap drips out. According to Cocco (1972), *yaraka* (*Phyllanthus piscatorum*) leaves are also used to treat injuries derived from poisoned arrow points. Although we could not observe this use, it was nonetheless confirmed by two older informants in Ocamo and by Helena Valero,[1] who related that she had been injured by a poisonous arrow, and was treated with *yaraka* leaves by the women of a village called *Hii-theri* (Valero, 1998). Apparently, the sap prevents swelling (*si yahetou*) and infection of the wounds. In addition, open wounds treated with *Phyllanthus piscatorum* are not infested with insect maggots from myiatic flies. In Hasupïwei the twigs of this shrub were occasionally burned in the evenings to prevent bites from vampire bats (*Desmodus rotundus*) during the night.

In Aratha, Ocamo, Hasupïwei, Irokai, and Mokaritha the leaves of *Phyllanthus piscatorum* are also used as tobacco (*Nicotiana tabacum*) (*pêê nahe*) substitute. The Yanomamï usually carry a wad of tobacco leaves in their lower lip. They do not smoke, snuff or drink (syrup) tobacco but merely employ wads. To smoke tobacco is generally regarded as extravagant. Consumption of tobacco is not restricted to anybody and even little children often use a tobacco wad (Zerries and Schuster, 1974). The fresh or dried leaves are moistened and mixed with ashes (*yupu ushipë*) from *Couratari* spp. (Lecythidaceae) and *Gustavia augusta* L. (Lecythidaceae) (which are probably rich in mineral salts) and then shaped into a wad that fits the lower lip.

[1] Helena Valero was kidnapped by a Yanomamï group in 1932 and lived with them for more than 30 years.

Tobacco consumption is of chief importance in Yanomamï culture and tobacco is an frequently used currency for trading. For a more detailed analysis of tobacco consumption among the Yanomamï in Venezuela also see Zerries (1964). Whenever real tobacco leaves get scarce or are lacking the Yanomamï look for substitutes. According to our observations, children and young women more often use tobacco substitutes, mainly because the possession and trade of tobacco leaves is prestigious and correlates with the overall status in the community. According to our informants in Hasupïwei, Irokai, and Mokaritha, selection criteria for tobacco substitutes seem not associated with resemblance in morphology, but any plant material, which anesthetizes the mouth or gives a hot or itching feeling. This raises the question of toxicity of certain substitutes. The majority of the substitutes recorded by us belong to the families Solanaceae, Gesneriaceae, and Rubiaceae, such as *Solanum* spp., *Besleria* spp., and *Psychotria* spp. The Yanomamï women and children occasionally employ the leaves and twigs of *Phyllanthus piscatorum* to make a wad when tobacco leaves are lacking. The preparation of the wad made with *Phyllanthus piscatorum* is analogous to the preparation of the regular tobacco. Upon chewing, the leaves of *Phyllanthus piscatorum* produce a slightly cool and anesthetizing feeling in the mouth. Interestingly, Milliken and Albert (1999) state that the Yanomamï of the Demini region in Brazil also use a plant called *yaraka henakï* as tobacco substitute. Because this plant was apparently not present in the area, they could not provide a botanical identification.

### 3.2. Antimicrobial effects

Because the ethnobotanical information pointed to a possible antiyeast or antifungal property of *Phyllanthus piscatorum*, we selected the yeast *Candida albicans*, as well as three pathogenic fungi, *Aspergillus fumigatus*, *Aspergillus flavus* and *Cryptococcus neoformans* as test strains. To assess a possible nonspecific antimicrobial activity of *Phyllanthus piscatorum* extracts, we have also included common Gram-negative and Gram-positive bacterial strains, as well as *Pseudomonas aeruginosa*. The antimicrobial activity of the extracts was quantitatively assessed by the presence or absence of inhibition zones and by measuring the diameter area around inoculation disks. The results of general antimicrobial activity (Table 1) show that the DCM, MeOH and water extracts of *Phyllanthus piscatorum* exhibit no inhibitory effects against *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* but significant activity against *Candida albicans*, *Aspergillus flavus* and *Aspergillus fumigatus*. MIC values for *Candida albicans*, *Candida flavus* and *Aspergillus fumigatus* obtained with extracts of *Phyllanthus piscatorum* show that the plant contains potent antiyeast and antifungal principles, respectively (Table 1). The DCM extract most strongly inhibited the growth of *Candida albicans* on TLC agar overlay and in agar culture broth, showing a MIC value of 20 μg/ml

Table 1
The antimicrobial activity of *Phyllanthus piscatorum* DCM, MeOH, and water extracts was measured by the diameter of the inhibition zone (+: ≥1 mm; ++: ≥5 mm; +++: ≥10 mm; ++++: ≥15 mm) and compared to positive controls

| Strains | Am | Ch | Mic | DCM (0.2 mg) | MeOH (0.2 mg) | Water (0.2 mg) |
|---|---|---|---|---|---|---|
| *Bacillus cereus* | ++++ | ++++ | – | + | ± | ± |
| *Escherichia coli* | – | – | – | – | – | – |
| *Staphylococcus aureus* | +++ | +++ | – | + | – | – |
| *Staphylococcus epidermidis* | +++ | +++ | – | – | + | ± |
| *Pseudomonas aeruginosa* | – | – | – | – | – | – |
| *Candida albicans* | – | – | ++++ | ++(+) | ++ | ++ |
| *Candida neoformans* | – | – | – | – | – | – |
| *Aspergillus fumigatus* | – | – | +++ | +(+) | + | + |
| *Aspergillus flavus* | | | +++ | +(+) | + | + |
| *Bacilus capitatus* | | | ++ | – | – | – |

Am: Ampicillin (10 μg/ml); Ch: Chloramphenicol (10 μg/ml); Mic: Miconazole-$NO_3$ (1 μg/ml).

Table 2
In vitro antifungal/antiyeast activity of *Phyllanthus piscatorum* extracts shown as minimum inhibitory concentration (MIC) in μg/ml (mean value of three experiments)

| DCM | MeOH | Water | Strain | Reference compounds[a,b] |
|---|---|---|---|---|
| ≥35 | ≥50 | ≥75 | *Aspergillus fumigatus* | ≥0.5[a]/≥0.4[b] |
| ≥15 | ≥20 | ≥40 | *Candida albicans* | ≥0.2[a] |
| ≥40 | ≥65 | ≥85 | *Aspergillus flavus* | ≥0.2[a] |
| ≥200 | ≥200 | ≥200 | *Bacillus capitatus* | ≥1[a] |
| ≥200 | ≥200 | ≥200 | *Candida neoformans* | – |

[a] Miconazole-$NO_3$.
[b] Amphotericin B.

for the DCM extract, 30 μg/ml for the MeOH extract, and 50 μg/ml for the water extract (Table 2).

### 3.3. Antiprotozoal effects

The DCM, MeOH and water extracts were subjected to antitrypanosomal and antiplasmodial testing in vitro. The DCM extract again showed moderate antitrypanosomal and antiplasmodial activity (Table 3) Especially the strain *Trypanosoma rhodesiense* (cause of African trypanosomiasis) was inhibited by *Phyllanthus piscatorum* extracts ($IC_{50}$ values in the lower μM range).

Table 3
Antiprotozoal activity of *Phyllanthus piscatorum* extracts, $IC_{50}$ values (μg/ml) ± S.D. ($n = 3$)

| DCM | MeOH | Water | Strain | Reference compounds[a,b,c] |
|---|---|---|---|---|
| 1.6 ± 0.2 | 3.4 ± 0.3 | 7.0 ± 0.2 | *Trypanosoma rhodesiense* | 0.003[a] |
| 9.1 ± 0.5 | 9.4 ± 1.1 | >20 | *Trypanosoma cruzi* | 0.27[b] |
| 3.2 ± 0.3 | 8.9 ± 0.2 | 9.9 ± 0.8 | *Plasmodium falciparum* K1 | 0.0007[c] |

[a] Melarsoprol.
[b] Benznidazol.
[c] Chloroquine.

Table 4
Cytotoxicity of *Phyllanthus piscatorum* extracts on neoplastic cell lines and peripheral blood mononuclear cells (PBMCs), $IC_{50}$ (μg/ml) after 72 h (mean of three experiments)

| Cell type | DCM | MeOH | Water | Reference componds[a] |
|---|---|---|---|---|
| Jurkat T cells | 30.5 | >50 | >50 | 1.0 |
| KB (Hela) | 6 | 18 | 26.5 | 0.5 |
| PBMCs | 38 | >50 | >50 | 1.5 |

[a] Helenalin.

### 3.4. Cytotoxic effects

Incubation of different concentrations (0.01–0.2 mg/ml) of *Phyllanthus piscatorum* DCM, MeOH and water extracts were incubated with leukemia Jurkat T, HeLa, and PBMCs. We found that within the first 48 h there was no significant cytotoxicity up to 0.2 mg/ml. However, after 72 h the DCM extract potently inhibited cell viability in all tested cell populations (Table 4). After 72 h the DCM extract inhibited 50% of HeLa cell growth at a concentration of 5 μg/ml. The MeOH and water extracts also exhibited weak cytotoxicity in HeLa cells ($IC_{50} = 10$ μg/ml). The cell viability of freshly isolated PBMCs was also weakly inhibited by the DCM extract ($IC_{50}$=30 μg/ml).

## 4. Conclusions

*Phyllanthus piscatorum* showed significant to potent antiyeast and antifungal activities, which point to a possible role in the treatment of vaginal candidiasis. In tropical habitats different forms of candidiasis are known to be a problem to women (Azzam-W et al., 2002). In addition, Yanomamï women repeatedly suffer from malaria infections and the resulting anemia is likely to make them more susceptible to vaginal candidiasis (Sofaer et al., 1982). Future studies on *Phyllanthus piscatorum* should also focus on *Trichomonas vaginalis*, which has been implicated in mycotic vulvovaginitis (Azzam-W et al., 2002).

The weak antibacterial activity might be due to polyphenols (tannins), which were detected in the water and MeOH

extracts with thin layer chromatography (TLC) spray reagents. This was partly also confirmed with the TLC agar overlay method, where the polar tannin bands were slightly bacteriostatic. The lack of significant antibacterial effects implies that *Phyllanthus piscatorum* is inactive against non-mycotic forms of vulvovagnitis.

The DCM, MeOH, and water extracts show no in vitro cytotoxicity with Jurkat and HeLa cell lines and PBMCs for up to 48 h incubation ($IC_{50}$ values $< 200\,\mu g/ml$). This 48 h delay in cytotoxicity is not commonly found with active extracts. Only after 72 h incubation a rapid increase in cell toxicity is observed. The use of the leaves and sometimes also twigs as tobacco substitute would imply that a certain amount of extract is swallowed and so taken up orally. This would make *Phyllanthus piscatorum* more effective against other pathologies. The antiprotozoal activity found for the extracts might be a another action of this medicinal plant. The extracts were found to exhibit weak activity against the trypomastigote form of *Trypanosoma rhodesiense* and little activity against *Trypanosoma cruzi* (Table 3). These effects should not be ignored since *Trypanosoma cruzi* is the causative agent of Chagas disease, which is also a problem in the Yanomamï territory.

Testing against *Plasmodium falciparum* also showed moderate activity (Table 3) and might be worth to be investigated further because in vivo activities were also reported from *Phyllanthus acuminatus* and *Phyllanthus niruri* extracts (Muñoz et al., 2000; Tona et al., 2001). Taken together, this data strongly support the view that the species *Phyllanthus piscatorum* exhibits a pharmacological potential that is still partially unexplored. To our knowledge, this is the first report on antifungal, antiprotozoal, and cytotoxic effects of *Phyllanthus piscatorum*.

We assume that *Phyllanthus piscatorum* is commonly known among all Yanomamï groups of southern Venezuela and north to the Sierra Tapirapecó under the cognate *yaraka kë henakï*. Differences in cultivation might be due to either availability, soil preferences of the plant or cultural need by the community. Other fishing techniques, such as the use of hooks and harpoons have partly replaced *Phyllanthus piscatorum* as a fish poison along the Orinoco. Furthermore, it is important to stress that *Phyllanthus piscatorum* is mainly cultivated as a fish poison and not primarily because of its medicinal properties. It has been stated previously that the medical system of the Yanomamï does not primarily rely on medicinal plants because general illness is associated to the spiritual world (Zerries and Schuster, 1974). Healing is therefore most often related to spirits (*hekurapë*), which are called upon by the shaman (*shapori* or *hekura*) during the healing rituals (*hekuramou*). Medicinal plants play a minor role in all communities visited and are employed very occasionally, especially when they are not associated to a spiritual function. However, the use of *Phyllanthus piscatorum* to treat fungal or yeast infections and wound healing shows that spiritual curing can be paralleled by experimental phytotherapy.

We are currently planning phytochemical and further pharmacological investigations on this species, including toxicological animal studies to assess the safety of its use. While *Phyllanthus piscatorum* is still used as a fish poison in the Yanomamï area, its use as medicine is being replaced by western medication provided by doctors and medical staff. In the more remote villages, however, the *yaraka* leaves are still cultivated and used. If it could be shown that *Phyllanthus piscatorum* is nontoxic to human beings it would be important to promote the use of this shrub and thus prevent the loss of this cultural heritage.

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# Pharmacopoeia in a shamanistic society: the Izoceño-Guaraní (Bolivian Chaco)

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**Abstract**

We present the results of an ethnopharmacological research within a Bolivian lowland ethnic group from the dry Chaco, the izoceño-guaraní. Izoceño-guaraní people belong to the extended Chiriguano group. They are actually organised in independent communities, settled down in south-east Bolivia. Struggling very soon for their rights, landowners of their territory, izoceño-guaraní appear to be well organised and maintain a still vivid culture. Medicine is in the hands of *Paye* who are recognised as specialists in their own group. Ethnopharmacological research leads us to collect approximately over 306 species, 189 of them having medicinal uses. We present here an overview of the izoceño-guaraní ethnomedicine and pharmacopoeia, based on vegetal and animal products.
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*Keywords:* Medicinal plants; Ethnopharmacology; Guaraní; Chaco; Bolivia; Traditional medicine; Shamanism

## 1. Introduction

Izoceño-Guaraní belongs to the Guaraní ethnic group. Guaraní people is encountered in North Argentina, Paraguay, and Bolivia and Brazil. In Bolivia, Guaraní are represented by the Guarayos, Sirionó, Yuki, Guarasug'we (now extinct), Tapieté and Chiriguano.

Chiriguano includes Ava, Simbas and Izoceños (our studied group). In Bolivia, Chiriguano are estimated around 50,000, 9000 being Izoceños. Actually, there is also a setting of some 6000 Chiriguano people around Santa Cruz, looking for a better future in the city suburb (Combes and Ros, 2001).

Chiriguano's history and ethnohistory has been well documented, (Combes and Saignes, 1991; Saignes et al., 1986; Langer, 1988; Metraux, 1928; Nordenskiold, 1916) and all authors comment the strong bellicose attitude of the Tupi-guarani migrants (from which the Chiriguano originated) heading West on their way for the mythic country of "Kandire", fighting, reducing in slavery and performing cannibalism over native tribes and especially over Chané groups speaker of an arawak language.

Later, Chiriguanos were considered by the Spanish conquerors as one of the most aggressive group of the colony, and an endless list of wars and encroachments occured extending to the republican period. Actually, a cultural distinction is recognised between the Izoceño and the Ava/Simba, among others, through language and some facts of material culture—actual Izoceño people gained his personality through an ancient long lasting and unique mixing up with Chané natives.

Contemporary izoceños are now settled down in some 20 independent communities in Izozog, between 19°–20° south, and 62°–63° west, spread over some 70 km, dwelling on both sides of the Parapetí river, which ends in a giant swamp called "bañados del izozog" (Fig. 1).

The Izoceño own their land territory, situated close by the Kaa-Iya (landlord of the forest) national Park, one of the largest in Bolivia. All this region belongs to the eco-region of the Chaco, and more precisely dry Chaco, characterised by 550–800 mm mean annual precipitation, an average temperature of 26 °C, and extreme temperature ranges (Navarro, 1999). Seasons are well marked—intense drought occurs during May–September (winter time) leading to the complete vanishing of the Parapetí river; *ad contrario*, during summer, the overflow of the river often causes severe inundation.

Izoceño society is organised around leaders (*Mburuvicha*), the top leader being the *Mburuvicha guasu* (high captain) which formerly shared his power with the *Paye* (shamans). It has to be mentioned that Izoceño-guaraní have always

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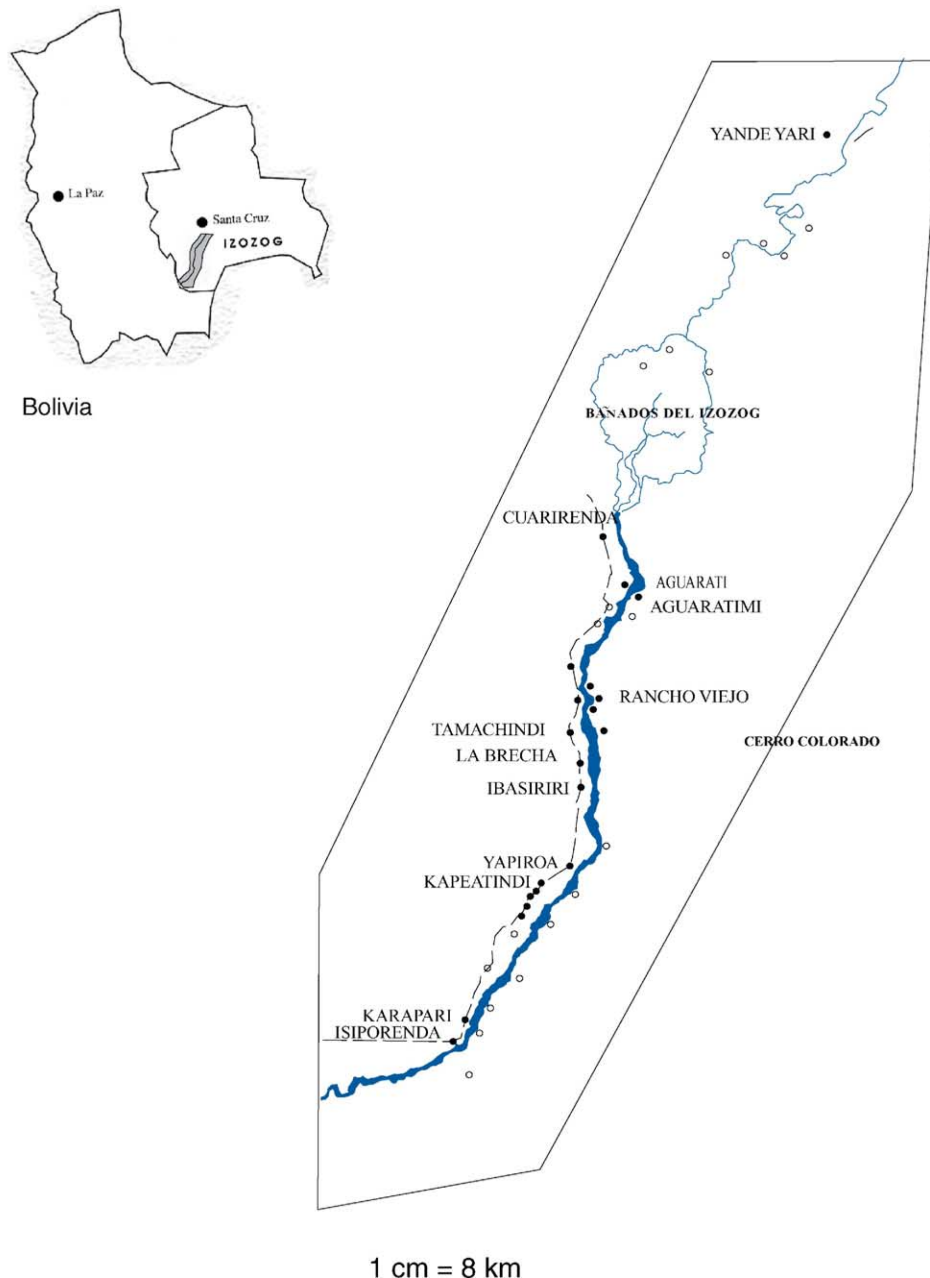


Fig. 1. Place of study.

strongly fought the setting of churches (Jesuits and Franciscans). It is only in 1923, that the first Canadian evangelists arrived. They did not stay long, and departed during the Chaco war. Nevertheless, actually, the evangelist church is more and more present (Hirsh and Zarzycki, 1995).

Chiriguanos always defined themselves as agricultures. Many myths refer to this condition, explaining that maize, considered as food by excellence, was offered to them by the "god" *Aguara Tumpa* himself, also teaching them its different ways of preparation, "Kägui" (a fermented drink) being the valued one (Giannecchini, 1996). "And", the same author concludes in 1898 "for them, hunting and fishing are second place activities". For some anthropologists, Chiriguano civilization is "maize civilisation", hence their name "maize eater" (Susnik, 1968). Apart from maize, traditional izoceño main staple vegetal food is beans (*Vigna unguiculata*), manioc, sweet potato, pumkin, together with *Prospis chilensis*, *Ziziphus mistol*, *Geoffrea decorticans*, *Bromelia serra*, etc. collected fruits or plant parts.

Albeit the ethnobotany of Guaraní ethnics groups from Paraguay or north Argentina have been well documented, (Arenas, 1981; Schmeda-Hirshmann, 1993, 1994) far less work have been conduced among Bolivian groups. A didactic guide for the medicinal use of chaqueñan medicinal plants have been published (De Lucca Droxkler and Zalles Asin, 1996). The ethnobotanical/ethnopharmacologi-

cal knowledge of the Isoceños have been approached. Gallo Toro (1996) briefly mentioned the uses of some 48 medicinal species, Saldias et al. (1993) and Montaño-Ortuño (1997) realised a preliminary inventory of most useful species in the same region, and some data can be picked up in booklets (Arauz and Chiraye, 2000). Some papers related with isoceño-guaraní ethnomedicine have been published (Hirsh and Zarzycki, 1995; Zolezzi and López, 1995; Riester, 1995). Preliminary to this paper, we have written two books for the Isoceño-Guaraní communities (Bourdy and Combes, 2001; Bourdy, 2002), dealing with isoceño plant's uses. In this paper, we present the ethnopharmacology of the Isoceño-Guaraní.

## 2. Methodology

We performed field work with the Isoceños between 1998 and 2001. Prior agreement with the CABI (Capitania del alto y bajo Izozog) and the *Paye*[1] (shamans), leads us to work with three *Paye* from the community of Ibasiriri, Aguaratimi and Cuarirenda. Other persons willing to share their knowledge were also invited to do so.

In the presence of the *Paye* (and sometimes their assistants), or the concerned informant, we collected vegetative samples in a variety of ecosystems such as:

- the forest alongside the Parapéti river were big trees such as *Maclura tinctoria*, *Pithecellobium scalare*, *Tecoma stans*, *Pisonia* sp., *Seguiera* sp., *Salix* sp., etc. can be found;
- the "algarrobales" (in local spanish), located a little more inland from the Parapéti river, characterised by a predominance of "algarrobo" or *Prosopis* spp., with *Vallesia glabra*, *Capparis salicifolia*, *Capparis tweediana*;
- the "sotales" (in local spanish), a forest formation a little bit more inland, characterised by the abundance of "soto", *Schinopsis cornuta*, with *Senna chloroclada* and *Bromelia serra*;
- the "choroquetales" (in local spanish), a shrubby and spiny formation of low trees rather distant from the river with *Ruprechtia trifolia* ("choroquete"), *Aspidosperma quebracho-blanco*, *Ziziphus mistol*, *Bulnesia* spp., *Cochlospermum tetraporum*, *Chorisia insignis*.

Also plant collection was done around houses, in cultivated fields and also in peculiar ecosystem such as the "bañados", and around the "Cerro Colorado", a low altitude formation far-east from the communities.

Then, back to the village, using the previously collected fresh samples we asked the *Paye* and other informants for the name(s) of the plant, and encourage them to speek freely about plant's uses or related information.

[1] *Paye* (in the singular form) and *Payereta* (plural form). For simplification we will keep the word *Paye* for both forms.

Four herbarium samples were collected per species to deposit in the National Herbarium of Bolivia (HNB), the Missouri Botanical Garden and specialists. Determination was made in HNB by the botanist of the team (Chāvez De Michel L.R.), and, for difficult cases specialists were consulted. At the termination of the study, we did organise a 3-day workshop with the *Paye* and other people, and we cross-checked our results with the informants.

## 3. Results and discussion

### 3.1. Overview of the isoceño ethnomedicine

It is impossible to speak about traditional isoceño medicine and pharmacopoeia, without mentioning the role played by the *Paye*. As said before, the traditional Izoceño society is structured around leaders (*Mburuvixa* or "capitán" in local spanish), acting and making decisions together with the *Paye*. Therefore, *Paye* not only play a spiritual role in the society, but also, have a strong social and political influence; this was already acutely observed by early spanish chroniclers during the 16th century, at the beginning of colonisation, and brilliantly highlighted by Clastres (1974).

The traditional function of the *Paye*, therefore is not limited to the medicine field; he is also in charge of the well being and prosperity of the community in its whole; it is why, until now, apart from curing physical disease, *Paye* are also requested to detain plagues or hazardous natural events such as drought, inundations, game vanishing, etc. *Paye* are also able to predict future and interpret dreams. In other words, the *Paye* is an intermediate between the present world and the supernatural world, a position shared by all shamans.

The *Paye* (or "good" shaman), is a person inhabited by a benevolent spirit, and has his counterpart in the person of the *Mbaekua* considered as the one from which evil happens; nevertheless this apparent simplification in roles is not that clear, as boundary *Paye*–*Mbaekua* is perceived wavering and are always questioned.

For the Izoceño, nature was put at the disposition of men but only in a limited form, and only in terms including reciprocity and respect (Riester, 1995). Animals and all form in the nature (rocks, rivers, mountains ...) have their landlord, *Iya*. *Iya* must be respected and treated right—for example, offences (as bad treatment to animals, overhunting) to the specific *Iya* who send game to the hunter, or patent lack of respect (through an offering of Coca leaves, tobacco or alcohol oversight) might cause total game vanishing and diseases.

When *Mbaekua* try to cause death, they introduce in their body victims what is designated in local spanish as "gusano/bicho" (worm), called in guaraní *Yzi*. Disease caused by above mentioned *Iya* spirits are generally considered as far more benign, as generally no "worm" is introduced in the body. Then depending upon the animal or object used as medium for transmitting *Yzi*, or the kind of

*Iya* spirit incriminated, the disease is classified as "simple" or "complicated".

Disease aetiology is highlighted thanks to the *Paye* powers in fact, its main and most important duty is to find the real origin of the disease or induced by some malevolent *Mbaekua*, or caused by some offended *Iya*, or "natural". Without doubt, isoceño ethnomedicine is of etiological essence, and as long as the very cause of the disease is not highlighted, no stable health improvement can be expected in this perspective it is not surprising that the treatment is influenced both by aetiology and symptoms.

In case of a *Mbaekua* disease the first task of the *Paye* is to extract the worm *Yzi* from the patients body. This is considered very often as being very hazardous for the *Paye* himself—in great risk of contamination. Also, in case of chronic severe disease many "extractions" must be performed, because the "mother of the worm" did have plenty time to reproduce inside the patient body, and so repeated sessions are done, until the final extract of the "mother".

The extraction of *Yzi* is performed in the presence of the family and relatives of the sick person. The *Paye* smokes restless local tobacco, wrapped in a maize leave; the smoke is directed over the patients body, for the *Paye* to read signs. Alcohol can also be used, swallowed and spitted over the patients body. No hallucinogenic plant is used during this session, nor actually, nor in past times. Despite differing considerably from other Bolivian ethnic group, Izoceños also chew Coca (*Erythroxylum coca* var. *coca*) leaves daily, which is much appreciated and was recorded as an object of a minor trade with the nearby Yuracaré since the 16th century. *Paye* do not use Coca at all for divination or healing purposes.

During the smoking session, the *Paye* sings in low voice ritual songs calling for allied spirits, aiming to help him in the diagnostic, calm the "worm" inside the patient and help for its extraction. The *Paye* extract the worm by sucking the patient's skin, then spits it out and deliver it to the relatives, which are in charge of burning it. For expulsion, some plants are most likely to be used by the *Paye*—among them, the milky sap of any *Kurupikai* (*Asclepias boliviensis*, *Asclepias curassavica* or *Sapium haematospermum*), is dropped in the patient nostrils in order to get rid of *Yzi* when located there. Later, these same species can be burnt around the patient bed to facilitate his recovery. *Mboiyu* (*Rhipsalis baccifera*) has a similar use, and *Kavopaye guasu* (*Senecio deferens*) is used by *Paye* after *Yzi* extraction—leaves are applied as poultice where sucking occurred, in order to help skin and health recovery. When the "mother of the worm" is extracted, the *Paye* give instructions for the treatment continuation, which is under the responsibility to his assistant. Sometimes, it is considered that the extraction of *Yzi* and the recitation of specifics songs (related with some animal origin for example) is sufficient for the sick to recover.

Despite knowing perfectly all plants and other kind of remedies from animal or mineral origin, the *Paye*, apart from giving instructions, do not handle any of them. In fact, remedies are prepared and administered by his assistant who generally knows few curatives or preventives songs and of course do not possess *Paye* powers.

Therefore, in contrast with the highly ritualised visionary performance of the *Paye*, fighting again evil forces with the help of invocations, ritual songs, smoke, and personal supernatural power, medicinal plants belong to the profane space. Their manipulation and use is allowed to anybody, their curative power is not related with any spiritual entity, and there is no restriction for the learning of the pharmacopoeia, as long as the person show interest.

### 3.2. Izoceño-guaraní pharmacopoeia

During this study, we collected about 980 herbarium samples, corresponding to about 306 different plant species (Chāvez De Michel et al., 2000). Two hundred and ninety seven of the species have an izoceño-guaraní name (we did not count here the obviously introduced species, having a "guaranised" name), and 298 (97%) were designated as useful. This high percentage can be explained by the fact that izoceños raise stock corrals (some cows, horses and many goats), very much appreciated as a sign of wealthiness or are also used to work in corrals, so most of the leafy species were designated as useful for fodder. One hundred and eighty nine species (61%) were identified as having medicinal uses and are listed in Table 1. Animal products used as medicinals (from Cuéllar, 2000 and our observations) are listed in Table 2.

#### 3.2.1. Repartition of uses

The majority of the species (104) are used to cure gastrointestinal disorders, such as diarrhoea (28), dysentery (23), "pasmo" (14) and stomach pain (14). "Pasmo" in local Bolivian-spanish has a different meaning depending upon the place where it is used. For the Izoceño, "pasmo" is characterised by difficulties to urinate and defecate, with the impression intestines are swollen up, whitish face and dizziness. The treatment may require the help of the *Paye*, and generally, plants are prepared in form of a poultice, eventually mixed up with caw feet grease, or better, jaguar or puma grease, if available.

Despite the high prevalence of vesicle problems, as stones or chronic inflammation (Kuñatuty, 2001), only five species were mentioned as useful for this type of disease but this apparent deficiency is counterbalanced by the fact that other animal medicinal product are used, such as tapir grated hoof, or turtles grated shell and avestrus gizzard.

Only one species (*Carica papaya* seeds) was indicated for the treatment of intestinal parasites, but it can be supposed that other plants used against diarrhoea/dysentery might also have a parasiticidal effect, thus indirectly treating this problem.

Then, dermatological problems appears of importance, because 56 species were indicated as cure for boils, wounds, infected spots and other various dermatosis presumably caused by fungal or yeast infection (29), as well as scabies

Table 1
Plant's uses

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Acanthaceae | | | | |
| guiraata mi | | | | |
| *Justicia dumetorum* Morong (GB1974) | Eye infection | Leaf and flower | Mashed for juice | Drops of juice in eye |
| *Ruellia ciliatiflora* Hook (AR685) | Haemorrhage (uterine, nasal, digestive), diarrhoea | Leaf | Decoction | Internal |
| *Ruellia coerulea* Morong (GB2115) | Diarrhoea | Leaf | In form of a tea | |
| Amaranthaceae | | | | |
| karuru guasu | | | | |
| *Amaranthus hybridus* L. (GB2099) | Wounds, cuts, dermatosis | Flower, leaf | Burnt for ashes | Local application |
| Anacardiaceae | | | | |
| ovaipi | | | | |
| *Astronium urundeuva* (Fr. Allem.) Engl. (AR712) | Wounds, cuts (stop blood) | Bark | Burnt for ashes | Ashes spread or mixed with animal grease |
| | Haemorrhage (nasal) | Bark | Decoction, until stays like jelly | Jelly over burning charcoal: inhalation |
| | Trauma, broken limb | Bark | Decoction, until stays like jelly | Around limb, as a plaster |
| urundei | | | | |
| *Schinopsis cornuta* Loesn. (AR548) | Susto | Stem | Burnt in houses | |
| | Haemorrhage (uterine) | Bark or leaf | Decoction | Internal |
| | Haemorraghe (nasal) | Leaf | Over burning charcoal | Inhalation |
| Annonaceae | | | | |
| aratiku | | | | |
| *Annona nutans* (R. E. Fries) R. E. Fries (AR591) | Ear pain | Leaf and root | Mashed for juice | Drops in ears |
| Apocynaceae | | | | |
| arakuarembiu | | | | |
| V*allesia glabra* (Cavanilles.) Link. (GB1992) | Dermatosis | Leaf | Burnt for ashes | Local application |
| | Heart pain | Young leaf | Few leaves squeezed in water | Internal |
| | Haemorrhage (vomiting blood) | Leaf | 20/30 leaves in 1/4 litre of cold water | Internal |
| | Eye infection | Fruit | Fruits squeezed for juice | Local application |
| | Rheumatic pain | Stem with leaves | Over fire, soaked in animal grease | Local application |
| | Rheumatic pain | Leaf | In form of a pomade | Local application |
| | Rheumatic pain | Leaf | Bath | |
| | Vesicle pain | Leaf | Few leaves squeezed in water | Internal |
| Iviraro guasu | | | | |
| *Aspidosperma quebracho-blanco* Schltdl. (GB1956) | Appendicitis | Bark | Decoction | Internal, small glass |
| | Dysentery | | | Not for pregnant woman or child under five |
| Aristolochiaceae | | | | |
| mboipina | | | | |
| *Aristolochia chiquitensis* Buc. (GB2042) | Appendicitis | Leaf | Squeezed in cold water | Internal + poultice |
| | | Leaf | Squeezed in cold water | Internal + poultice |
| takareoreo | | | | |
| *Aristolochia* sp. | Haemorrhage (uterine) | Leaf and flower | Decoction | Internal |
| | Appendicitis | Root | Decoction | Internal |
| | Whooping cough | Leaf and flower | Squeeze in cold water | Internal |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Asclepiadaceae | | | | |
| kurupicay* | | | | |
| *Asclepias boliviensis* Fourn. and (RM2734). *Asclepias curassavica* L. (AR507) | *Paye*'s use<br>*Paye*'s use | | | |
| supuakati* | | | | |
| *Philibertia gracilis* D.Don (AR667) and *Sarcostemma gracile* Decaisne in DC. (GB2147) | Dermatosis, wounds | Sap | | Local application |
| | Eye infection | Sap | | Drops in eye |
| | Headache | Aerial part | Mashed | Local application |
| supuakoroi* | | | | |
| *Morrenia brachystephana* Griseb. (RM2741) and *Sarcostemma clausum* (Jacq.) Roem. & Schltdl. (AR598) | Wounds, cuts | Sap, or ashes | | Local application |
| | Uterine haemorrhage | Aerial part | Decoction | Internal |
| supuaroki | | | | |
| *Morrenia odorata* (Hook & Arn.) Lindl. (GB2023) | Teeth pain | Milky sap | | Local application |
| | Diarrhoea, dysentery | Leaf | Squeezed in cold water | Internal |
| | Headache | Aerial part | Pomade | Local application |
| Asteraceae | | | | |
| chorochikea | | | | |
| *Austrobrickellia patens* (Don ex Hook & Harn). K. R. (AR684) | Fever | Whole plant burnt over charcoal | Whole plant burnt over charcoal | Inhalation of smoke |
| eirakuañeti | | | | |
| *Pectis odorata* Griseb. (RM2699) | Intestinal spasm | Aerial parts | In form of a tea | Internal |
| | Coloured urine | Aerial parts | In form of a tea | Internal |
| | Kidney ailment (pain, inflammation) | Aerial parts | In form of a tea | Internal |
| | General malaise | Aerial parts | In form of a tea | Internal |
| guirae | | | | |
| *Tessaria integrifolia* Ruíz et Pavón (GB2059) | Dizziness | Flowers | In form of a tea | Internal |
| îvira käti | | | | |
| *Porophyllum lanceolatum* DC. (GB2108) | Lung ailment with fever | Aerial part | Strong decoction | Internal |
| kaami | | | | |
| *Egletes viscosa* (L.) Less. | Blood vomits | Entire plant | Decoction | Internal |
| kaane guasu | | | | |
| *Tagetes minuta* L. (GB2081) | Liver pain, with fever | Leaf | As a tea | Internal |
| | Pasmo | Entire plant | Squeeze juice in cold water and prepare in poultice mashed plants | Internal and local application |
| | Stomach pain | Leaf | As a tea | Internal |
| | Rheumatic pain | Entire plant | Leaf | Pomade |
| | Kidney ailment (pain, inflammation) | Entire plant | As a tea | Internal |
| | Digestion problem | Entire plant | As a tea | Internal |
| karaguapua* | | | | |
| *Mikania cordifolia* (L.f.) Willd. (RM2580) and *Mikonia congesta* DC. (RM2581) | Pasmo | Entire plant | Mashed | Local application |
| kavopaye guasu | | | | |
| *Pluchea sagittalis* (Lam.) Cabr. (RM2612) | Pasmo | Aerial parts | Pomade | Local application |
| | Vesicle pain | Flower | As "poro" | internal |
| | Swelling of body, with fever | Aerial parts | As bath, or as poultice | Local application |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| kavopaye mi | | | | |
| *Senecio deferens* Griseb. (GB1984) | Rheumatic pain | Leaf | Heat leaf over flame | Local application |
| | Rheumatic pain | Leaf | Pomade | Local application |
| | *Paye*'s use | Leaf | | |
| ñatiuna | | | | |
| *Bidens cynapiifolia* Kunth. (RM2756) | Pain when urinate | Entire plant | As a tea | Internal |
| | Kidney pain | Entire plant | As a tea | Internal |
| piki kii | | | | |
| *Vernonia cincta* (Griseb.) H. Robinson (GB1973) | Scabies | Leaf | Mashed in cold water | Local application (bath) |
| | Eye infection | Flower/sap | | Local application |
| Bignoniaceae | | | | |
| aguarakigua | | | | |
| *Pithecoctenium cynanthoides* DC. (GB1953) | Contraceptive effect | Fruit | Decoction | Internal |
| guirapitiyu | | | | |
| *Tecoma stans* (L.) Juss. ex H. B. K. | Arrebato | Leaf | Vapour steam bath | Inhalation |
| | Dysentery | Leaf | Squeezed in cold water | |
| karapi | | | | |
| *Arrabidaea candicans* (L. Rich.) DC. (AR663) | Haemorrhage (vomiting blood) | Leaf, flower | Squeezed in water | Internal |
| *Arrabidaea truncata* (Sprague) Sandw. (GB1929) | Dermatosis | Sap | Local application | |
| Bombacaceae | | | | |
| samou | | | | |
| *Chorisia insignis* H.B.K.(GB1961) | Sunstroke in child | Leaf | Bath | |
| Boraginaceae | | | | |
| mandiyu mi | | | | |
| *Heliotropium procumbens* Mill. | Feverish child | Leaf | Bath | |
| Bromeliaceae | | | | |
| karaguata | | | | |
| *Bromelia serra* Griseb. (AR586) | Kidney inflammation | Leaf | Mashed with water for juice | Internal |
| *Aloe vera* (L.) Burm. f. (GB2140) | Dermatosis | Leaf | Yellow sap | Local application |
| taperachi* | | | | |
| *Tillandsia* spp. (same recipes for all *Tillandsia*) | | | | |
| *Tillandsia bryoides* Griseb. (GB2129) | Pasmo | Leaf | Pomade with animal grease | Local application |
| *Tillandsia duratii* Vis. (GB1958) | – | Entire plant | Plant mashed in poultice | Local application |
| *Tillandsia loliacea* Mart. ex Schult. F. (GB2129bis) | Kidney inflammation | Leaf | Pomade with animal grease | Local application |
| *Tillandsia recurvata* L. (AR551) | | | | |
| *Tillandsia vernicosa* Baker (GB1959) | | | | |
| Cactaceae | | | | |
| amenda karu | | | | |
| *Quiabentia verticillata* (Vpl.) Vpl. (RM2608) | Infected purulent spots, fungus | Leaf | Peeled off, internal part used | Local application |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| guayapa | | | | |
| *Pereskia sacharosa* Griseb. (RM2527) | Boils, atlet foot, fungus, shingles | Leaf | Emptied (only cuticle used) | Local application |
| | Diarrhoea, dysentery | Leaf | Squeezed in water | Juice drunk |
| | Shingles | Leaf | Mashed | Local application |
| mboiyu | | | | |
| *Rhipsalis baccifera* (Mill.) Stern. (RM2561) | Snake bite | Aerial parts | Chew plant and swallow juice | Internal |
| | Fever | Aerial parts | Mash plant for juice | Internal |
| | For Payé use | Aerial parts | | |
| sainimbe | | | | |
| *Opuntia* sp. (GB2136) | Rheumatic pain | Fruit | Cut in 2 parts | Local application |
| *Opuntia paraguariensis* Schuman (GB1964) | Cough, vomits (linked with special observance rules during first menstruation) | Fruit | | Eaten raw |
| sipepe | | | | |
| *Cereus* sp. (GB2132) | Rheumatic pain | Stem | Slice of peeled stem | Local application |
| Cannaceae | | | | |
| tarope | | | | |
| *Canna glauca* L. (GB2104) | Ear pain | Flower | Squeeze juice | Local application |
| Capparidaceae | | | | |
| ivaguasu | | | | |
| *Capparis salicifolia* Griseb. (AR606) | Infected spots with pus, all over body, scabies, shingles | Bark, leaf, sap, root | In decoction, pomade, poultice | Local application |
| | Any kind of dermatosis | | | |
| | Dysentery | Bark and leaf | Decoction | Internal |
| ivaguasu mi | | | | |
| *Capparis tweediania* Eichl. (GB1975) | Scabies | Leaf | Squeezed in water | Bath |
| | Constipation | Leaf | Squeezed in water | Internal |
| | Susto | Leaf | Squeezed in water | Bath |
| ivoviguasu | | | | |
| *Capparis speciosa* Griseb. | Stomach pain | Fruit | Soak in alcohol | Cloth on stomach |
| | – | – | Prepared in form of a syrup | Internal (to induce vomits) |
| | Headache | Leaf | Mashed or soak in alcohol | Applied locally |
| | – | – | Squeeze leaves in water | Bath |
| | Susto | Leaf | Susto | |
| | Rheumatic pain | Leaf | Squeeze leaves in water | Bath |
| ivovimi | | | | |
| *Capparis speciosa* Griseb. | Susto | Leaf | Bath, or burnt in house | |
| | Headache | Leaf | Mashed or soak in alcohol | Applied locally |
| j'ati j'atia | | | | |
| *Cleome parviflora* H. B. K. (GB2086) | Eye infection | Leaf | Juice from leaf | Applied locally |
| tararakimi | | | | |
| *Cleome serrata* Jacq. (GB2096) | Dermatosis | Leaf | Squeeze in water | External |
| | Sunstroke | Leaf | Decoction | Bath + drink |
| | Rheumatic pain | Leaf | Decoction | Bath + drink |
| Caricaceae | | | | |
| mbapaya | | | | |
| *Carica papaya* L. (AR647) | Worms | Seeds | Mashed in powder | Internal |
| | Diarrhoea | Flower (male or female) | As a tea | Internal |
| | Cough | Flower (male or female) | As a syrup | Internal |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Celastraceae | | | | |
| chorimimi | | | | |
| *Maytenus scutioides* (Griseb.) Lourt. & O'Donell (RM2544) | Diarrhoea, dysentery | Leaf | Mashed in water | Internal |
| | – | Leaf and bark | Decoction | Internal |
| | – | Fruit | Decoction | Internal |
| | Haemorrhage (any kind) | Leaf and bark | Decoction | Internal |
| Chenopodiaceae | | | | |
| kaane mi | | | | |
| *Chenopodium ambrosioides* L. (GB2152) | Liver pain, with fever | Leaf | As a tea | Internal |
| | Pasmo | Entire plant | Squeeze juice in cold water and prepare in poultice mashed plants | Internal and local application |
| | Stomach pain | Leaf | As a tea | Internal |
| | Rheumatic pain | Entire plant | Leaf | Pomade |
| | Kidney ailment (pain, inflammation) | Entire plant | As a tea | Internal |
| | Digestion problem | | As a tea | Internal |
| Commelinaceae | | | | |
| yaîmbara matia* | | | | |
| *Commelina diffusa* N.L. Burm. and *Commelina erecta* L. (GB2066) | Eye infection | Flower | Water from inside flower | Local application |
| Convolvulaceae | | | | |
| tararaki | | | | |
| *Ipomoea carnea* ssp. *fistulosa* (Mart. ex Choisy) D. Austin (GB2057) | Dermatosis | Leaf | Squeeze in water | External |
| | Sunstroke | Leaf | Decoction | Bath + drink |
| | Rheumatic pain | Leaf | Decoction | Bath + drink |
| tareiyokua* | | | | |
| *Ipomoea cairica* (L.) Sweet (RM2519) and *Ipomoea dumetorum* Willdenow ex Roemer & Schultes (GB2159) | Dermatosis | Sap | | Local application |
| Cruciferaceae | | | | |
| kaanemi | | | | |
| *Coronopus didymus* (L.) Smith (AR587) | Pasmo | Entire plant | Poultice, pomade | Local application |
| tupeisa | | | | |
| *Lepidium bonariense* L. (GB2070) | Sunstroke in child | Aerial parts | Decoction | Bath |
| Cucurbitacae | | | | |
| aguarasandia | | | | |
| *Cayaponia* sp. (GB2903) | Scabies | Leaf | Mashed in poultices | Local application |
| andai | | | | |
| *Cucurbita moschata* Duchesne (AR637) | Ringworm | Sap from fruit | | Applied on skin |
| naeyoeko | | | | |
| *Luffa cylindrica* (L.) Roem (AR673) | Anuria | Leaf | Squeeze in water | Internal |
| yacaratia | | | | |
| *Momordica charantia* L. (GB2004) | Fortifiant | Entire plant | Soaked in water | Bath |
| | Scabies | Leaf | Mashed | Poultice |
| | Sunstroke | Leaf | Mashed in water | Bath |
| | Malaria | Aerial parts | Mashed in water | Bath |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Equisetaceae | | | | |
| kavayuruguai | | | | |
| *Equisetum giganteum* L. (AR508) | Lack of appetite, weariness | Entire plant | Squeeze stem for juice | Internal |
| | Kidney ailment (pain, inflammation) | Entire plant | Decoction | Internal |
| pikikiimi | | | | |
| *Chamaesyce hirta* (L.) Millsp. (GB2003) | Scabies | Leaf | Mashed in water | Bath |
| | Eye infection | Leaf | Sap | In the eye |
| Euphorbiaceae | | | | |
| ivirati | | | | |
| *Julocroton* sp. (GB2109) | Appendicitis | Stem with leaf | Decoction | Internal |
| kaape | | | | |
| *Chamaesyce serpens* (H. B. K.) Small (AR576) | Rheumatic pain | Entire plant | Pomade | Local application |
| kavujuo | | | | |
| *Ricinus communis* L. (GB1989) | Muscular cramps | Seed | Powdered | Rubbing |
| | Fever | Leaf or seed | Pomade | Local application |
| | Headache | Leaf or seed | Pomade | Local application |
| kurupicayguasu | | | | |
| *Sapium haematospermum* Muell. Arg. (GB1969) | Boils in breast (breast feeding woman) | Leaf mashed | Poultice | Local application |
| maniira* | | | | |
| Same recipes for all "maniira" | | | | |
| *Jatropha curcas* L. (GB2106) | Dermatosis | Sap | | Local application |
| *Jatropha excisa* Griseb. (GB1926) | Headache | Leaf | Soaked in grease | Local application |
| *Jatropha flavopirens* Pax. & Hoffm. (GB2126) | Shingles | Sap | | Local application |
| *Jatropha hieronymi* O. Ktze. (AR630) | Tooth pain | Sap | | Local application |
| *Jatropha papyrifera* Pax & Hoffm. (GB1960) | | | | |
| *Jatropha* sp. (GB2146) | | | | |
| piki kii | | | | |
| *Chamaesyce hirta* (L.) Millsp. (GB2003) | Scabies | Leaf | Mashed in cold water | Local application (bath) |
| | Eye infection | Flower/sap | | Local application |
| | Tooth pain | Sap | | Local application |
| pino | | | | |
| *Cnidoscolus tubulosus* (Muell. Arg.) Johnst. (GB1947) | Bruises | Sap + inside of stem grated | Sap + inside of stem grated | Local application |
| | Boils | Sap + inside of stem grated | Sap + inside of stem grated | Local application |
| | Tooth pain | Sap | | Local application |
| | *Paye*'s use | | | |
| urukuya* | | | | |
| *Croton lachnostachyus* Baill. (GB2062)and *Croton sarcopetalus* Muell. Arg. (GB1935) | Scabies, white spots on skin | Sap | | Applied on skin |
| | Wounds, cuts | Leaf burnt for ashes | | Applied on skin |
| | Rheumatic pain | Leaf | Pomade | Local application |
| | Headache | Leaf | Pomade | Local application |
| | Eye infection | Sap | | In the eye |
| Fabaceae | | | | |
| iguopei | | | | |
| *Prosopis chilensis* (Molina) Stuntz (GB2131) | Cough, respiratory ailment | Flower, leaf | Syrup/in form of a pomade | Internal + external |
| *Prosopis flexuosa* DC. (GB2045) | Cough, respiratory ailment | Flower, leaf | Syrup/in form of a pomade | Internal + external |
| | Diarrhoea | Leaf | Decoction | Internal |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| iguopere | | | | |
| *Acacia aroma* Gill. (GB1983) | Infected wounds, boils | Bark | Grated in decoction | Internal |
| | Dysentery | | Bark soaked in water | Internal |
| ivirayepiro | | | | |
| *Caesalpinia paraguariensis* (Parodi) Burkart (GB2020) | Wounds, boils, dermatosis | Bark | Strong decoction | External |
| | | Fruits | Burnt in ashes | External |
| | – | Sap of petiole | | External |
| | Fever | Bark and leaf | Decoction | Internal |
| | Diarrhoea, dysentery | Bark | Decoction | Internal |
| | Fatigue | Bark and leaf | Decoction | Internal |
| | Stomach pain | Bark and leaf | Decoction | Internal |
| kumandamanda* | | | | |
| *Vigna adenantha* (G. F. Meyer) Mar. Merech & Stain (AR683) and *Calliandra* sp. (RM2649) | Vomits | Leaf | Bath | Flower |
| kumbaru | | | | |
| *Geoffroea decorticans* (Gill. ex Hook. & Arn.) Burk. (GB2036) | Cough | Flower | As a tea | Internal |
| | General fatigue | Leaf | As a tea | Internal |
| | Diarrhoea, stomach ache | Bark | Decoction | Internal |
| | Diarrhoea, stomach ache | Bark | Maceration | Internal |
| kurusapoi | | | | |
| *Pterogyne nitens* Tul. ex Benth (GB2015) | Anuria | Leaf and bark | As a tea | |
| | Scabies | Leaf and bark | Strong decoction | Bath |
| | Scabies | Leaf and bark | Burnt as ashes | Local application |
| mbuijae | | | | |
| *Senna chloroclada* (Harms.) I. & B. (GB1942) | Chagas | Flower | As a tea | Internal |
| | Kidney ailment (pain, inflammation) | Root | Decoction | Internal |
| taperivai | | | | |
| *Senna spectabilis* (DC.) I. & B. var. *spectabilis* (GB1978) | Headache | Leaf | As a poultice, and as a tea | Internal, external |
| | Whooping cough | Leaf | As a tea | Internal |
| taperivai mi | | | | |
| *Senna pendula* Willd. var. *praeandina* Irwin & Barneby (GB2113) | Rheumatic pain | Leaf | Pomade | Local application |
| tatare | | | | |
| *Acacia albicorticata* Burkart (GB1981) | Cough, bronchitis | Flower | Preparation of a syrup | Internal |
| | Wounds, cuts | Bark | Powdered | Local application |
| | | Bark | Burnt in ashes | Local application |
| tatarera | | | | |
| *Pithecellobium scalare* C. Martius (AR510) | Cough, respiratory ailment | Bark | Decoction | Internal |
| timboy wata | | | | |
| *Carthomion polyanthum* (Spreng.) Burk.(GB2046) | Intestinal problems (without diarrhoea) | Bark or leaf | Decoction | Internal |
| topei | | | | |
| *Senna morongii* (Britton) Irwin & Barneby (RM2706) | Atlet foot, fungus | Leaf | Decoction | Internal, external |
| töte kumanda* | | | | |
| *Senna occidentalis* (L. ) Link. (GB1942) and *Senna obtusifolia* (L.) Irwin & Barneby (AR681) | Rheumatic pain | Leaf | Pomade | Local application |
| | Pasmo | Leaf | Pomade | Local application |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| yukeri | | | | |
| *Acacia praecox* Griseb. | Flower | Kidney inflammation, anuria | As a tea | Internal |
| no recorded name | | | | |
| *Parkinsonia aculeata* L. | Fever | Leaf | Squeeze for juice | Internal |
| no recorded name | | | | |
| *Sesbania virgata* (Cav.) Pers. (RM2733) | Vesicle plain or kidney pain | Leaf | Squeeze in water or in decoction | Internal |
| Loranthaceae | | | | |
| nderirembiu* | | | | |
| *Phoradendron fallax* Kuijt.(AR527) | Appendicitis | Leaf | Squeeze in water and also mashed in paste | Internal |
| *Phoradendron liga* (Gill.) Eichl. (AR659) | | | | Applied as poultice |
| *Psittacanthus cordatus* (Hoffmsegg.) Blume (RM2700) | Eye infection | Leaf | Squeeze for juice | In the eye |
| Malpighiaceae | | | | |
| isipoaviyu* | | | | |
| *Janusia guaranitica* (St. Hil.) Adr. Juss. (GB2014) and *Mascagnia brevifolia* Griseb. (GB2040) | Fever, vomits, headache | Leaf | Decoction | Internal |
| | Fever, vomits, headache | Leaf | Pomade | Local application |
| isipopita | | | | |
| *Heteropterys dumetorum* (Griseb) Nied. (GB2112) | Muscular cramps | Leaf, macerate with alcohol | Local application | |
| Malvaceae | | | | |
| guaichi* | | | | |
| *Abutilon herzogianum* R. E. Fries (AR675) | Diarrhoea, dysentery | Young leaf | Squeeze in water | Internal |
| *Gaya tarijensis* R. E. Fries (GB2155) | Fever | Young leaf | Squeeze in water | Internal |
| *Malvastrum coromandelianum* (L.) Garcke (AR695) | | | | Bath for children |
| *Melochia pyramidata* L. (RM27213) | | | | |
| *Sida cordifolia* L.(GB2145) | | | | |
| *Sida santaramensis* Monteiri (GB2147 bis) | | | | |
| *Spermacoce tenuior* L. (RM2722) | | | | |
| *Wissadula wissadulifolia* (Gris.) Krap. (GB1949) | | | | |
| *Wissadula* sp. (AR608) | | | | |
| Menispermaceae | | | | |
| takumbokumbomi | | | | |
| *Cissampelops pareira* L. (GB2103) | Blood vomiting, nasal haemorrhage | Stem with leaf and fruit | Decoction | Internal |
| Moraceae | | | | |
| tatayiua | | | | |
| *Maclura tinctoria* (L.) Don ex Steudel (GB2043) | Tooth pain | Sap | | Local application |
| | Uterine haemorrhage | Bark and leaf | Decoction | Internal |
| Nycktaginaceae | | | | |
| chunurembiu | | | | |
| *Boerhaavia erecta* L.(GB1944) | Kidney pain & inflammation | Leaf | Squeezed for juice | Internal |
| | Sciatic pain | Root | Decoction | Internal |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Olacaceae | | | | |
| guasukea | | | | |
| *Ximenia americana* L. (GB2130) | Diarrhoea | Dried bark | Decoction | Internal |
| | – | Leaf | As tea | Internal |
| Onagraceae | | | | |
| tupeicha | | | | |
| *Ludwigia octavalvis* (Jacq.) Raven (GB2067) | Stomach pain | Leaf | As a tea | Internal |
| | Liver pain | Leaf | As a tea | Internal |
| Papaveraceae | | | | |
| jati jati'a | | | | |
| *Argemone subfusiformis* Ownbey (GB1971) | Cough, cold, flu | Flower | As a tea | Internal |
| | Liver pain | Leaf | As a tea | Internal |
| Passifloraceae | | | | |
| mburukuya | | | | |
| *Passiflora cincinnata* Mast. (AR583) | Against vomits in child | Flower | Squeeze in water | Internal |
| | Scalp dermatosis in child | Flower | Squeeze in water | Wash, flower as poultice |
| | Scalp dermatosis in child | Sap | | Local application |
| | Uterine haemorrhage | Leaf | Squeeze in water for juice | Internal |
| Phytolacaceae | | | | |
| chipi | | | | |
| *Petiveria alliacea* L. (RM2629) | Tapped nose | Root | Grated | Inhaled, and applied on head |
| | Malaria | Entire plant | In boiling water | Inhale vapours |
| iviravuku | | | | |
| *Seguiera* sp. (RM2758) | Dermatosis | All tree burnt | Preparation of a soap | Local application |
| Plumbaginaceae | | | | |
| isipoporomboyi | | | | |
| *Plumbago coerulea* H. B. K. (RM2540) | Tooth pain | Root | Induce destruction of tooth | Local application |
| Poaceae | | | | |
| kapii pururu | | | | |
| *Digitaria insularis* (L.) Mez ex Ekam (AR572) | Cough, flu, cold | Entire plant | Pound and leave macerate | Internal |
| | Bronchitis | – | Maceration | – |
| | Nasal haemorrhage | Flower | Squeezed | Applied on skin |
| kapii* | | | | |
| *Eragrostis ciliaris* (L.) R. Brown | General malaise, with fever, chills and headache | Root and aerial part | Decoction | Internal |
| *Eriochloa punctata* (L.) Desv. (GB2118) | | | | |
| *Panicum scabridum* Doll. (RM2622) | | | | |
| *Panicum trichanthum* Nees. (RM2589) | | | | |
| *Pennisectum purpureum* Schumach (GB2117) | | | | |
| Polygonaceae | | | | |
| choroque | | | | |
| *Ruprechtia triflora* Griseb. (GB1965) | Whooping cough | Bark | Strong decoction | Internal |
| | Intestinal cramps, diarrhoea | Leaf or bark | Decoction or diarrhoea | Internal |
| isipopita | | | | |
| *Muehlenbeckia tamnifolia* (H.B.K.) Meissn. (GB2119) | Liver pain | Leaf | Pomade | Local application |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Ranunculaceae | | | | |
| kavara rendivaa | | | | |
| *Clematis denticulata* Velloso (GB1968) | Tapped nose | Young seeds | Crushed | Breezed |
| | Cough | Leaf without petiole | As a tea/pomade | Internal and external |
| | Scabies, scalp excema/dermatosis | Aerial part | Squeeze plant in water | As a wash |
| | Malaria | Aerial part | Burnt over flame | Inhalation of smoke |
| Rhamnaceae | | | | |
| yuai | | | | |
| *Zizyphus mistol* Griseb.(RM2707) | Diarrhoea | Bark | Pounded in water, maceration | Internal |
| | | Leaf | Decoction | Internal |
| | Wounds, cuts | Bark | Grated in water | As a wash |
| Rubiaceae | | | | |
| guaichi | | | | |
| *Spermacoce tenuior* L. (RM2722) | Diarrhoea, dysentery | Young leaf | Squeeze in water | Internal |
| | Fever | Young leaf | Squeeze in water | Internal<br>Bath for children |
| Rutaceae | | | | |
| pomelo | | | | |
| *Citrus paradisi* Macf. (AR621) | Heart problems | Leaf | Maceration in alcohol | Internal |
| | Weariness | Leaf | Steam bath | External |
| Salicaceae | | | | |
| guirapuku | | | | |
| *Salix humboldtiana* Willd. (GB2078) | Fever | Leaf | Squeezed in water | Local application |
| | Dysentery | Leaf | Squeezed in water | Internal |
| Sterculiaceae | | | | |
| guirapinda | | | | |
| *Byttneria filipes* mart. ex. K. Schum. (GB2100) | Muscular cramps, pain in all muscles | Leaf | As a tea | Internal |
| Sapindaceae | | | | |
| täguïro* | | | | |
| *Paullinia neglecta* Radlk. (GB2056) | Eye pain, conjunctivitis | Leaf | Squeeze for juice | Local application |
| *Serjania caracasana* (Jacq.) Willd. (AR629) | Stomach pain | Leaf | Squeeze for juice | Internal |
| *Serjania marginata* Casar. (RM2601) | | | | |
| *Serjania perulacea* Radlk. (GB2032) | | | | |
| *Urvillea chacoensis* A. T. Hunz. (RM2767) | | | | |
| Sapotaceae | | | | |
| guira rira | | | | |
| *Bumelia obtusifolia* Roem. & Schultes (GB2122) | Diarrhoea | Leaf, or bark | Decoction | Internal |
| Scrophulariaceae | | | | |
| tupeicha mi* | | | | |
| *Capraria biflora* L. and *Scoparia dulcis* L. (GB2029), (GB2073) | Diarrhoea, dysentery | Leaf | Squeezed in water | Internal |
| | Fever/sunstroke in child | Leaf | Squeezed in water | Bath |
| | Stomach ache | Leaf | Squeezed in water | Internal |
| Simaroubaceae | | | | |
| chorimimi | | | | |
| *Castelca coccinea* Griseb. (AR541) | Diarrhoea, dysentery | Leaf | Mashed in water | Internal |
| | – | Leaf and bark | Decoction | Internal |
| | – | Fruit | Decoction | Internal |
| | Haemorrhage (any kind) | Leaf and bark | Decoction | Internal |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Solanaceae | | | | |
| kaoveti | | | | |
| *Nicotiana glauca* R. Grah.(GB1936) | Headache | Leaf | Soaked in grease | Local application |
| | Tooth pain | Leaf | Soaked in alcohol | Local application |
| | – | Leaf | Decoction in salted water | Local application |
| | Fever | Leaf | Crude, as poultice | Local application |
| kamambu* | | | | |
| *Physalis* spp. *Physalis angulata* L. (GB2006) *Physalis maxima* Mill. (RM2533) *Physalis viscosa* L. (GB2153) | Intestinal cramps | Aerial part | As a tea | Internal |
| guiravevi | | | | |
| *Solanum glaucophyllum* Desf. (GB2090) | Diarrhoea with vomits | Leaf | Decoction | Internal + bath |
| guirakiyo* | | | | |
| *Solanum amygdalifolium* Steud.(GB2101) | Dermatosis | Leaf | Pomade | Local application |
| *Solanum lorentzii* Bitter (GB2111) *Solanum physaloides* Dunal (RM2769) | Fever | Aerial part | | As a bath |
| mbotovovo | | | | |
| *Lycianthes asarifolia* (Kunth & Bouche) Bitter (GB2054) | Boils, abscess | Leaf | Mashed leaves as poultice | Local application |
| | Tooth pain | Leaf | Juice from leaf | On gums |
| pïriri* | | | | |
| *Solanum pseudocapsicum* L. (RM2747) and *Solanum stuckertii* Bitter (RM2582) | Vesicle pain | Leaf | Squeeze in water | Internal |
| | Whooping cough | Aerial parts | Squeeze in water | Internal |
| | Kidney ailment | Aerial parts | Squeeze in water | Internal |
| tutia* | | | | |
| *Solanum conditum* C. Morton (GB2082) | Scabies | Fruit | Crushed | Local application |
| *Solanum hieronymi* Kuntze (RM2742) | Kidney pain | Leaf | Squeezed for juice | Internal |
| *Solanum palinacanthum* Dunal. (RM2504) | Heart pain | Leaf | As a tea | Internal |
| *Solanum sisymbrifolium* Lam. (GB1976) | Nasal haemorrhage | Leaf | Squeezed in cold water | Internal |
| yakurembiu | | | | |
| *Solanum argentinum* Bitter & Lillo (GB2017) | Anuria | Flower and fruits | Pomade | Local application |
| | Scabies | Fruits | Crushed | As poultice |
| | Tooth pain | Root | Grated | Local application |
| | Swellings | Leaf | Soaked in grease | Local application |
| | Headache | Leaf | | Local application |
| | Rheumatic pain | Leaf | | In bath |
| yandipa* | | | | |
| *Cestrum parqui* L'Herit. (AR638) and *Cestrum strigillatum* Ruíz & Pavón (RM2547) | Fever | Leaf | Squeezed in cold water | Bath |
| | Headache | Leaf | Soaked in grease | Local application |
| | Rheumatic pain | – | – | – |
| | Scabies, dermatosis | Leaf mashed in water | | Local application |
| | Diarrhoea | Leaf squeezed for juice | | Internal |
| | Metrorraghia | Young leaves | Juice | Local application and internal |
| Sterculiaceae | | | | |
| kurundil | | | | |
| *Guazuma ulmifolia* Lam. (GB1972) | Cough, with fever | Leaf and bark | Decoction | Internal |
| | Lungs ailment with fever | Bark | Decoction | Internal |

Table 1 (*Continued*)

| Family isoceño-guaraní name scientific determination (voucher number) | Medicinal indication | Part used | Mode of preparation | Way of administration |
|---|---|---|---|---|
| Ulmaceae | | | | |
| ñuguäsi* | | | | |
| *Celtis pubescens* Spreng.(GB1950) and *Celtis spinosa* Spreng. (RM2604) | Digestion | Leaf | As a tea | Internal |
| | Diarrhoea | Leaf | As a tea | Internal |
| | – | – | Squeezed- in maceration | Internal |
| Urticaceae | | | | |
| pino* | | | | |
| *Urera aurantiaca* Wedd. (RM2748) and *Urera baccifera* (L.) Guad.(RM2745) | Bruises | Sap + grated stem | | Local application |
| | Boils, abscess | Sap + grated stem | | Local application |
| | Tooth pain | Sap | | Local application |
| Verbenaceae | | | | |
| ivirakati | | | | |
| *Lippia alba* (Mill.) N. E. Br. (GB2114) | Respiratory ailment | Leaf and stem | Squeezed in water | Internal |
| yapurundi mi | | | | |
| *Lantana camara* L.(GB1988) | Weariness, fever | Leaf | Squeezed in water | Bath |
| no recorded name | | | | |
| *Recordia boliviana* Moldenke (GB2091) | Nasal haemorrhage | Leaf and stem | Decoction | Internal + water on head |
| Vitaceae | | | | |
| mbokere* | | | | |
| *Cissus palmata* Poiret (GB2055) | Muscular cramps | Aerial part | Mashed in water/pomade | Internal + external |
| *Cissus verticillata* (l.) Nicholson + C. E. Jarvis subsp. *laciniata* (GB1996) | Acute inflammation in knees/ankle | Aerial part | Mashed/poultice | External |
| *Cissus verticillata* (l.) Nicholson + C. E. Jarvis subsp. *verticillata* (RM2770) | – | Root | Sliced soaked in alcohol | External |
| Zygophyllaceae | | | | |
| guiraita | | | | |
| *Bulnesia bonariensis* Griseb. (GB2011) | Pasmo | Leaf | As a tea | Internal |
| | Gastritis (to induce vomits) | Dried bark | Decoction | Internal |

supuakati*: same recipes for all species listed under

(15 species). Seven species were said to be used after the intervention of the *Paye*, for curing a disease called *Iyateu* (Lord of the Tick), characterised by spreading of blisters on the skin, causing strong itching and fever. In addition, Cuéllar (2000), quoted the widespread use of undetermined fishes grease against skin problems, inflammations and wounds, together with turtle grease (for whitish spot on skin) and grated armadillo shell.

Twenty seven species were indicated as febrifuges, only three species being specifically mentioned as antimalarial. For the izoceño, a distinction is made between "proper" fever "from inside, with chills" (22 species), what is perceived as a simple "superficial body heating" (1), and a "sunstroke fever" (5 species) characterised by a superficially feverish body, said to be caused by an excess of sun together with general aching, swelling bone pain. *Kuarai mbaerasi* (sun disease) is taken very seriously, because it is said that if worsening, bloody vomiting or nasal haemorrhage will occur.

Twenty one species were then indicated in case of urinary problems.

Twenty one species are used to treat rheumatic pain, all administered externally, except two species, administered in the form of a tea. Nevertheless, it must be added that favourite treatment are generally those made out from some animal grease (from avestrus, tapir underib, puma and porcupine), rubbed on the affected area.

The rest of the species were mentioned to treat headache (17), eye infections (20 species of plants together with

Table 2
Animals medicinal's uses (from Cuéllar, 2000 and our observations)

| Vernacular name of animal (*Latin name*) Part of animal used | Medicinal use |
| --- | --- |
| **Mammals** | |
| mborevi (*Tapirus terrestris*) | |
| Grease | Fever |
| – | Muscular pain |
| – | Cough, respiratory problems |
| Penis | Impotency |
| Stone from stomach | Heart pain |
| Nails | Heart pain |
| – | Vesicle pain |
| – | Rheumatic pain |
| guaju (*Mazama gouazoubira*) | |
| Grated horn | Ear pain |
| guasukaka (*Lama guanicoe*) | |
| Hair | Ear pain |
| yagua (*Felis onca*) and yaguapïta (*Puma concolor*) | |
| Grease | Muscular pain |
| – | Colds |
| tatuakuti (*Dasypus novemcinctus*) | |
| Grease | Diarrhoea |
| taturapua (*Tolypeutes matacus*) | |
| Grease | Bronchitis |
| – | Pain in the ribs |
| Shell | Cough, bronchitis |
| – | Wounds |
| tatuaguaju (*Euphractus sexcinctus*) | |
| Grease | Snake bite |
| – | Asthma |
| – | Cough |
| – | Colds |
| – | Eye irritation |
| kua (*Coendou prehensilis*) | |
| Grease | Rheumatic pain |
| aguara (*Pseudalopex gymnocercus*) | |
| Grease | Paralysis |
| Flesh | Heart attack |
| anguyatutu (*Ctenomys* sp.) | |
| Grease | Whooping cough |
| – | Bronchitis |
| Hair (as tea) | Whooping cough |
| Teeth in powder | For extraction of spines in skin |
| **Birds** | |
| äräkuä (*Ortalis canicolis*) | |
| Grease | Muscular pain |
| – | Teeth pain |
| pato negro (*Cairina moschata*) | |
| Grease | Sunstroke |
| guaïjaïja (*Amazoneta brasiliensis*) | |
| Grease | Eye irritation |
| yandu (*Rhea americana*) | |
| Egg shell | Haemorrhages |
| Feathers | Haemorrhages |
| Gizzard | Ulcers in stomach |
| Gizzard | Stone in vesicle |
| Gizzard | Muscular pain |
| Grease | Snake bite |
| – | For extraction of spines in skin |
| tüka (*Ramphastos toco*) | |
| Unspecified part | Haemorrhages |
| **Reptiles** | |
| mboitini (*Crotalus durissus*) | |
| Flesh | Snake bite |
| Rattle | Snake bite |
| Flesh | To improve sight |
| Grease | To improve sight |
| teyuguajupïta (*Tupinambus rufescens*) | |
| Skin from tail | Tooth pain |
| – | Tooth pain |
| – | Appendicitis |
| – | Ear pain |
| – | Back pain |
| – | Snake bite |
| – | Eye irritation |
| karumbe (*Chelonoides carbonaria*) and karumbepërëre (*Chelonoides chilensis*) | |
| Grease | Eye pain |
| – | Spots on skin |
| Shell | Vesicle |
| Gall | Varicose veins |
| kotokoto (*Bufo paracnemis*) | |
| Live animal | Erysipelas |
| Unspecified part | Broken limb |
| Fishes (all species) | |
| Grease | Dermatosis |
| – | Burns |
| – | Swelling |
| – | Inflammation |
| – | Snake bite |
| – | Bronchitis |

turtle grease, duck and lizard grease), followed by respiratory disease (20 species) including cough, whooping cough ( 5), serious lung ailment with high fever (3) and other respiratory diseases (8).

Respiratory problems are also treated with the help of some animal product like tapir or puma grease, applied externally, or administered internally, grated armadillos shell and grease and fox grease (our observations and Cuéllar, 2000).

A quite high number of species (17) was designated as useful in case of haemorrhages, (nasal or digestive), and 10 species were also indicated for uterine haemorrhage occurring during pregnancy, metrorrhagias and menorrhagias. From this batch of species, four (*Castelca coccinea*, *Maytenus scutioides*, *Ruellia ciliatiflora*, *Schinopsis cornuta*) are used whatever the kind of haemorrhage succeeding. Nasal or digestive haemorrhage are always considered seriously, as they can result from the introduction of *Yzi* in concerned organs. In minor cases, haemorrhage is associated with sunstroke or physical stroke. Haemorrhages can also be treated by ingestion of a tea from avestrus or toucan feather or egg shell powder.

Chagas disease, despite a high prevalence in this zone (Noireau et al., 2000), is mentioned just once, maybe be-

cause it is quite asymptomatic in its chronic form and also because any serious heart illness is always related to the effect of malevolent *Mbaekua*. Nevertheless, "heart problem" related or not with terminal chagas disease can be alleviated by seven species, together with tapir grated stomach stone or nails.

Surprisingly, despite the presence of highly venomous snakes (*Crotalus durissus*, *Micrurus pyrrhocryotus*) dwelling in the arid Chaco, only two plants were mentioned to be used in case of snake bites. Again, other animal recipe are in use, one with the flesh of the same snake itself, chopped and applied tightly on the bite. Other recipes mention the use of the flesh of a lizard or armadillo, avestrus and fishes grease (Cuéllar, 2000 and our observations).

Also, only one plant was mentioned to be used for what is called in the Chaco region "arrebato" (local spanish), though our informants all said that no treatment was available in the hospital. This disease, caused by sudden temperatures changes or the impact of strong emotional ups and downs is characterised by a strong pick of fever, with chills, general itching and weariness.

Finally, it must be advised that during this survey, we never worked with midwives—thus, we collected just one species (a contraceptive) related with reproductive life. Therefore, we do consider our data in this specialised area needs to be completed.

A survey performed by the Swiss Red Cross (settled down in the zone since over 20 years managing a small hospital and two dispensaries) indicated that during the years 1998–1999, acute respiratory problems was the first cause for consultation (72%), followed by diarrhoeas (20%), then scabies (6%), chickenpox, sexual transmitted disease, acute chagas and snake bites. This repartition of uses must be understood as a panorama of confirmed cases, with a priority given to mother and child, thus not reflecting the exact epidemiological situation of the zone. As far as we know, no other specific epidemiological report have been made in the zone.

### 3.2.2. Preparation and posology of remedies

*3.2.2.1. Preparations for internal uses.* Most of the plant administered internally are prepared in form of decoction, infusion or the juice of fresh leaves is extracted, after mashing the plant with some water. Some aromatic flowers (*Pectis odorata*, *Pluchea sagittalis*) are prepared in what is called a "poro" (local spanish name). Poro is a name designating both the recipient and the drink prepared in it. The recipient is a small fruit of a selected variety of *Lagenaria siceraria* (Cucurbitaceae), opened at one end, emptied and left to dry well. Some spoons of purchased "Yerba" (finely chopped dried *Ilex paraguariensis* Aquifoliaceae leaves and stem) are put in the "poro", eventually with some sugar, and completed with warm (not boiling) water. "Poro" drink is the equivalent of coffee or tea drink, and is sipped through a special straw all over the day; so are the "poros" prepared with medicinal plants.

Besides these main preparations, some special tips have been recorded, for example, in antidiarrhoeal preparations, the activity of the remedy can be enforced by putting in the warm medicinal liquid a small piece live of charcoal previously soaked in sugar. For respiratory ailments, we noted the preparation of medicinal syrups, with honey, added not only as a sweetener, but also because of its medicinal activity. It must be pointed out that honey, one of the most appreciated wild forest products, has also many medicinal indications.

*3.2.2.2. Preparations for external uses.* For external uses such as dermatological problems, rheumatic pain and also for some gastro-intestinal problem, poultice is the preferred form of application of plants. They are made from plants or parts of plants, smashed or plain. In one recipe, the watery leaf of *Quiabentia verticillata* is peeled, then applied on the skin, also all Cactaceae are sliced up, then peeled to remove spines and applied without dressing.

Previous to application, leaves can also be warmed up over a fire, then soaked in some oil or animal grease for this last purpose "maize grease" is much valued. "Maize grease" is obtained when preparing the *Kägui* drink for special occasions. *Kägui* fermented drink is made from maize seeds, soaked in water and cooked twice. After the second run, once the preparation has cooled down, a grease film floats. It is kept apart and traditionally added over the *Kägui* glass of important guests in order to honour them. As said before, this product is also claimed to have medicinal properties, and is swallowed pure, or used for massages, soaking of leaves or as an excipient for pomade preparation.

Animal grease is also widely used alone or mixed with fresh plants, in pomades.

Generally speaking, all animal greases are said to be good for inflammation processes, for any kind of fever, but some greases are used for specific purposes and some are more valued than others because they are more difficult to obtain. When a plant is meant to be mixed with some animal grease in order to prepare an ointment, a bunch of leaves is carefully pounded, then mixed and stirred up well with animal grease over a light fire for a while, the resulting mass is filtered through a strainer and when cold kept in a container (this is the way of preparation of all plants listed to be used as "pomade" in Table 1).

As briefly quoted above, animal grease and other products derived from animal sources are very much in use and their properties are various. In the study undertaken by Cuéllar (2000), 23 species of wild animal were identified for medicinal purposes—11 mammals, 5 birds and 5 reptiles—a part from grease, all animals parts can be used, such as hairs, spines, egg shell, nails, feathers, flesh, skin and even live animals such as toads against erysipelas (Table 2).

A notable use of ashes from calcinated plants (*Acacia albicortica*, *Caesalpinia paraguariensis*, etc.) was recorded as useful in wounds and skin infections such as infected or exuding excema, or other dermatosis. Powdered ashes are spread over the skin and are said to induce a quick

"drying" of the skin. Sometimes, for the same results, *Acacia albicortica* papyraceous bark is grated in fine powder and applied directly.

Medicinal soaps are still prepared against scabies and dermatosis, from *Seguiera* sp. and *Enterolobium contortisiliquum* bark, trees are burnt and boiling water is poured over the ashes. Resulting soda is mixed with caw grease, eventually a little of sulphur, and left to dry, then moulded in form of a small ball and kept in maize leaf.

Another recipes for external uses are based on the direct application of sap, juice, latex, on the skin—in one recipe, *Capraria salicifolia* roots are left soaking for a while in water until fermentation occurs, this enhancing dramatically their antisarcotpic activity.

Other notable preparation is the direct inhalation of the smoke of medicinal plants heated over live charcoal recorded for *Clematis denticulata* (against malaria) and *Schinopsis cornuta* (against nasal haemorrhage). Inhalation of steam from medicinal plants thrown in boiling water is also performed in cases of strong fever caused either by malarial attack (*Petiveria alliacea*) or by "arrebato" (*Tecoma stans*).

Finally, baths with medicinal plants seem to be destined for feverish children or those suffering with sunstroke.

The posology of the remedies varies from small cups (for preparation accounted with some toxicity) up to one liter of medicinal beverage prepared in advance, to drink all day long ad libitum when thirsty. "Poro" might be drunk any time of the day. In case of haemorrhages or other serious conditions, small quantities of liquid are administered regularly to the patient until its condition improves. The main notable feature in the administration of isoceño remedies, in fact, is its versatility and plurality—for one species and one use, different preparation modes can be recorded, coupled with different administration ways. Thus, it is quite common that remedies are administered at the same time orally, in form of decoction or infusion, and externally, in form of poultice or pomade, also at the same time, some animal grease will be used for massage or in local application.

## 4. Conclusion

In a survey directed to three isoceño communities, Lowrey (1999) found that in one of the communities, 45% of the interviewed people said they will consult only the *Paye* in case of disease, 24% admitted they will refer to both *Paye* experience and hospital services and 16% said they will only go to the hospital and not seek for *Paye* assistance. Also, 16% said that they do prepare traditional remedies and never consult the *Paye*. Therefore there is an average of 89% of the interviewed people using "traditional medicine" in one form or another for healthcare.

Going further in her enquiry, Lowrey found out that the average number of medicinal plants known by the interviewed people was 2.5 *per capita* (medicinal animal products were not included). Twenty six percent of the interviewed said that they did not know a single remedy. Only three people mentioned over six remedies. In the other communities, the number of well known remedies may vary from three (Ibasiriri) to four in Rancho viejo. Also, without surprise, most cited vegetal remedies are from plants living in the domestic space and most of them being also food source—*Ximenia americana*, *Castelca coccinea*, *Aspidosperma quebracho-blanco*, *Celtis* spp., *Zizyphus mistol*, *Ruprechtia triflora*.

Altogether, these preliminary results might seem contradictory—most of the people say they use traditional medicine but when asked about the plant remedies, very few could mention some or some very "ordinary" ones.

Logically, Lowrey's conclusion is that average isoceño men or women have a poor knowledge and poor use of medicinal plants from the wildness (designated as "the forest"), and also of traditional remedies in general. From her point of view, this could be due to many factors—first, the isoceño in its very essence is an agricultor, and perceive the "wildness" as mostly the place of the game, and also, the dwelling place of all bad spirits *Iyareta*. "Forest" is strongly in opposition with cultivated fields. Secondly, massive seasonal migrations in cane field outside the zone occurring since decades could also increase the loss of knowledge on medicinal plants. Finally, concludes Lowrey, it has to be pointed out that the relation of the isoceño with their surrounding ecosystem have been dramatically perturbed since the 19th century, because of the settling down of cattle ranches in the zone, inducing much damage to plants.

Obviously, if we compare the knowledge of "ordinary" isoceño people (a part from few notable exceptions), to the general knowledge of people from an amazonian ethnic group we studied well (Bourdy et al., 2000), we totally agree with Lowrey: plant's name and plant's uses are poorly known. Besides that, indeed, there is a batch of most common remedies (made out from plants growing nearby houses) that are quoted by the majority. In fact, it is the *Paye* and its assistant that have the most complete and intimate knowledge about plant remedies. Our suggestion is that isoceño people strongly believed that the use of the pharmacopoeia or other curative techniques will be of no benefice at all if the sick is not set free from the evil force by the *Paye*. As said before, health recovery is totally dependant upon the *Paye* performance, including diagnostic and extraction of the disease personified by *Yzi*. Therefore, the pharmacopoeia comes in the second place and is not valued for itself—in this therapeutic act, "symbolic effectiveness" primes. In the same way Susnik (1983) quoted "this society is, beyond everything, a paternalist and shamanistic society", we would emphasise that isoceño medicine, is beyond everything, shamansitic.

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# Antidiabetic and antihyperlipidaemic effect of alcoholic *Syzigium cumini* seeds in alloxan induced diabetic albino rats

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**Abstract**

*Syzigium cumini*, commonly known as 'jamun', is widely used in different parts of India for the treatment of diabetes mellitus. The present study was designed to evaluate the antidiabetic and antihyperlipidaemic effect of an alcoholic extract of *Syzigium cumini* seeds (JSEt) in alloxan diabetic rats. Diabetes was induced by single intraperitoneal injection of alloxan (150 mg kg$^{-1}$ body weight). Oral administration of alcoholic JSEt to diabetic rats at a dose of 100 mg kg$^{-1}$ body weight resulted in a significant reduction in blood glucose and urine sugar and lipids in serum and tissues in alloxan diabetic rats. The extract also increases total haemoglobin. The extract brought back all the parameters to normal levels. The effect of alcoholic JSEt was similar to that of insulin. Thus, our investigation clearly shows that alcoholic JSEt has both antidiabetic and antihyperlipidaemic effects.
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*Keywords: Syzigium cumini*; Alloxan diabetes; Seed extract; Antidiabetic; Antihyperlipidaemic

## 1. Introduction

Diabetes mellitus was known to ancient Indian physicians as 'madumeha'. Many herbal products including several metals and minerals have been described for the care of diabetes mellitus in ancient literature (Nadkarni, 1992). Ayurveda has been the first to give an elaborate description of this disease, its clinical features and the patterns and its management by herbal or herbomineral drugs. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Momin, 1987).

The Jamun tree which is native to India, thrives easily in tropical climate and is found in many parts of our sub-continent as well as countries of Asia and Eastern Africa. The Jamun tree is a large evergreen, grown widely in the Indogangetic plains and also in the Cauvery delta of Tamilnadu (Indira and Mohan Ram, 1992). The Jamun belongs to the family Myrtaceae and is botanically identified as *Syzigium cumini* Linn. (Samba-Murthy and Subrahmanyam, 1989). Jamun seeds have been used by natives in the treatment of diabetes (Chopra et al., 1958).

The Jamun seeds have been considered as an indigenous source of medicines with hypoglycaemic (Mahapatra et al., 1985), antipyretic (Ghosh et al., 1985) and antiinflammatory (Chaudhuri et al., 1990) actions. We have studied the hypoglycaemic (Stanely Mainzen Prince et al., 1998a) and antioxidant (Stanely Mainzen Prince and Menon, 1998b) properties of an aqueous extract of *Syzigium cumini* seeds in experimental diabetic rats. In this communication, we made an attempt to evaluate the antidiabetic and antihyperlipidaemic actions of alcoholic extract of Jamun seed extract at different doses on blood glucose, serum and tissue lipids in alloxan diabetic rats.

## 2. Materials and methods

### 2.1. Plant material

*Syzigium cumini* (Jamun) seeds were collected fresh from Nagercoil, Kanyakumari District, Tamil Nadu, India and dried. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (number 367) was deposited in the Botany Department of Annamalai University. The dried seeds were ground by an electrical mill (mesh number 50)

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and the powdered seeds were kept separately in airtight containers in a deep freeze until the time of use.

### 2.2. Preparation of alcoholic Jamun seed extract (JSEt)

The powdered seeds were extracted in a soxlet with 95% ethanol at a temperature of 50 °C for 12 h. The resultant extract was filtered. The filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40 °C. The dried mass was stored in a refrigerator and considered as the extract. The yield of the extract was 4.75% (w/w, in terms of dried starting material).

### 2.3. Preparation of diabetic animals

Rats of original Wistar strain bred in the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University were used in this study. Experiments were carried out in male rats weighing between 130 and 160 g. They were housed (six per cage) in plastic cages (47 cm × 34 cm × 18 cm) lined with husk renewed every 24 h. The rats were fed on pellet diet (Hindustan Lever, India). Drinking water was allowed ad libitum.

Diabetes was induced in the rats by a single intraperitoneal injection of alloxan (150 mg $kg^{-1}$ body weight). Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15–20 ml) intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia (Stanely Mainzen Prince et al., 1998a).

After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycaemia with blood glucose range of 200–270 mg $dl^{-1}$ were used for the study. Blood was collected from the eyes (venous pool).

### 2.4. Experimental design

In the experiment, a total of 36 rats (30 diabetic surviving rats + 6 normal rats) were used. Diabetes was induced in rats, 2 weeks before starting the treatment. The rats were divided into six groups as follows, after the induction of alloxan diabetes and each containing six rats.

Group 1, control rats given 2 ml of saline; Group 2, diabetic rats given 2 ml of saline; Group 3, diabetic rats given alcoholic JSEt (25 mg $kg^{-1}$) suspended in saline daily using an intragastric tube for 42 days; Group 4, diabetic rats given JSEt (50 mg $kg^{-1}$) daily for 42 days; Group 5, diabetic rats given JSEt (100 mg $kg^{-1}$) daily for 42 days; Group 6, diabetic rats given protamine zinc insulin (6 U $kg^{-1}$) intraperitoneally daily for 42 days.

During the second, fourth and sixth week of JSEt treatment, blood glucose and urine sugar of all the rats were determined. Prior to sacrifice, rats were deprived of food for 12 h, but allowed free access to drinking water. After 42 days of treatment, the rats were kept in metabolic cages and urine samples were collected. The urine sugar was estimated by Benedict's method. The rats were then sacrificed by cervical dislocation. Blood was collected in two separate tubes. One tube containing potassium oxalate and sodium fluoride was used for the estimation of glucose. The other tube containing the blood was allowed to clot at room temperature and the serum obtained after centrifugation was used for lipids estimation. Liver, kidney and heart tissues were collected in ice cold containers for lipids estimation.

### 2.5. Estimation of blood glucose and total haemoglobin

Fasting blood glucose and total haemoglobin were estimated by the methods of Sasaki et al. (1972) and Drabkin and Austin (1932), respectively.

### 2.6. Estimation of cholesterol

Cholesterol in serum and tissues were estimated by the method of Zak et al. (1953).

### 2.7. Estimation of phospholipids

Phospholipids in serum and tissues were estimated by the method of Zilversmit and Davies (1950).

### 2.8. Estimation of free fatty acids (FFAs)

Free fatty acids in serum and tissues were estimated by the method of Falholt et al. (1973).

### 2.9. Statistical analysis

All the grouped data were statistically evaluated and the significance of various treatments were calculated using Student's *t*-test. All the results were expressed as mean ± S.D. from six rats in each group.

## 3. Results

The dose-dependent effect of alcoholic JSEt on blood glucose and urine sugar in normal and experimental rats are depicted in Table 1. The blood glucose and urine sugar were significantly elevated in diabetic rats as compared to normal rats. Oral administration of alcoholic JSEt at 25 and 50 mg $kg^{-1}$ body weight significantly lowered the blood glucose and urine sugar as compared to untreated diabetic rats. JSEt at a dose of 100 mg $kg^{-1}$ body weight restored the blood glucose and urine sugar to normal levels. Since 100 mg $kg^{-1}$ body weight of JSEt showed the highest blood glucose lowering effect, we have taken only 100 mg $kg^{-1}$ body weight of JSEt for our further studies.

Table 1
Dose-dependent effect of alcoholic JSEt on blood glucose and urine sugar in diabetic rats

| Group | Blood glucose ($mg\,dl^{-1}$) | | Urine sugar |
|---|---|---|---|
| | Initial | Final | |
| Normal | 76.5 ± 4.3 | 84.3 ± 3.4 | + |
| Diabetic control | 262.4 ± 7.2 | 326.1 ± 6.5## | +++ |
| Diabetic + JSEt (25 mg) | 258.7 ± 5.6 | 206.6 ± 5.9** | ++ |
| Diabetic + JSEt (50 mg) | 264.0 ± 4.6 | 135.3 ± 3.9** | + |
| Diabetic + JSEt (100 mg) | 265.7 ± 3.9 | 85.7 ± 3.6** | − |
| Diabetic + insulin | 268.7 ± 3.8 | 85.6 ± 4.9** | − |

Values are given as mean ± S.D. for six rats in each group.
Diabetic control is compared with normal.
Experimental groups are compared with diabetic control.
(+) Indicates 0.25% sugar and (+++) indicates more than 2% sugar.
Values are statistically significant at $^{**}P < 0.001$ as compared with diabetic control; $^{\#\#}P < 0.001$ as compared with normal.

Table 2
Effect of alcoholic JSEt on total haemoglobin and change in body weight in diabetic rats

| Group | Total Hb ($g\,dl^{-1}$) | Change in body weight (g) |
|---|---|---|
| Normal | 14.6 ± 1.3 | 37.3 ± 1.5 |
| Diabetic control | 10.8 ± 0.8## | −18.9 ± 0.9## |
| Diabetic + JSEt (100 mg) | 14.1 ± 0.9** | 10.5 ± 0.3** |
| Diabetic + insulin | 14.2 ± 1.1** | 10.6 ± 0.4** |

Values are given as mean ± S.D. for six rats in each group.
Diabetic control is compared with normal.
Experimental groups are compared with diabetic control.
Values are statistically significant at $^{**}P < 0.001$ as compared with diabetic control; $^{\#\#}P < 0.001$ as compared with normal.

The levels of total haemoglobin and change in body weight in normal and experimental rats are given in Table 2. The total haemoglobin and body weight were significantly lowered in diabetic rats as compared to normal rats. Oral administration of alcoholic JSEt significantly increased total haemoglobin and body weight as compared to untreated diabetic rats.

The levels of serum and tissue cholesterol, phospholipids and free fatty acids are shown in Table 3. The levels of serum and tissue cholesterol, phospholipids and free fatty acids were significantly higher in diabetic rats as compared to normal rats. Oral administration of alcoholic JSEt lowered the serum and tissue lipid levels as compared to untreated diabetic rats.

JSEt at a dose of 25, 50 and 100 $mg\,kg^{-1}$ showed significant blood glucose lowering effect. JSEt at a dose of 100 $mg\,kg^{-1}$ restored all the parameters to normal levels. The effect of JSEt was similar to that of insulin.

## 4. Discussion

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia

Table 3
Effect of alcoholic JSEt on serum and tissue lipids in diabetic rats

| Group | Serum ($mg\,dl^{-1}$) | | | Liver (mg per 100 g wet tissue) | | | Heart (mg per 100 g wet tissue) | | | Kidney (mg per 100 g wet tissue) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cholesterol | Phospholipids | Free fatty acids | Cholesterol | Phospholipids | Free fatty acids | Cholesterol | Phospholipids | Free fatty acids | Cholesterol | Phospholipids | Free fatty acids |
| Normal | 81.3 ± 1.9 | 102.6 ± 2.6 | 78.6 ± 2.6 | 318.6 ± 17.6 | 1798.4 ± 83.6 | 717.6 ± 33.4 | 170.9 ± 9.3 | 985.4 ± 43.6 | 495.6 ± 23.6 | 504.7 ± 23.7 | 1460.0 ± 71.2 | 732.6 ± 31.9 |
| Diabetic control | 167.0 ± 4.9* | 156.3 ± 2.9* | 173.5 ± 4.5* | 535.6 ± 30.6* | 3412.9 ± 147.4* | 1270.5 ± 60.6* | 360.4 ± 16.9* | 1895.4 ± 90.4* | 1962.7 ± 95.9* | 778.9 ± 41.2* | 3312.4 ± 147.9* | 1702.8 ± 86.6* |
| Diabetic + JSEt (100 mg) | 82.8 ± 1.8** | 103.3 ± 2.1** | 79.6 ± 2.5** | 320.2 ± 18.2** | 1799.6 ± 89.6** | 718.5 ± 35.9** | 171.9 ± 8.7** | 986.6 ± 45.5** | 496.8 ± 25.6** | 506.4 ± 26.9** | 1464.0 ± 73.9** | 733.9 ± 34.8** |
| Diabetic + insulin | 82.6 ± 2.3** | 103.6 ± 2.2** | 79.4 ± 3.5** | 320.1 ± 21.1** | 1799.5 ± 87.6** | 718.8 ± 34.4** | 171.2 ± 8.2** | 986.4 ± 46.2** | 496.9 ± 23.5** | 506.3 ± 28.3** | 1463.3 ± 75.8** | 732.9 ± 32.6** |

Values are given as mean ± S.D. for six rats in each group.
Diabetic control is compared with normal.
Experimental groups are compared with diabetic control.
Values are statistically significant at $^{*}P < 0.001$ as compared with normal; $^{**}P < 0.001$ as compared with diabetic control.

(Chattopadhyay et al., 1997). In our study, we have found that administration of JSEt to diabetic rats reversed their blood glucose which was also reflected in their urine sugar levels. The possible mechanism by which JSEt brings about its hypoglycaemic action may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or its release from the bound form (Stanely Mainzen Prince et al., 1998a).

We have registered a decrease in body weight in alloxan diabetic rats. When JSEt was administered to animals given alloxan, the weight loss was reversed. The ability of JSEt to protect body weight loss seems to be as a result of its ability to reduce hyperglycaemia.

We have also noted lowered levels of total haemoglobin in alloxan diabetic rats. During diabetes, the excess glucose present in the blood reacts with haemoglobin to form glycosylated haemoglobin. So the total haemoglobin level is lowered in alloxan diabetic rats (Sheela and Augusti, 1992). Administration of JSEt reversed the total haemoglobin levels in alloxan diabetic rats.

We have noticed elevated serum lipids in alloxan diabetic rats. Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease (Al-Shamaony et al., 1994). Lowering of serum lipids levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease (Rhoads et al., 1976). The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-Shamaony et al., 1994).

In our study, we have also observed an increase in the concentration of cholesterol, phospholipids and free fatty acids in liver, kidney and heart in alloxan diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus (Sharma et al., 1996), demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids (Chorvathova et al., 1993; Bopanna et al., 1997; Stanely Mainzen Prince et al., 1999). Administration of alcoholic JSEt normalized serum and tissue lipids, secondary to the diabetic state. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose (Krishnakumar et al., 2000). The regression of the diabetic state on JSEt administration increases the utilization of glucose, thereby depressing the mobilization of fat.

In our previous study, we have observed that the aqueous extract of JSEt has hypoglycaemic effect (Stanely Mainzen Prince et al., 1998a). In this communication by using alcoholic JSEt, we have observed antidiabetic and antihyperlipidaemic effects, but the alcoholic extract showed better effect than the aqueous extract. The effect of alcoholic JSEt at a dose of $100\,\mathrm{mg\,kg^{-1}}$ body weight restored all the deranged parameters in alloxan-induced rats to normal levels and its effect is similar to that of insulin.

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# Anti-inflammatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae)

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**Abstract**

An aqueous extract of the aerial part of *Peperomia pellucida* (L.) HBK (Piperaceae) was tested for anti-inflammatory (paw edema induced by carrageenin and arachidonic acid) and analgesic activity (abdominal writhes and hot plate) in rats and mice, respectively. Oral administration of 200 and 400 mg/kg of the aqueous extract exhibited an anti-inflammatory activity in the carrageenin test, which was based on interference with prostaglandin synthesis, as confirmed by the arachidonic acid test. In the abdominal writhing test induced by acetic acid, 400 mg/kg of the plant extract had the highest analgesic activity, whereas in the hot-plate test the best dose was 100 mg/kg. The $LD_{50}$ showed that *Peperomia pellucida* (5000 mg/kg) presented low toxicity.
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*Keywords: Peperomia pellucida* (L.) HBK; Anti-inflammatory effect; Analgesic

## 1. Introduction

*Peperomia pellucida* (L.) HBK is popularly known in northeastern Brazil as "coraçãozinho" (little heart), "língua de sapo" (toad's tongue), "erva-de-vidro" (glass grass), and "erva-de-jaboti" (purpoise grass). It is a herbaceous plant with succulent, alternate oval leaves and inflorescences in terminal spikes, axillary and opposite to the leaves, which grows well in loose and humid soils under the shade of trees.

In folk medicine, this species is used to treat abscesses, boils and skin wounds, and eye inflammation (conjunctivitis) (Bojo et al., 1994). Literature data have confirmed the antimicrobial effect of the species (Bojo et al., 1994), whereas other activities of the species, such as its anti-inflammatory properties, have not been proved. Other medicinal properties attributed to *Peperomia pellucida*, varies depending on the region, are to lower blood cholesterol level (in Northeastern Brazil), against proteinuria, and as a diuretic (in Guyana) (May, 1982).

On this basis, the objective of the present investigation was to study the anti-inflammatory and analgesic effect of an aqueous extract of *Peperomia pellucida*.

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## 2. Materials and methods

*Peperomia pellucida* specimens were collected on the University Campus of the Federal University of Sergipe, Brazil, in May 1998, and the entire aerial part was used to prepare the aqueous extract. A voucher specimen was deposited at the University of Sergipe Herbarium.

### 2.1. Preparation of the aqueous extract

Dried *Peperomia pellucida* aerial parts were ground to a fine powder in blender and extracted with distilled water (3:10, w/v) under continuous shaking for 4 h at 35 °C, and then was filtered (pH 6.0). The filtrate was lyophilized and stored at 5 °C until use. The yield of the aqueous extract was 11.2%. The crude extract was diluted with distilled water just before administration to animals.

### 2.2. Animals

The study used male and female albino Wistar rats weighing 160–240 g and Swiss mice weighing 20–30 g, were supplied by the Central Animal House of the Federal University of Sergipe. The animals received Purina ration and water ad libitum throughout the experiment. The animals submitted

to oral administration of the extracts or drugs were fasted for 16 h before the experiment (water was available).

### 2.3. Anti-inflammatory activity

#### 2.3.1. Carrageenin-induced rat paw edema

The test was used to determine the anti-inflammatory action of the extract by the method of Winter et al. (1962). Groups of 10 animals received by the intragastric route either 10 mg/kg indomethacin (reference drug) or the crude aqueous extract of *Peperomia pellucida* (100, 200, and 400 mg/kg). One hour before they had a subplantar injection into the right hind paw of 0.1 ml/paw of carrageenin solution (200 μg/kg) suspended in distilled water. Paw volume was measured by dislocation of the water column in a plethysmograph (model 7150, Ugo Basile) immediately after carrageenin application (time zero) and 1, 2, 3, and 4 h after the stimulus.

#### 2.3.2. Arachidonic acid-induced rat paw edema

Rat paw edema was induced in six animals by subplantar injection into the right hind paw of 0.1 ml 0.5% arachidonic acid dissolved in carbonate buffer, pH 8.5. Norhydroguaiaretic acid (NDGA, 100 mg/kg) as reference and *Peperomia pellucida* aqueous extract (400 mg/kg) were administered intraperitonally 30 min before arachidonic acid injection. Edema volume was measured by a plethysmography immediately after arachidonic acid injection and at 15 min intervals thereafter for a period of 2 h (DiMartino et al., 1987).

### 2.4. Analgesic activity

Analgesic activity was assessed by abdominal writhing test ($n = 10$) using acetic acid (Koster et al., 1959) and by hot-plate test ($n = 8$) applied to mice of both sexes.

In the writhing test, 0.6% acetic acid was injected intraperitoneally and the number of writhes was counted starting 10 min after injection for a period of 20 min. Indomethacin (10 mg/kg) and morphine (2.5 mg/kg), were used as reference drugs, and the *Peperomia pellucida* extracts (100, 200, and 400 mg/kg) were administered by the intragastric route 1 h before acetic acid injection.

The hot-plate test was performed at a fixed temperature of $55 \pm 0.5\,^{\circ}C$, with a maximum duration estimated at 30 s. Reaction time (paw licking, jumping, etc.) was measured 30, 45, 60, and 90 min after intraperitoneal injection of 10 mg/kg morphine as a reference drug and of *Peperomia pellucida* extracts (100, 200, and 400 mg/kg).

### 2.5. Toxicity ($LD_{50}$)

Acute toxicity was tested in Swiss mice ($n = 10$) of both sexes according to the method of Brito (1994). The animals received a *Peperomia pellucida* extract (5000 mg/kg) and vehicle (water) by the intragastric route and the mortality rate was observed for 48 h, followed by daily weight monitoring for 14 days.

### 2.6. Statistical analysis

The results were reported as means±S.E.M. and analyzed by one-way ANOVA and Student's *t*-test. A value of $P < 0.05$ was considered significant.

## 3. Results and discussion

The aqueous extract of *Peperomia pellucida* presented a dose-dependent anti-inflammatory activity at all concentrations tested, with no significant difference at the concentration of 100 mg/kg 4 h after carrageenin injection (Table 1).

The paw edema induced by carrageenin has been extensively studied in the assessment of the anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins (Vinegar et al., 1987). The fact that the *Peperomia pellucida* extract inhibited edema starting from the first hour and during all phases of inflammation suggests that it probably inhibited different aspects and chemical mediators of inflammation.

The paw edema induced by arachidonic acid is a widely used method for distinguishing between 5-lipooxygenase and cyclooxygenase inhibitors (Griswold et al., 1987). Subplantar injection of arachidonic acid produced significant edema as early as after 15 min and reached a peak at 75 min.

Table 1
Effect of *Peperomia pellucida* aqueous extract (AE) on carrageenin-induced rat paw edema ($n = 8$)

| Treatment (mg/kg) | Mean ± S.E.M. (ml) | | | | Percent edema inhibition[a] |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| Control | 0.28 ± 0.04 | 0.45 ± 0.03 | 0.53 ± 0.02 | 0.39 ± 0.02 | – |
| Indomethacin 10 | 0.13 ± 0.02** | 0.18 ± 0.02** | 0.22 ± 0.02** | 0.16 ± 0.02** | 59.08 |
| AE 100 | 0.17 ± 0.02** | 0.37 ± 0.03* | 0.45 ± 0.03** | 0.36 ± 0.04 | 18.18 |
| AE 200 | 0.13 ± 0.03** | 0.20 ± 0.03** | 0.26 ± 0.03** | 0.25 ± 0.05** | 49.15 |
| AE 400 | 0.09 ± 0.03** | 0.24 ± 0.07** | 0.27 ± 0.07** | 0.24 ± 0.06** | 51.09 |

[a] Percent inhibition of total edema response.
* $P < 0.05$ vs. control.
** $P < 0.01$ vs. control.

Table 2
Effect of *Peperomia pellucida* aqueous extract (AE) on arachidonic acid-induced rat paw edema ($n = 8$)

| Time (min) | Mean ± S.E.M. (ml) | | |
|---|---|---|---|
| | Control | NDGA (100 mg/kg) | AE (400 mg/kg) |
| 15 | 0.30 ± 0.03 | 0.05 ± 0.01** | 0.18 ± 0.02* |
| 30 | 0.48 ± 0.03 | 0.09 ± 0.02 ** | 0.50 ± 0.04 |
| 45 | 0.51 ± 0.04 | 0.17 ± 0.01** | 0.52 ± 0.04 |
| 60 | 0.54 ± 0.04 | 0.21 ± 0.03** | 0.55 ± 0.04 |
| 75 | 0.55 ± 0.04 | 0.21 ± 0.02** | 0.53 ± 0.04 |
| 90 | 0.52 ± 0.04 | 0.21 ± 0.22** | 0.49 ± 0.03 |
| 105 | 0.52 ± 0.04 | 0.21 ± 0.02** | 0.49 ± 0.03 |
| 120 | 0.52 ± 0.04 | 0.21 ± 0.02** | 0.49 ± 0.03 |
| Percent edema inhibition[a] | – | 65.8 | 4.7 |

[a] Percent inhibition of total edema response.
* $P < 0.05$ vs. control.
** $P < 0.01$ vs. control.

Table 3
Analgesic effect of the aqueous extract (AE) of *Peperomia pellucida* on abdominal acetic acid (0.6%)-induced writhes in mice ($n = 8$)

| Treatment (mg/kg) | Number of writhes (mean ± S.E.M.) | Percent inhibition of writhes |
|---|---|---|
| Control | 29.8 ± 4.2 | – |
| Indomethacin 10 | 12.9 ± 1.9** | 56.7 |
| Morphine 2.5 | 6.8 ± 3.2** | 77.2 |
| AE 100 | 23.2 ± 3.2 | 22.1 |
| AE 200 | 21.5 ± 2.3 | 27.8 |
| AE 400 | 14.9 ± 1.1** | 50.1 |

** $P < 0.01$ vs. control.

The aqueous extract of *Peperomia pellucida* did not block edema formation when administered intraperitoneally, but edema was inhibited by NDGA (Table 2). The rat paw edema induced by arachidonic acid is perceptibly reduced by inhibitors of arachidonic acid metabolism and by corticosteroids and is insensitive to selective cyclooxygenase inhibitors (DiMartino et al., 1987). On the basis of the present results, we may propose that the aqueous extract of *Peperomia pellucida* has an anti-inflammatory action interfering with prostaglandin synthesis.

In the acetic acid-induced writhing test, the *Peperomia pellucida* extract demonstrated a significant analgesic effect at 400 mg/kg dose, inhibiting pain by 50.07% compared to control, while at lower doses, 100 and 200 mg/kg, the inhibition was not significant, 22.15 and 27.85%, respectively (Table 3). This result suggests that the analgesic effect of *Peperomia pellucida* is related to the mechanism of prostaglandin synthesis as is the case for the anti-inflammatory process induced by carrageenin, indicating the presence of an inflammatory pain process (Duarte et al., 1988). Using the same test, Aziba et al. (2001) obtained higher inhibition percentages (78.3%) by using methanolic extract of *Peperomia pellucida* (210 mg/kg). These differences can be explained by the use of different extracts, climatic conditions for plant growing and plant origin.

In the hot-plate test, an analgesic effect was observed at concentrations of 100 and 200 mg/kg (Table 4), indicating that the extract possesses an activity related to both inflammatory and non-inflammatory pain.

Animals treated with 5000 mg/kg of the aqueous extract of *Peperomia pellucida* were observed and weighed daily for 14 days and showed no changes in behavior or weight, a fact indicating low toxicity of the extract. According to Lorke (1983), substances are considered to be of low toxicity when the $LD_{50}$ reaches levels above 5000 mg/kg.

## 4. Conclusions

The aqueous extract of *Peperomia pellucida* showed an anti-inflammatory and analgesic activity by the models used

Table 4
Analgesic effect of the aqueous extract (AE) of *Peperomia pellucida on the latency of paw withdrawal* in the hot-plate test in mice ($n = 8$)

| Treatment (mg/kg) | Latency time (s) (mean ± S.E.M.) | | | | Percent inhibition[a] |
|---|---|---|---|---|---|
| | 30 min | 45 min | 60 min | 90 min | |
| Control | 7.06 ± 1.16 | 7.06 ± 1.54 | 6.49 ± 1.28 | 5.55 ± 0.66 | – |
| Morphine 10 | 12.66 ± 1.12* | 11.32 ± 1.17* | 11.88 ± 1.25* | 11.32 ± 0.79* | 80.4 |
| AE 100 | 10.85 ± 0.86* | 10.71 ± 0.55* | 9.01 ± 0.91 | 9.56 ± 0.99* | 53.4 |
| AE 200 | 9.82 ± 0.87 | 9.80 ± 1.24 | 9.15 ± 1.23 | 9.30 ± 0.49* | 48.2 |
| AE 400 | 9.34 ± 1.18 | 7.34 ± 1.54 | 8.30 ± 0.95 | 8.60 ± 0.87* | 28.3 |

[a] Percent inhibition of total hot-plate response.
* $P < 0.05$ vs. control.

in the present study. This fact suggests that the aqueous extract has an anti-inflammatory action interfering with prostaglandin synthesis.

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# Diuretic and nephroprotective effect of Jawarish Zarooni Sada—a polyherbal unani formulation

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**Abstract**

A number of drugs, both single and compound preparations are used widely in Tibb-e-Unani (Unani medicine) in the management of renal diseases. But such drugs mostly, have not been investigated for their described effects. Jawarish Zarooni Sada (JZS) is one such polyherbal preparation containing 15 ingredients, mainly described to be diuretic and nephroprotective. Therefore, in the present study ethanol and water extracts of JZS (300 mg each) were investigated for diuretic activity by measuring the total urine output over a period of 6 h. Sodium and potassium level in urine sample was also estimated. Nephroprotective activity of JZS against gentamicin-induced nephrotoxicity was investigated by administering JZS along with high dose of gentamicin (40 mg/kg) and elevation of serum urea and serum creatinine was taken as the index of nephrotoxicity. JZS showed significant diuretic and nephroprotective effect.
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*Keywords:* Diuretic; Nephroprotective; Nephrotoxicity; Unani medicine

## 1. Introduction

Tibb-e-Unani (Unani medicine) claims to possess many safe and effective drugs, useful in various renal disorders. But most of them despite being widely used in the management of renal ailments have not been scientifically studied for their pharmacological effects. Only few Unani drugs such as Bisahri (*Aerva lanata* Juss.) (Amin et al., 1994), Revand chini (*Rheum emodi*) (Azhar, 2000) and Banadequl Buzoor (a polyherbal composition) (Anwar et al., 1999) have been investigated for their diuretic, nephroprotective and associated effects. Similarly, very few drugs used in various other traditional systems of medicine have been reported to possess such pharmacological effects, which may be used in renal diseases. *Clerodendron trichotomum* Thumb. has been shown to produce diuretic, natriuretic and kaliuretic activity (Lu Wei et al., 1994), quercetin isolated from vegetable sources has been shown to produce protective effect against cisplatin-induced nephrotoxicity (Priya and Devi, 1999) and curcumin isolated from turmeric has been shown to produce protective effect against adriamycin-induced nephrotoxity (Venkatesan et al., 2000). These reports are although, of preliminary nature, however, show great potential of herbal/traditional drugs in offering safe and effective drugs for renal diseases.

Jawarish Zarooni Sada (JZS) is a reputed polyherbal preparation containing 15 ingredients (Table 1). It is a standardized (Afzal et al., 2003) pharmacopoeal preparation that has been described in Unani literature, to be diuretic, tonic to kidney and nephroprotective, useful in burning micturation, nephritis, and nephrotic syndrome like conditions (Anonymous, 1972, 1993; Husain, 1914; Said, 1997). Although, some of its ingredients have been studied for various pharmacological effects, viz. *Apium graveolens*, L. (Singh and Handa, 1995), *Daucus carota*, L. (Bishayee et al., 1995) and *Crocus sativus* (Verma and Bordia, 1998), etc. but neither of these ingredients has been investigated for pharmacological effects associated with kidney diseases nor the compound as a whole investigated scientifically for its reported actions. Therefore, in the present study it was investigated for diuretic, natriuretic and kaliuretic activity. It was also investigated for its nephroprotective activity against gentamicin-induced nephrotoxicity.

## 2. Materials and methods

### 2.1. Preparation of aqueous and ethanol extracts of JZS

All the ingredients of JZS (Table 1) were procured from Dawakhana Tibbiya College, Aligarh Muslim University (AMU), Aligarh. Professor S.H. Afaq (Pharmacognosist), Department of Ilmul Advia, Ajmal Khan Tibbiya College,

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Table 1
Ingredients of JZS

| Unani name | Scientific name | Parts used | Weight (g) |
|---|---|---|---|
| Maghz-Tukhm-Kharpazah | *Cucumis melo* Linn. | Seed | 30 |
| Maghz-Tukhm-Kheyar | *Cucumis sativus* Linn. | Seed | 30 |
| Tukhm-e-Karafs | *Apium graveolens* Linn. | Seed | 30 |
| Post-Beekh-e-Karafs | *Apium graveolens* Linn. | Root bark | 30 |
| Tukhm-e-Gazar | *Daucus carota* Linn. | Seed | 30 |
| Nankhah | *Trachyspermum ami* Sprague. | Seed | 30 |
| Badiyan | *Feniculum vulgare* Mill. | Seed | 30 |
| Qaranfal | *Syzygium aromaticum* Merr and Perry | Flowers and buds | 30 |
| Filfi siyah | *Piper nigrum* Linn. | Fruit | 30 |
| Aqarqarha | *Anacyclus pyrethrum* D.C. | Root | 10 |
| Darchini | *Cinnamomum zeylanicum* Blume. | Stem bark | 10 |
| Mastagi | *Pistacia lentiscus* Linn. | Resin | 10 |
| Zafran | *Crocus sativus* Linn. | Stigma and style | 10 |
| Ood Hindi (Agar) | *Aquilaria agallocha* Roxb. | Wood | 10 |
| Bisbasa | *Myristica fragrans* Houtt. | Aril of fruit | 10 |

AMU, Aligarh confirmed the identity of drugs. The voucher specimens (No. AF/2000/1-15) have been deposited in the museum of the department of Ilmul Advia, A.K. Tibbiya College, AMU, Aligarh.

All the ingredients except saffron and mastic were dried at room temperature and reduced to coarse powder by grinding, while saffron and mastic were powdered in a China clay mortar, separately and then mixed with the other powdered ingredients. Powdered drug was divided into two equal parts (165 g each); one part was immersed in 1.65 l of distilled water while the other in 1.65 l of 70% ethanol, separately, and left for 12 h at room temperature and then extracted for 6 h in soxhlet apparatus. The two filtrates, after filtration through filter paper were concentrated using a hot plate and water bath, respectively. The yield of both the extracts was found to be 1/5 of crude drug (w/w).

### 2.2. Experimental animals

Wistar albino rats of either sex weighing 100–150 g, divided into four groups of six animals each were used. They were maintained on standard diet and water ad libitum unless stated otherwise, and housed in clean polypropylene cages at room temperature (30 ± 2 °C) with a 12-h light:12-h dark cycle.

### 2.3. Treatment schedule

Dose for albino rats was calculated by multiplying the human therapeutic dose, described and practiced in Unani medicine (Kabiruddin, 1978; Anonymous, 1993), by conversion factor of 7 (Freirich, 1968), and the animals were treated as follows.

Group I (control) — Animals in this group were administered with 30 ml/kg of 0.9% normal saline, intragastrically by gastric canula

Group II (Test A) — Concentrated aqueous extract was reconstituted in normal saline (30 mg/3 ml, w/v) and was administered intragastrically in the dose of 300 mg/kg by gastric canula

Group III (Test B) — Ethanol extract was also reconstituted and administered in the same way as aqueous extract

Group IV (control standard) — Animals in this group were treated with some known/standard drug (mentioned with each test)

### 2.4. Experiments

#### 2.4.1. Test for nephroprotective effect

Group I was given vehicle (normal saline) in the dose of 30 ml/kg, twice a day, for 7 days. Groups II and III were given aqueous and ethanol extracts (as mentioned in the treatment schedule) along with gentamicin (GM) in the dose of 40 mg/kg, twice a day for 7 days. The animals in group IV were treated with GM only (40 mg/kg) twice a day for 7 days. GM was administered by intramuscular injection at quarters (Anwar et al., 1999).

On eighth day 12 h after the vehicle/drug administration all the animals were sacrificed by overdosing of anaesthetic ether and blood was collected by cervical decapitation. Serum was separated from the blood and the level of urea and creatinine was estimated by a diagnostic kit from J. Mitra Pvt. Ltd., New Delhi. Elevation of urea and creatinine level in the serum was taken as the index of nephrotoxicity (Anwar et al., 1999; Bennit et al., 1982; Ali et al., 2001).

#### 2.4.2. Test for diuretic activity

Group I served as plain control and administered with vehicle, groups II and III were given single dose aqueous and ethanol extract, respectively (in the same way as in previous test), while the animals in group IV were treated with furosemide in the dose of 4 mg/kg, dissolved in normal saline

and administered intragastrically by gastric canula. Food and water were withdrawn 8 h before the administration of drug (Taylor and Topliss, 1962; Amin et al., 1994).

Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by the animals over a period of 6 h was collected in a jar. Total urine output was measured and concentration of sodium and potassium was determined by 'flame photometer.'

### 2.5. Statistical analysis

The results were given as mean ± S.E.M. Significance was determined by using the Student's *t*-test. *P*-value equal to or less than 0.05 showed significance.

## 3. Results

### 3.1. Nephroprotective effect

Rats treated with GM only (group IV) showed a significant ($P < 0.001$) increase in two serum markers of the kidney function, viz. serum urea and serum creatinine, when compared with control group, showing strong nephrotoxic effect. While the groups (II and III) of animals received aqueous and ethanol extracts along with GM produced a significant ($P < 0.01$) decrease in the level of serum markers of the kidney function when compared with group IV, demonstrating significant nephroprotective effect (Table 2).

### 3.2. Diuretic effect

#### 3.2.1. Total urine output

Both aqueous and ethanol extracts of JZS at a dose of 300 mg/kg induced significant ($P < 0.01$) increase in urine volume, as compared to control group. The diuresis was almost equal to that induced by furosemide (Table 3).

#### 3.2.2. Electrolytes

*3.2.2.1. Urinary sodium.* Both aqueous and ethanol extracts were found to produce significantly ($P < 0.001$) increased natriuresis. Furosemide-treated animals also produced significant ($P < 0.01$) increase in natriuresis, but the maximum effect was produced by aqueous extract (Table 3).

*3.2.2.2. Urinary potassium.* Both aqueous and ethanol extracts have been shown to increase the excretion of the potassium in urine, significantly ($P < 0.001$ and $P < 0.01$, respectively) as compared to the control group. Furosemide also increased the excretion of potassium significantly, however, amount of potassium excreted by it was found to be little less as compared to the excretion exhibited by aqueous extract (Table 3).

## 4. Discussion

This study shows that JZS produced striking increase in total urine output over a period of 6 h. It also increased the

Table 2
Effect of JZS on serum creatinine and serum urea

| Groups | Serum creatinine (mg/dl) (mean ± S.E.M.) | Serum urea (mg/dl) (mean ± S.E.M.) |
|---|---|---|
| Group 1 (control) | 0.938 ± 0.289 | 35.69 ± 1.204 |
| Group II (treated with aqueous extract) | 1.811 ± 0.273* | 72.46 ± 6.08** |
| Group III (treated with alcoholic extract) | 1.87 ± 0.177* | 63.86 ± 2.63* |
| Group IV (treated with gentamicin) | 3.375 ± 0.395** | 94.18 ± 7.18** |

$n = 6$.
* $P < 0.01$.
** $P < 0.001$.

Table 3
Effect of JZS on urine volume and sodium and potassium excretion

| Groups | Urine volume (ml) (mean ± S.E.M.) | Sodium excretion (ppm) (mean ± S.E.M.) | Potassium excretion (ppm) (mean ± S.E.M.) |
|---|---|---|---|
| Group 1 (control) | 0.808 ± 0.085 | 1908.33 ± 24.46 | 478.33 ± 15.88 |
| Group II (treated with aqueous extract) | 3.06 ± 0.17** | 2268.33 ± 24.20** | 691.66 ± 19.12** |
| Group III (treated with alcoholic extract) | 3.08 ± 0.15** | 2240.00 ± 19.72* | 680.83 ± 19.30* |
| Group IV (treated with furosemide) | 3.80 ± 0.52** | 2246.66 ± 31.94** | 678.33 ± 24.87* |

$n = 6$.
* $P < 0.01$.
** $P < 0.001$.

excretion of sodium and potassium significantly. Therefore, JZS has been shown to possess significant diuretic, natriuretic, and kaliuretic effects which may be one of the basis of its therapeutic application in various renal ailments, such as nephritis, burning micturation, etc. and different oedematous diseases. Its diuretic effect has been shown to be more or less equal to that produced by furosemide. Test drug has also been shown to produce remarkable nephroprotective effect against GM-induced nephrotoxicity. Elevation of serum creatinine and serum urea has been considered as the most important manifestation of severe tubular necrosis of kidney (Gilman et al., 1992; Bennit et al., 1982; Ali et al., 2001). GM has been reported to produce nephrotoxicity even at normal therapeutic dose level (Smith et al., 1977, 1980) mainly because it accumulate in the proximal tubular cells and cause local necrosis (Lietman and Smith, 1983; Bennit et al., 1982) and aggravate the toxicity further at high dose level (Aronoff et al., 1983). But concomitant administration of the test drug along with high dose of GM did not produce nephrotoxicity at all, as the serum urea and serum creatinine level has been found in the normal limit. It clearly, indicated that the test drug protected the kidney from the toxic effect of gentamicin.

Further, significant elevation of serum urea and serum creatinine in group of rats treated with GM only, demonstrated a severe toxicity as compared to control group, which is an indication of severe tubular necrosis. Having protected the kidney at this dose, test drug has been shown to possess striking nephroprotective effect. The nephroprotective effect of JZS may be attributed to the ability of the test drug to facilitate the excretion of toxic substances along with increased diuresis. But, since JZS did not allow two important serum markers of kidney function, to elevate significantly above the normal level it therefore, may be inferred that the test drug has strong protective effect against aversive effect of GM.

Diuretics in general are reported to augment the toxicity of gentamicin hence, causing severe tubular necrosis on simultaneous administration (Mitchell et al., 1977). JZS in spite of being a potent diuretic not only did not increase the degree of toxicity but also protected the kidney even from the minimum toxic effect that was likely to arise after the administration of GM. This further indicates its striking nephroprotective effect.

Thus, the findings of present study validate the claim and the age-old use of Jawarish Zarooni Sada in Unani system of medicine for the management of renal diseases.

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# Inductive effect of the leaf mixture extract of *Aloe buettneri*, *Justicia insularis*, *Dicliptera verticillata* and *Hibiscus macranthus* on in vitro production of estradiol

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**Abstract**

In the course of a preliminary clarification of the mechanisms of the leaf mixture extract of *Aloe buettneri*, *Justicia insularis*, *Dicliptera verticillata* and *Hibiscus macranthus*, locally used to regulate the menstrual cycle and to treat dysmenorrhea or cases of infertility in women, pieces of proestrus rat ovary were incubated in the presence of increasing concentration of the plant extract and/or human Chorionic Gonadotropin (hCG). The in vitro production of estradiol and progesterone by ovarian cells of proestrus rat was significantly increased in the presence of various concentration of hCG ($P < 0.05$). The different concentration of the plant extract increased the production of estradiol by twofold. In addition, the in vitro production of estradiol by ovarian cells increased by 13-fold when they were incubated with hCG (0.1 IU/ml) and a concentration of 130 μg/ml of the plant extract. These results clearly attest the direct effects of some chemical components of the leaf mixture of the plants on ovarian steroidogenesis.
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*Keywords:* *Aloe buettneri*; *Justicia insularis*; *Dicliptera verticillata*; *Hibiscus macranthus*; Estradiol; In vitro; Female rats

## 1. Introduction

*Aloe buettneri* (Liliaceae), *Dicliptera verticillata* (Acanthaceae) and *Justicia insularis* (Acanthaceae) are herbaceous plants highly encountered in African tropic (Adjanohoun et al., 1989). Ethnopharmacological studies have demonstrated the implication of these medicinal plants, alone or in association, in reducing labor pains (Berhaut, 1971) and curing disturbed menstrual functions and functional sterility (Bhaduri et al., 1968; Gupta, 1972; Telefo et al., 1998).

During studies on the biochemical and physiological changes in some parameters of reproduction of immature rats treated for 20 days with various doses of an aqueous extract of *Aloe buettneri*, *Justicia insularis*, *Dicliptera verticillata* and *Hibiscus macranthus*; significant increase in uterine and ovarian weight as well as serum estradiol were obtained (Telefo et al., 1998). These results were a clear indication of the presence of estrogenic compounds in the plant extracts.

Many plants derived compounds (flavonoids, lignans, coumestans) have been proven to mimic the biologic effects of endogenous hormones by binding to their nuclear receptor or regulating the activities of key enzymes of their metabolisms: cytochrome P450 aromatase; 17β-hydroxysteroid deshydrogenase (Kurzer and Xu, 1997). The estrogenic effects of some of these compounds are often related to the stimulation of the hypothalamus–pituitary complex with the resulting increase in Follicle Stimulating Hormone (FSH), which will thereafter induce ovarian steroidogenesis and estradiol synthesis (Bep, 1986).

Whether the estrogenic potential of the aqueous mixture of plants obtained in our previous work was limited to a direct activity of its "estrogen-like" compounds on their target organs or to an indirect effect through stimulation of FSH production or through the activity of "FSH-like" compounds in the extract of plants is unknown. As flavanoids with proven estrogenic potential have been also reported to inhibit aromatase activity in various tissue (Pelissero et al., 1996; Ibrahim and Abul-Hajj, 1990), the inductive (FSH-like) or

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inhibitory (estrogen-like) effect of the plant extract constituents on in vitro production of estradiol will help in obtaining preliminary explanation on the site of action of the extract of plants.

## 2. Methodology

### 2.1. Preparation of Krebs–Henseleit solution

This solution was prepared as described by Benford and Hubard (1987). The following reagents were used: $CaCl_2$ (3.2 mM), $NaHCO_3$ (24 mM), KCl (4.8 mM), $MgSO_4$,7·$H_2O$ (1.2 mM), $KH_2PO_4$ (1 mM), HEPES (2.5 mM), glucose (SIGMA: 1 g/l), penicillin (SIGMA: 500 U/ml) and streptomycin (SIGMA: 50 μg/ml).

### 2.2. Preparation of the extract

The fresh leaves of the four medicinal plants *Aloe buettneri* A. Berger (Liliaceae), *Justicia insularis* T. Anders (Acanthaceae), *Dicliptera verticillata* G.J.H. Amshoff (Acanthaceae) and *Hibiscus macranthus* Hochst ex A. Rich (Malvaceae) were collected in August 1994 in Batoufam village (western province of Cameroon) and identified in the National Herbarium (IRAD, Cameroon), respectively, under voucher specimen numbers 52232, 34997, 20387 and 41881. They were mixed as described previously (Telefo et al., 1998), washed and dried at 50 °C in a ventilated oven for 48 h. The dried mixture was ground in a mortar and 3.6 g of the powder was used to prepare the initial extract by infusion, using 1 l of distilled water for 30 min. The concentration of the initial extract (1.3 mg/ml) was computed after its lyophilization. The different working concentrations of the plant extract (13, 26, 52, 130 μg/ml) were obtained by diluting the initial extract at 1/100, 1/50, 1/25 and 1/10, respectively.

### 2.3. Phytochemical tests

Phytochemical tests for alkaloids, flavonoids, glycosides, coumarins and quinones were carried out on the dried powdered leaves of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis* (Table 1). The detection procedure and solvent systems used were as described by Wagner and Bladt (1996).

### 2.4. Experimental design

The experiments were conducted on pieces of ovary weighing 25 mg. The ovaries were obtained from adult rats (3–4 months old) at the proestrus stage of their cycle and which have initially presented three consecutive or regular 4-day cycles.

During the first phase of the experiment, pieces of ovary were distributed in culture tubes placed on ice and containing 500 μl of Krebs solution and increasing concentration of either human Chorionic Gonadotropin (hCG; 0, 0.1, 1, 10 IU/ml) or plant extracts (0, 13, 26, 52, 130 μg/ml). Three culture tubes were used in each concentration of hCG and plant extract. The tubes were then incubated at 37 °C during 2 h in an atmosphere of 5% $CO_2$/95% $O_2$. Incubation was stopped by freezing the tubes on carbon ice. The tube content was then homogenized and steroid hormones (estradiol, progesterone) extracted and measured using $^3$H-RIA kit as described by a World Health Organization Technical Manual (WHO, 1990).

During the second phase of the study, pieces of ovary were incubated in culture tubes containing the Krebs medium, the extract of plants concentrated at 130 μg/ml and the same increasing concentration of hCG. The next steps of the experiment were conducted as described above.

### 2.5. Statistical analysis

All the data were analyzed using Khruskal–Wallis test for their normal distribution. Mann–Withney test was then used for comparison between means (Schwartz, 1991).

## 3. Results

### 3.1. Effects of various concentration of hCG or extract of plants on the in vitro production of estradiol and progesterone

The in vitro production of progesterone and estradiol by pieces of proestrus rat ovary incubated during 2 h in the presence of increasing amounts of hCG are shown in

Table 1
Preliminary phytochemical analysis of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis*[a]

| Medicinal plants | Chemical constituents | | | | |
|---|---|---|---|---|---|
| | Alkaloids | Coumarins | Glycosides | Flavonoids | Quinones |
| *Aloe buettneri* | − | Traces | + | − | + |
| *Dicliptera verticillata* | + | + | + | + | − |
| *Hibiscus macranthus* | + | + | + | + | Traces |
| *Justicia insularis* | + | − | + | + | − |

[a] (+) present; (−) absent.

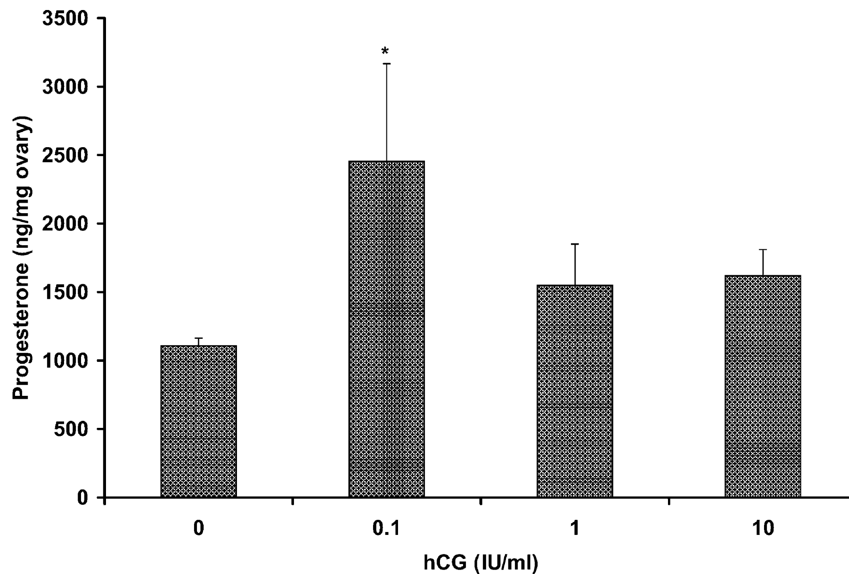


Fig. 1. Production of progesterone in Krebs–Henseleit medium by ovarian slices from proestrus adult rat. The ovarian slices were incubated during 2 h in the absence (control) or presence of increasing doses of hCG. Each bar represents the mean ± S.D. of three determinations. Value statistically different from that of the control: $^{*}P < 0.05$ (Mann–Whitney test).

Figs. 1 and 2, respectively. A significant amount of progesterone was produced by the ovarian cells when incubated with 0.1 IU/ml hCG ($P < 0.05$). At higher concentrations of hCG (1, 10 IU/ml), progesterone secretion was induced at a lesser extend compare to that with the lower concentration. The production of estradiol increased by threefold compare to that of the medium without hCG and whatever the concentration of hCG used.

In the presence of the different dilution of the extract of plants, no significant inductive effect on the production of progesterone was noticed at lower concentrations. However, an increase of 39% compare to the amount of progesterone secreted in the control medium was observed at the highest concentration of the plant extract (Fig. 3). The different concentration of the extract of plants significantly increased the production of estradiol (Fig. 4). At concentrations of 13 and 130 μg/ml of plant extract, the production of estradiol was respectively tripled and doubled compare to that from the medium without the extract of plants.

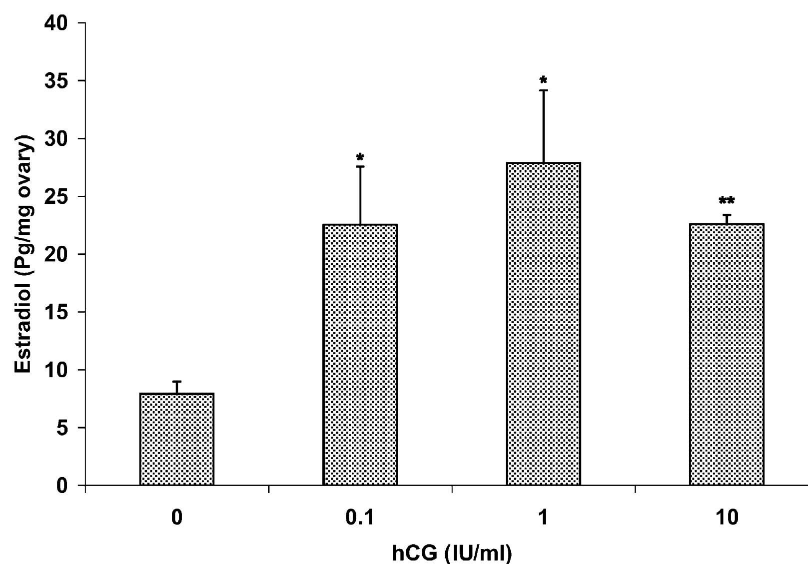


Fig. 2. Production of estradiol in Krebs–Henseleit medium by ovarian slices from proestrus adult rat. The ovarian slices were incubated during 2 h in the absence (control) or presence of increasing doses of hCG. Each bar represents the mean ± S.D. of three determinations. Value statistically different from that of the control: $^{*}P < 0.05$, $^{**}P < 0.01$ (Mann–Whitney test).

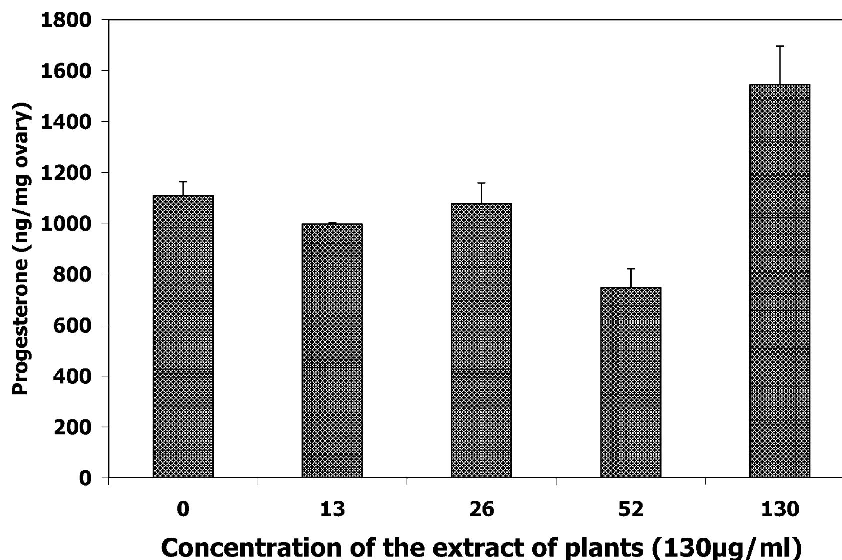


Fig. 3. Production of progesterone by ovarian slices of proestrus adult rat incubated during 2 h in Krebs–Henseleit medium containing increasing concentration of the aqueous extract of plants. Each bar represents the mean ± S.D. of three determinations.

### 3.2. *Combined effect of a concentration of 130 μg/ml plant extract and various concentration of hCG on in vitro production of estradiol and progesterone*

Incubation of ovarian cells obtained from rats at the proestrus stage of their cycle with the extract of plants (130 μg/ml) and increasing amounts of hCG resulted in no significant variation in the production of progesterone compare to the medium containing only the extract of plants (130 μg/ml) and no hCG. The presence of the extract rather resulted in a decrease of 27 and 60% of the amount of progesterone produced compare to that produced in medium containing only 0.1 and 1 IU/ml hCG, respectively (Fig. 5).

A significant inductive effect of the plant extract (130 μg/ml) on the production of estradiol, when incubated in the presence of 0.1 IU/ml hCG, was observed ($P < 0.001$). The amount of estradiol produced in this incubating condition was five times higher than that resulting from the medium containing only the extract of plants (130 μg/ml) or hCG (0.1 IU/ml). With higher concentration of hCG (1, 10 IU/ml), no variation in the stimulation of estradiol production by the extract of plants (130 μg/ml) was noticed (Fig. 6).

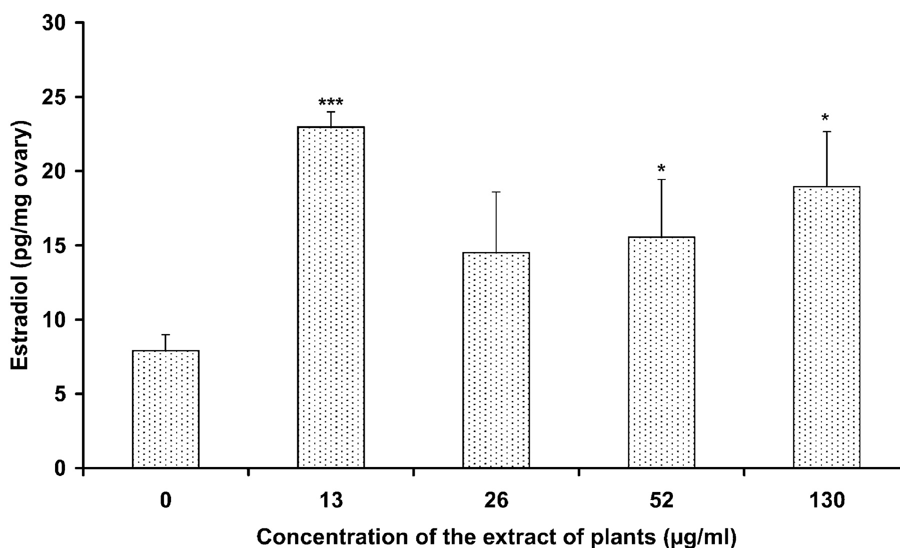


Fig. 4. Production of estradiol by ovarian slices of proestrus adult rat incubated during 2 h in Krebs–Henseleit medium containing increasing concentration of the aqueous extract of plants. Each bar represents the mean ± S.D. of three determinations. Value statistically different from that of the control: $^{*}P < 0.05$, $^{***}P < 0.001$ (Mann–Whitney test).

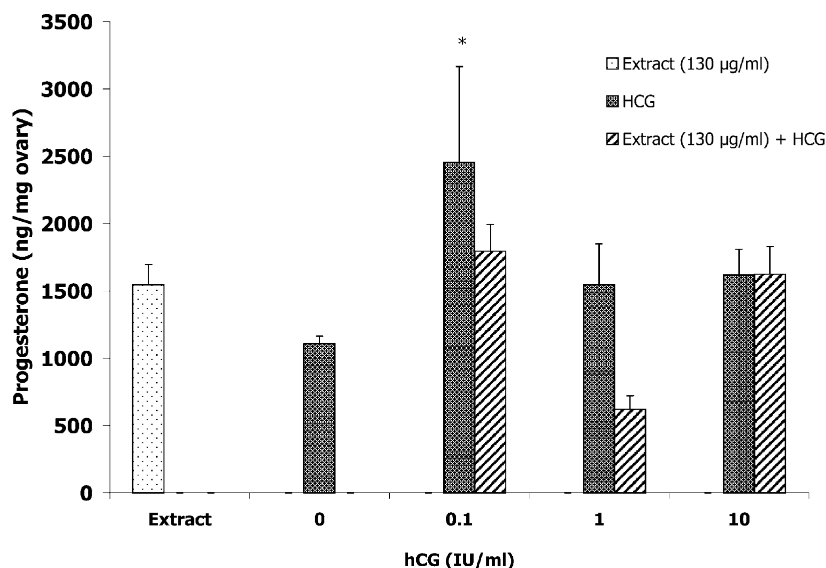


Fig. 5. Combined effect of the aqueous extract of plants (130 µg/ml) and increasing concentration of hCG on in vitro production of progesterone by ovarian slices of proestrus adult rat. Each bar represents the mean ± S.D. of three determinations. Value statistically different from that of the control: $^{*}P < 0.05$ (Mann–Whitney test).

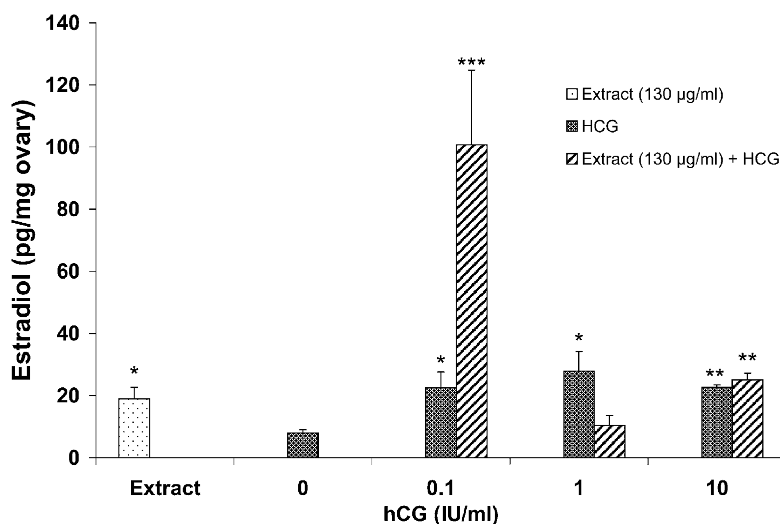


Fig. 6. Combined effect of the aqueous extract of plants (130 µg/ml) and increasing concentration of hCG on in vitro production of estradiol by ovarian slices of proestrus adult rat. Each bar represents the mean ± S.D. of three determinations. Value statistically different from that of the control: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Mann–Whitney test).

## 4. Discussion and conclusion

The present results show the inducing effect of increasing levels of hCG and of the extract of plants (alone or in combination with hCG) on in vitro production of estradiol by adult rat preovulatory follicles. The stimulating effect of hCG was observed on both progesterone and estradiol production.

The hCG is a glycoprotein hormone composed of two non-identical subunits α and β. Its structural analogy to LH has for long been established (Barkley and Zelinski-wooten, 1999). In binding to their common and specific LH/hCG receptor on luteal and theca interstitial cells, hCG molecules will stimulate the cholesterol side-chain cleavage and the activity of 17α-hydroxylase/$C_{17-20}$ lyase system. These enzymatic activations could justify the high amount of progesterone and estradiol secreted by preovulatory follicles in the presence of increasing amount of hCG. Similar inducing effects of hCG on in vitro synthesis of estradiol and progesterone production have been obtained with immature rat granulosa cells (Overes et al., 1992).

In the presence of increasing concentration of the plant extract, significant increases in estradiol secretion were obtained whatever the plant extract dilution used. This observation may result from the direct inducing effect of some chemical compounds in the extract of plants on the synthesis

of estradiol. This direct inducing effect of the compounds on estradiol synthesis was more effective when the plant extract (130 μg/ml) was combined to hCG (0.1 IU/ml) during the 2 h of incubation. In these conditions, estradiol production increased by 13-fold compare to the medium without hCG and the plant extract and by fivefold compare to the medium containing only the plant extract (130 μg/ml) or hCG (0.1 IU/ml) (Fig. 6). The presence of hCG in the medium has increased the amount of androgens (androstenedione, testosterone) which in turn were metabolized by aromatase into estradiol (Erickson, 1993; Hillier et al., 1994). However, the combined use of high concentration of hCG (1 or 10 IU/ml) and the plant extract (130 μg/ml) did not produce any inducing effect on estradiol synthesis. This may result to the loss of specific LH/hCG receptors on preovulatory follicles at these concentrations of hCG.

Modulation by estradiol of progesterone synthesis in defined human luteal cells have been demonstrated (Vega et al., 1994; Fish et al., 1994). The data of both studies indicate direct inhibition of 3β-hydroxysteroid deshydrogenase activity by estradiol. The high amount of estradiol produced when the preovulatory follicles were incubated with various concentration of the plant extract (alone or combine with hCG) may have also resulted in the inhibition of progesterone synthesis (Figs. 3 and 5).

Results so far obtained attest the steroidogenic potential of the extract of plants and precisely its direct effect on estradiol synthesis. The high amount of estradiol produced when the preovulatory follicles were incubated in the presence of the extract of plants (130 μg/ml) and hCG (0.1 IU/ml) may be linked to the activation of aromatase by some chemical compounds of the plants.

FSH is the most effective inducing compound of aromatase activity (Hillier et al., 1994; Smyth et al., 1995). Phytochemical analysis of each plants used in the leaf mixture extract preparation indicated the presence of flavanoids, glycosides, alkaloids, etc. As far as the inhibitory effect of flavonoids on in vitro production of estradiol is clearly established (Pelissero et al., 1996; Kao et al., 1998); the above observation may likely result to the action of "FSH-like" compounds of the extract of plants.

However, future work on in vitro effects of the extract of combined or separated plants on aromatase activity; FSH, LH and estradiol ovarian receptors as well as tests for estrogenicity and anti-estrogenicity will help in clarifying its mechanism of action.

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# Cholesterol lowering effect of SG-GN3, the extract of salted and fermented small shrimps, *Acetes japonicus*, in Triton WR-1339 or high cholesterol-diet induced hypercholesterolemic rats

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**Abstract**

The cholesterol lowering effect of SG-GN3, the extract of salted and fermented small shrimps, *Acetes japonicus*, was investigated in hypercholesterolemic animal models. Hypercholesterolemia was induced with Triton WR-1339 (nonionic detergent) or high cholesterol (HC)-diet. SG-GN3 significantly decreased total cholesterol (TC) in Triton WR-1339 model at 30 post-treatment hour (549.80 ± 152.46 mg/dl) compared to the control which induced by only Triton WR-1339 (798.84 ± 94.98 mg/dl), whereas high-density lipoprotein (HDL) content did not decrease ($P < 0.05$). In HC-diet model, TC content significantly decreased by SG-GN3 treatment at 3 post-treatment day ($P < 0.05$). These results suggest that SG-GN3 effectively decreased serum TC level in hypercholesterolemic animal models.
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*Keywords:* SG-GN3; *Acetes japonicus*; Hypercholesterolemia; Total cholesterol (TC); High-density lipoprotein (HDL)

## 1. Introduction

Salted and fermented small shrimps are most commonly used in Kimchi (Korean fermented vegetable product) (Cheigh and Park, 1994), an important sp. food of the Korean food culture. A recent study revealed that the serum cholesterol concentration was significantly lower in mice fed shrimp and squid with 0.1% cholesterol diet when compared with that in mice fed a commercial diet (Tanaka et al., 1998). The shrimps, *Acetes japonicus*, used for salted and fermented small shrimps are mainly caught in the Yellow Sea in February, May, June, and October. They are abundant in nitrogen sources including protein and amino acids, which accelerate the fermentation process; in particular, nitrogen sources in baby shrimps speed up the fermentation process. In this study we sought to verify the usefulness of the hypercholesterolemia rat models induced by Triton WR-1339 (nonionic detergent) and high cholesterol (HC)-diet, using lovastatin, a well-known cholesterol lowering drug. Secondly, the cholesterol lowering effect (reduction in both plasma triglycerides and cholesterol) of SG-GN3, the extract of salted small shrimps, was evaluated using the same models.

## 2. Materials and methods

### 2.1. Preparation of salted and fermented small shrimps extract

One hundred grams of SG-GN3 (Korean patents No. 2001-37965) extract was obtained by extracting the salted and fermented small shrimps, *Acetes japonicus* (1.5 kg), three times with ethanol (6 l). It was dried up and then suspended in $H_2O$ and centrifugated at $12{,}000 \times g$ for 10 min. The pellets were resuspended in $H_2O$ at 10% (w/v), homogenized using a glass homogenizer (Wheaton, USA), and ultracentrifugated (Bachmann, USA; SW41Ti rotar) at $124{,}100 \times g$ for 2 h. The pellets were used in this study.

### 2.2. Animals and diets

All animal experiments were performed under protocols approved by Institutional Animal Care and Use Committee of Seoul National University. All efforts were made to minimize animal suffering. Sprague–Dawley male rats were

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Table 1
Descriptive data of the number and weight of each group

| | Triton WR-1339 model | | | | HC-diet model | | | |
|---|---|---|---|---|---|---|---|---|
| | Number | W(g)1 | W(g)2 | W(g)3 | Number | W(g)1 | W(g)2 | W(g)3 |
| SG-GN3 | 5 | 80.50 ± 2.92[a] | 117.80 ± 5.69 | 121.20 ± 7.77 | 5 | 54.60 ± 2.77 | 94.90 ± 5.46 | 116.60 ± 5.81 |
| Lovastatin | 5 | 82.90 ± 5.35 | 118.40 ± 5.97 | 121.10 ± 5.80 | 5 | 51.20 ± 5.25 | 90.00 ± 8.21 | 112.20 ± 7.54 |
| Triton/HC-diet (positive control) | 5 | 84.90 ± 1.85 | 125.30 ± 2.59 | 131.20 ± 2.84 | 5 | 49.70 ± 4.78 | 88.50 ± 8.04 | 107.90 ± 8.42 |
| Saline (control) | 5 | 82.10 ± 2.41 | 117.90 ± 4.92 | 134.40 ± 5.98 | 5 | 51.70 ± 4.09 | 87.70 ± 5.25 | 106.60 ± 9.24 |

[a] Values are mean ± S.D. Abbreviations: W(g)1, weight (g) at the acclimation period; W(g)2, weight (g) at the beginning of study period; W(g)3, weight (g) at the end of study period.

purchased from Slc Ltd. (Japan), with mean body weight of 51.8 ± 4.37 g (HC-diet model) and 82.6 ± 3.50 g (Triton WR-1339 model) at the start of the study period. The animals were kept at a constant temperature (20–22 °C) and humidity (50–55%), and were fed with commercial diet (Samyang, Korea). Water was allowed ad libitum.

### 2.3. Inducing hypercholesterolemic rats

#### 2.3.1. Triton WR-1339 model

Twenty rats (5-week-old) were randomly assigned to four groups. After 1 week of acclimation, all groups except the fourth one were injected i.p. with a 10% aqueous solution of Triton WR-1339 (Sigma, USA) at 60 mg/100 g BW Food was withdrawn 10 h prior to the blood sampling just before Triton WR-1339 injection. To the first group, SG-GN3 (0.2 g) in saline (1 ml) was administered by means of a stomach tube 2 h before Triton WR-1339 injection. Using the same method, lovastatin (Sigma; 40 mg/kg BW) was administered to the second group. The third group (Triton positive control) was only administered with Triton WR-1339. The fourth group was injected with only normal saline i.p. Blood was collected from the retro-orbital sinus just before and every 10 h after the administration of Triton WR-1339.

Fig. 1. Total cholesterol (TC) of the rats treated with Triton WR-1339 or HC-diet. Results are expressed as mean ± S.D. $^{*}P < 0.05$, comparison of values at different times with respect to time 0 in the respective groups. (A) TC of Triton-inducing model. $^{a}P < 0.05$, SG-GN3 vs. Triton positive control; $^{b}P < 0.05$, lovastatin vs. Triton positive control. At 30 post-treatment hour. (B) TC of HC-diet-inducing model. Abbreviations: Lov, lovastatin; Triton, Triton WR-1339; HC-diet, high cholesterol-diet; Con, saline control.

#### 2.3.2. High cholesterol (HC)-diet model

Four-week-old rats were given 1.5% cholesterol (Sigma) and 0.5% cholic acid (Sigma) with standard equilibrated diet (Samyang, Korea) for 6 days. Fifteen hypercholesterolemic rats were selected and were separated into three groups. HC-diet was withdrawn 10 h prior to blood sampling on the seventh day of experiment period, at the same time, the first group was administered with SG-GN3 (0.1 g) dissolved in saline (1 ml) by means of a stomach tube. The standard diet was withdrawn 10 h prior to every blood sampling, but water was allowed ad libitum. Using the same method, lovastatin (40 mg/kg BW) was administered to the second group. The third group was HC-diet positive control, and the fourth group was administered with only normal saline. Blood was collected from the retro-orbital sinus once daily after drug administration.

### 2.4. Determination of serum lipoproteins

Serum samples were assayed for total cholesterol, high-density lipoprotein and triglyceride using standard enzymatic assay kits: total cholesterol Kit, HDL-Cholestase Kit, Cleantech TG-S Kit (*Asan Pharmacy* Ltd., Korea).

### 2.5. Statistical analysis of data

Data were expressed as mean ± S.D. Repeated-measures Statistical Analysis System (SAS) was used for statistical analysis of TC, HDL, TC/HDL ratio, TG, and body weight. The significant difference between each group was analyzed through the *Duncan's* multiple range test at a level of $P < 0.05$.

## 3. Results

### 3.1. Clinical signs and weight change

During the experimental period, no peculiar clinical signs nor significant changes in body weight were observed among the groups (Table 1).

### 3.2. Effect of the extract, SG-GN3 on serum TC, HDL and TG

#### 3.2.1. Triton WR-1339 model

Initial values for serum TC were similar among all groups. In the group administered with only Triton WR-1339,

Fig. 2. High-density lipoprotein (HDL) contents of rats treated with Triton WR-1339 or HC-diet. Results are expressed as mean ± S.D. $^{*}P < 0.05$, comparison of values at different times with respect to time 0 in respective groups. (A) HDL of Triton-inducing model. $^{a}P < 0.05$, SG-GN3 vs. Triton positive control. (B) HDL of HC-diet-inducing model. $^{b}P < 0.05$, lovastatin vs. HC-diet positive control. For abbreviations see Fig. 1.

the TC value gradually increased up to 30 post-treatment hour (798.84 ± 94.98 mg/dl). The groups administered with SG-GN3 and lovastatin showed significant decreases in TC compared with the Triton WR-1339 administered group (SG-GN3: 549.80 ± 152.46 mg/dl; lovastatin group: 512.18 ± 42.97 mg/dl) at 30 post-treatment hour (Fig. 1A).

The HDL value increased in SG-GN3 group at 50 post-treatment hour (Triton only: 19.18 ± 2.34 mg/dl; SG-GN3 30.28 ± 4.87 mg/dl) (Fig. 2A). Initial TC/HDL ratio was not different among groups, but significantly decreased in SG-GN3 group, compared with Triton WR-1339-treated group from 30 to 50 post-treatment hours (at 30 post-treatment hour: Triton only TC/HDL ratio: 39.80 ± 7.82; SG-GN3: 28.40 ± 6.17, at 40 post-treatment hour: Triton only: 33.82 ± 4.75; SG-GN3: 21.74 ± 6.15, at 50 post-treatment hour: Triton only: 31.36 ± 5.45; SG-GN3: 18.18 ± 3.77) (Fig. 3A).

TG value increases were too high in the Triton WR-1339 Model to be calculated into the data (the value of TG was over 3000 mg/dl, data not shown).

*3.2.2. HC-diet model*

The TC value of HC-diet treatment group (283.80 ± 147.97 mg/dl) was two times higher than that of the saline-treated control group (106.22 ± 29.72 mg/dl) at 3 post-treatment day. Furthermore, TC values of SG-GN3 (182.22 ± 22.74 mg/dl) and lovastatin groups (220.78 ± 28.46 mg/dl) decreased at 3 post-treatment day (Fig. 1B).

No significant changes were observed in HDL values among all groups (Fig. 2B). Although not significantly, TC/HDL ratio decreased in SG-GN3 and lovastatin groups compared with the control group treated with only HC-diet at 3 post-treatment day (Fig. 3B).

Initial TG values were different among groups; however, from 3 post-treatment day, no significant differences were observed. At 5 post-treatment day, significant increases in TG values were observed in SG-GN3 treatment groups (only HC-diet control: 78.38 ± 5.49 mg/dl versus SG-GN3: 116.63 ± 9.71 mg/dl) (data not shown).

## 4. Discussion

Triton WR-1339 has been reported to block the removal of TG from plasma (Catanozi et al., 2001). Injection of Triton WR-1339 into rabbits resulted in the increased plasma cholesterol and TG concentrations for up to 36–48 h (Mitropoulos et al., 1994). Triton WR-1339 was also reported to interact preferentially with HDL, changing the size and density of lipoprotein. When Triton-treated

Fig. 3. TC/HDL ratio of rats treated with Triton WR-1339 or HC-diet. Results are expressed as mean ± S.D. $^{*}P < 0.05$, comparison of values at different times with respect to time 0 in respective groups. (A) TC/HDL ratio of Triton-inducing model. $^{a}P < 0.05$, SG-GN3 vs. Triton positive control. (B) HDL of HC-diet-inducing model. $^{b}P < 0.05$, lovastatin vs. HC-diet positive control. For abbreviations see Fig. 1.

HDL particles were used as substrates for the enzyme LCAT (Lecitin; cholesterol acyltransferase), enzyme activity decreased in parallel to the displacement of apo A-1 (Yamamoto et al., 1984). In our study, the HDL values decreased slightly in Triton WR-1339-treated group compared with the control group (Fig. 2A). On the other hand, the HDL value of SG-GN3 group significantly increased at 50 post-treatment hour. In other studies, Triton WR-1339 suppressed intravascular lipolysis (Chirieac et al., 2000), and lipoprotein lipase activity was significantly inhibited 2 h after the injection of Triton (Borensztajn et al., 1976). The TG value in our Triton WR-1339 Model greatly increased up to 3000 mg/dl, an indication that TG was not hydrolyzed by the lipoprotein lipase.

TC values increased in Triton WR-1339-treated groups up to 40 post-treatment hour, and decreased thereafter. Therefore, Triton WR-1339 experiment was performed within 40–50 post-treatment hour, as confirmed through the effectiveness of Triton on TC in the course of time.

The TC/HDL ratio is a better indicator of coronary heart disease risk than individual lipoprotein concentration (Stampfer et al., 1991; Kinosian et al., 1995; Kailash, 1999). Therefore, it may be more appropriate to study the effect of cholesterol on the TC/HDL than on individual lipoprotein concentration. In this study, the TC/HDL ratios of SG-GN3-treated groups steeply decreased in both hypercholesterolemic models compared with the TC values of Triton WR-1339 and HC-diet Models at 50 post-treatment hour and 5 post-treatment day, respectively, an indication that the HDL values of SG-GN3-treated groups did not decrease in both hypercholesterolemic models.

Altogether the experiments show that the cholesterol lowering effect of SG-GN3 and it would then provide the opportunity to develop new drugs and food adducts for the prevention and treatment of hypercholesterolemia.

In conclusion, our results confirmed Triton WR-1339 model, except for the high TG values observed and HC-diet model, as effective hypercholesterolemia rat models. Secondly, our investigations demonstrated that SG-GN3, significantly decreased serum TC but did not decrease HDL in Triton WR-1339 model. The serum TC-lowering effect was observed in the HC-diet model. Further studies related to the actions of SG-GN3 on the mechanism may provide fresh insights into new cholesterol lowering materials.

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# Anti-inflammatory activity of methanolic extracts from *Ventilago harmandiana* Pierre

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**Abstract**

Methanolic extracts from the heart wood, stem bark, and stem wood of *Ventilago harmandiana* Pierre (Family Rhamnaceae) were assessed for anti-inflammatory effects using both acute and chronic inflammatory models. Analgesic and antipyretic activities of the extracts were also evaluated. It was found that all extracts possessed strong inhibitory effects on the acute phase of inflammation as seen in ethyl phenylpropiolate (EPP)- and arachidonic acid (AA)-induced ear edema as well as in carrageenin-induced paw edema in rats. The extracts elicited only weak inhibitory activity on cotton pellet-induced granuloma formation, a subchronic inflammatory model. In the analgesic test, all extracts exerted pronounced inhibitory activity in acetic acid-induced writhing response but showed only weak effects in the tail-flick test. The extracts also showed excellent antipyretic activity on yeast-induced hyperthermia in rats.
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*Keywords:* *Ventilago harmandiana* Pierre; Inflammation; Anti-inflammatory; Analgesic and antipyretic activities

## 1. Introduction

Plants of the genus *Ventilago*, e.g. *Ventilago leiocarpa* Bunge, have been used in Taiwan for the treatment of cough, rheumatism, and contused wounds (Hsu, 1972; Kan, 1980). In the development and evaluation of Taiwan folk medicines, a previous study showed that an ethanolic extract from the stem of *Ventilago leiocarpa* possessed anti-inflammatory activity (Lin et al., 1995). *Ventilago harmandiana* is a rare liane species of *Ventilago* found in the forests of Thailand. The water decoction of heart wood and stem bark of the plant are used in Thai traditional medicine for the treatment of diabetes as well as wound and chronic inflammation. Typically, 100 g of the powdered plant material was boiled with 300 ml of water for about 1 h and about 50 ml of the decoction was consumed twice daily. Interestingly, most plants with anti-inflammatory property lack an ulcerogenic effect, which is the most common side effect of nonsteroidal anti-inflammatory drugs (NSAIDs). Some even possess anti-ulcerogenic activity, e.g. *Curcuma longa* Linn. (Merhra et al., 1984), *Garcinia kola* (Bradide, 1993; Ibriroke et al., 1997), *Turnera ulmifolia* (Antonio and Souza Brito, 1998), and *Zingiber officinale* Roscoe (Manonmani et al., 1994; Sharma et al., 1994). It was therefore of interest to investigate the activity of *Ventilago harmandiana* in the hope that it would have anti-inflammatory and related activities without or minimal common side effects of NSIADs, in a similar fashion to other proven anti-inflammatory plants. Only six species of the *Ventilago* plants have been investigated chemically, i.e. *Ventilago bombaiensis*, *Ventilago calyculata*, *Ventilago goughii*, *Ventilago viminalis*, and *Ventilago vitiensis* (Ali et al., 1994).

The purpose of this work was thus to study the anti-inflammatory activity of the extracts of *Ventilago harmandiana* in some inflammatory models. Other related activities, i.e. analgesic and antipyretic effect, were also included.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats weighing 40–60, 100–120, and 200–250 g, and male Swiss albino mice weighing 30–40 g were purchased from the National Laboratory

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Animal Center, Nakorn Pathom. All animals had free access to water and food and were acclimatized at least 1 week before starting the experiments.

### 2.2. Plant material and plant extracts

The heart wood, stem bark, and stem wood of *Ventilago harmandiana* were collected from Pangnga Province of Thailand. A voucher specimen (BKF no. 352013) of *Ventilago harmandiana* has been deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand. The methanol extracts from the heart wood (VHHW; 600 g yields 44 g extract), stem bark (VHBK; 9.7 kg yields 770 g extract), and stem wood (VHSW; 10 kg yields 520 g extract) were prepared by percolation with methanol at room temperature. The solvent was removed under reduced pressure and the residue was lyophilized in each case.

### 2.3. Preparation of test drugs

All test drugs were suspended in 5% Tween 80, except in the ear edema model, where they were dissolved in acetone. All test drugs were intraperitoneally administered in an equivalent volume of 0.25 ml/100 g body weight of the animal. For the ear edema model, a local application of test drug to the ear was performed. Control groups received vehicle only in the same volume and by the same routes.

### 2.4. Ethyl phenylpropiolate (EPP)- and arachidonic acid (AA)-induced ear edema in rats

The methods of Brattsand et al. (1982) and Young et al. (1983) were used. Male rats weighing 40–60 g were used. Ear edema was induced by the topical application of either EPP or AA dissolved in acetone to the inner and outer surfaces of both ears by means of an automatic microliter pipette. Test drugs, were dissolved in acetone and applied topically in a volume of 20 μl to the inner and outer surfaces of the ear just before the irritants. The control group received acetone.

### 2.5. Carrageenin-induced hind paw edema in rats

Male rats of 100–120 g body weight were divided into groups of six animals. Test drugs were given intraperitoneally 1 h prior to carrageenin injection. The control group received 5% Tween 80 only. A volume of 0.05 ml of 1% carrageenin was injected intradermally into the plantar side of the right hind paw of rats by the method of Winter et al. (1962). Foot volumes of animals were determined by means of a volume displacement technique using a plethysmometer.

### 2.6. Cotton pellet-induced granuloma formation in rats

Adsorbent cotton wool was cut into pieces weighing 20 + 1 mg and made up to a pellet. The pellets were then sterilized in a hot air oven at 120 °C for 2 h. Two pellets were implanted subcutaneously, one on each side of the abdomen of the animal under light ether anesthesia and sterile technique according to the method of Swingle and Shideman (1972). Test drugs were administered in a once daily dosage regimen throughout the experimental period of 7 days whereas the control group received 5% Tween 80 only. On the 8th day after implantation, rats were killed by a large dose of pentobarbital sodium. The implanted pellets were dissected out and carefully removed from the surrounding tissues and weighed immediately for the wet weight. The thymus was also dissected out. Both cotton pellets and thymuses were dried at 60 °C for 18 h and their dry weight determined. The change in body weight of the animals from the first and the last day of the experiment was also recorded.

### 2.7. Acetic acid-induced writhing response in mice

Male Swiss albino mice weighing 30–40 g were used. The study was carried out as described by Collier et al. (1968) and Nakamura et al. (1986). A writhing response was produced by injection of an aqueous solution of 0.75% acetic acid in a volume of 0.1 ml/10 g body weight into the peritoneal cavity and the animals were then placed in a transparent plastic box. The number of writhes, a response consisting of contraction of an abdominal wall, pelvic rotation followed by hind limb extension, was counted during continuous observation for 15 min beginning from 5 min after the acetic acid injection. Test drugs and control vehicle were administered 30 min before the acetic acid injection.

### 2.8. Tail-flick test in rats

Male rats weighing 200–250 g were used. The method described by Gray et al. (1970) was used. The rat's tail was placed to cover a flush mounted photocell window of the Tail Flick Apparatus (model Ds-20m, Ugo Basile, Italy). Heat was applied by the infrared lamp (100 W bulb) mounted in a reflector. The light intensity was adjusted to give a normal reaction time of 2–4 s. The timer was activated when the lamp was turned on. When the rat felt pain and flicked its tail, the light fell on the photocell and automatically stopped the timer. The cut-off time of 10 s was the maximum time which an unflicked tail can be exposed to the heat without damage. The control reaction time was first determined. Test drugs were given 30 min before re-exposure to the heat. The analgesia was quantified as the percentage of maximum possible response time.

### 2.9. Antipyretic study

Male rats weighing 200–250 g were used. Initial rectal temperatures were recorded using a 10-channel electric thermometer. Thereafter, hyperthermia was induced in rats according to the method of Teotino et al. (1963) by subcutaneous injection of 1 ml/100 g body weight of 20% yeast

Table 1
Inhibitory effects of extracts from *Ventilago harmandiana* on EPP-induced ear edema

| Drug | Dose (mg/kg) | Edema thickness (μm) | | | | Edema inhibition (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 1 h | 2 h | 15 min | 30 min | 1 h | 2 h |
| Control | – | 223 ± 8 | 306 ± 10 | 320 ± 13 | 330 ± 13 | – | – | – | – |
| Phenylbutazone | 1 | 46 ± 10*** | 86 ± 10*** | 106 ± 10*** | 120 ± 13*** | 79 | 72 | 67 | 64 |
| VHHW | 1 | 100 ± 13*** | 163 ± 15*** | 216 ± 23*** | 230 ± 17*** | 55 | 47 | 33 | 30 |
| | 2 | 73 ± 16*** | 110 ± 11*** | 156 ± 15*** | 190 ± 11*** | 67 | 64 | 51 | 42 |
| | 4 | 56 ± 8*** | 90 ± 11*** | 113 ± 10 | 163 ± 15*** | 75 | 71 | 65 | 51 |
| VHBK | 1 | 153 ± 10*** | 228 ± 22** | 243 ± 15** | 263 ± 15** | 31 | 26 | 24 | 20 |
| | 2 | 100 ± 19*** | 200 ± 13*** | 220 ± 18*** | 246 ± 16*** | 55 | 35 | 31 | 26 |
| | 4 | 73 ± 18*** | 103 ± 15*** | 150 ± 11*** | 206 ± 16*** | 67 | 66 | 53 | 38 |
| VHSW | 1 | 183 ± 23** | 236 ± 23** | 256 ± 23** | 276 ± 15** | 17 | 23 | 20 | 16 |
| | 2 | 140 ± 18*** | 206 ± 10*** | 243 ± 15** | 253 ± 10*** | 37 | 33 | 24 | 23 |
| | 4 | 80 ± 13*** | 173 ± 16*** | 223 ± 20*** | 246 ± 10*** | 64 | 44 | 30 | 25 |

Values are expressed as mean ± S.E.M. ($n = 5$).
Statistically significant from control group: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
VHHW: *Ventilago harmandiana* heart wood, VHBK: *Ventilago harmandiana* bark, VHSW: *Ventilago harmandiana* stem wood.

in NSS. Those animals which showed a rise in rectal temperature of more than 1 °C were used. Test drugs were then administered and the rectal temperatures of animals were recorded at 30-min interval for 2 h following drug treatment.

### 2.10. Statistical analysis

Results were expressed as mean + standard error of mean (S.E.M.). Student's *t*-test was applied to the results to evaluate the significance. *P* values less than 0.05 were considered significant.

### 2.11. Chemicals

Aspirin (Vidhyasom Co., Ltd., Bangkok, Thailand), pentobarbital sodium injection U.S.P. (Nembutal®, Abbott Laboratories, North Chicago, USA), prednisolone (Scherisone®, Schering Bangkok Ltd., Nonthaburi, Thailand), phenidone (Riedel-de Haen AG, D-3016 Seelze 1, Germany), phenylbutazone (Sigma Chemical Company, St. Louis, USA), morphine sulfate injection U.S.P. (The Government Pharmaceutical Organization, Bangkok, Thailand), Lambda carrageenin (Sigma Chemical Company), AA (Sigma Chemical Company), and EPP (Fluka Chemie AG, Switzerland).

## 3. Results and discussion

The three methanolic extracts from *Ventilago harmandiana* possessed varying dose-dependent topical and systemic anti-inflammatory activity in different experimental models of acute and chronic inflammation. Among the three extracts, VHHW from the heart wood exerted strongest inhibitory activity on the EPP- and AA-induced rat ear edema and on carrageenin-induced rat paw edema.

Of the long list of mediators, including histamine, 5-HT, the kinins, complement, etc., the metabolites of AA have

Table 2
Inhibitory effects of extracts from *Ventilago harmandiana* on AA-induced ear edema

| Drug | Dose (mg/kg) | Edema thickness (μm) | | | | Edema inhibition (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 1 h | 2 h | 15 min | 30 min | 1 h | 2 h |
| Control | – | 188 ± 11 | 226 ± 10 | 300 ± 13 | 340 ± 11 | – | – | – | – |
| Phenidone | 1 | 53 ± 10*** | 76 ± 15*** | 113 ± 10*** | 130 ± 11*** | 72 | 66 | 62 | 62 |
| Phenylbutazone | 1 | 177 ± 15 | 223 ± 15 | 300 ± 12 | 336 ± 15 | 6 | 1 | 0 | 1 |
| VHHW | 1 | 116 ± 15*** | 143 ± 8*** | 210 ± 11*** | 280 ± 18** | 38 | 37 | 30 | 18 |
| | 2 | 80 ± 13*** | 116 ± 15*** | 173 ± 16*** | 220 ± 18*** | 57 | 49 | 42 | 35 |
| | 4 | 66 ± 10*** | 93 ± 16*** | 150 ± 11*** | 203 ± 15*** | 65 | 59 | 50 | 40 |
| VHBK | 1 | 130 ± 11*** | 166 ± 10** | 250 ± 11** | 330 ± 9** | 31 | 27 | 17 | 3 |
| | 2 | 90 ± 11*** | 130 ± 11*** | 206 ± 10*** | 233 ± 15*** | 52 | 42 | 31 | 31 |
| | 4 | 76 ± 15*** | 110 ± 11*** | 173 ± 16*** | 223 ± 20*** | 62 | 51 | 42 | 34 |
| VHSW | 1 | 146 ± 21*** | 180 ± 18** | 262 ± 14** | 330 ± 11 | 22 | 20 | 13 | 3 |
| | 2 | 100 ± 18*** | 146 ± 21*** | 223 ± 16** | 260 ± 13*** | 47 | 35 | 26 | 24 |
| | 4 | 83 ± 8*** | 116 ± 8*** | 206 ± 16*** | 233 ± 16*** | 56 | 49 | 31 | 31 |

Values are expressed as mean ± S.E.M. ($n = 5$).
Statistically significant from control group: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
VHHW: *Ventilago harmandiana* heart wood, VHBK: *Ventilago harmandiana* bark, VHSW: *Ventilago harmandiana* stem wood.

Table 3
Effect of test extracts from *Ventilago harmandiana* and aspirin on carrageenin-induced paw edema in rats

| Drug | Dose (mg/kg) | Time after carrageenin injection | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 h | | 3 h | | 5 h | |
| | | EV (ml) | EI (%) | EV (ml) | EI (%) | EV (ml) | EI (%) |
| Control | – | 0.63 ± 0.05 | – | 0.99 ± 0.02 | – | 1.12 ± 0.06 | – |
| Aspirin | 150 | 0.20 ± 0.02*** | 68 | 0.26 ± 0.04*** | 74 | 0.35 ± 0.05*** | 69 |
| VHHW | 75 | 0.39 ± 0.05*** | 38 | 0.66 ± 0.04*** | 33 | 0.86 ± 0.03*** | 23 |
| | 150 | 0.29 ± 0.03*** | 54 | 0.52 ± 0.04*** | 47 | 0.63 ± 0.03*** | 44 |
| | 300 | 0.23 ± 0.02*** | 63 | 0.41 ± 0.02*** | 59 | 0.59 ± 0.02*** | 47 |
| VHBK | 75 | 0.56 ± 0.10* | 11 | 0.82 ± 0.05** | 17 | 1.01 ± 0.04 | 10 |
| | 150 | 0.44 ± 0.04*** | 30 | 0.69 ± 0.04*** | 30 | 0.75 ± 0.01*** | 33 |
| | 300 | 0.40 ± 0.02*** | 37 | 0.51 ± 0.02*** | 48 | 0.75 ± 0.04*** | 33 |
| VHSW | 75 | 0.58 ± 0.05 | 8 | 0.92 ± 0.04 | 7 | 1.04 ± 0.05 | 7 |
| | 150 | 0.53 ± 0.05*** | 16 | 0.78 ± 0.05*** | 21 | 0.80 ± 0.03*** | 28 |
| | 300 | 0.48 ± 0.03*** | 24 | 0.68 ± 0.02*** | 31 | 0.83 ± 0.04*** | 25 |

Values are expressed as mean ± S.E.M. ($n = 5$).
Statistically significant from control group: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

become the recent focus of attention. Alone or in appropriate combination AA products of the cyclooxygenase (COX) and lipoxygenase (LOX) pathways are capable of producing the characteristic signs of inflammation: vasodilatation, hyperemia, pain, edema, and cellular filtration (Lewis and Austen, 1981; Issekutz and Movat, 1982). The cyclooxygenase products, particularly protaglandin $E_2$ ($PGE_2$), contribute to increased blood flow through a vasodilatation action, but the lipoxygenase pathway is necessary for vascular leakage and edema consequent on cellular infiltration (Wedmore and Williums, 1981).

The skin in particular exhibits a wide variety of inflammatory reactions and contains significant amounts of both COX and LOX enzymes (Ziboh et al., 1984). Edema caused by topically applied EPP is due to vasodilatation and increased vascular permeability. This event is caused by the release of various inflammatory mediators, such as histamine, 5-HT, and prostaglandins (Brattsand et al., 1982). All test extracts exerted a dose-dependent inhibition of ear edema formation induced by EPP (Table 1). It is suggested that test extracts probably possess anti-inflammatory activity by inhibition of the release or synthesis of various inflammatory mediators.

AA produced an intense inflammatory reaction in mouse ear and subsequent experiments demonstrated that this response can be ameliorated by putative LOX inhibitors (Chang et al., 1986). All test extracts exhibited significant inhibition of the ear edema formation in this inflammatory model in a dose-dependent relation (Table 2). It is therefore possible that the test extracts possess anti-inflammatory activity in part by inhibition of LOX pathway.

In the carrageenin-induced rat paw edema model, all extracts showed significant inhibitory effect on the edema formation at the third hour after carrageenin injection (Table 3). The results suggest that the main mechanism of action of the test extracts may involve prostaglandin biosynthesis pathway and also influence other mediators of inflammation.

Inflammation is a complex chronic process, which makes the study of it difficult. In order to study its many aspects various test models have been developed. Among them cotton pellet-induced granuloma formation is a typical feature of an established chronic inflammatory reaction and can serve as a subchronic and chronic inflammatory test model for investigation of anti-arthritic substances (Spector, 1969). This model has been employed to assess the transudative and proliferative components of chronic inflammation. The fluid

Table 4
Effect of test extracts from *Ventilago harmandiana* and reference drugs on cotton pellet-induced granuloma formation in rats

| Drug | Dose (mg/kg) | Body weight (g) | | | Dry thymus weight (mg/100 g) | Transudative weight (mg) | Dry granuloma weight (mg/mg cotton) | Granuloma inhibition (%) |
|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Gain | | | | |
| Control | – | 234 ± 2 | 258 ± 2 | 24 ± 1 | 82.6 ± 1.9 | 249.8 ± 1.6 | 2.3 ± 0.0 | – |
| Aspirin | 150 | 236 ± 3 | 254 ± 4 | 18 ± 2* | 80.8 ± 2.0 | 236.8 ± 0.1* | 2.1 ± 0.0 | 8 |
| Prednisolone | 2.5 | 226 ± 2 | 238 ± 2 | 12 ± 0*** | 50.2 ± 2.1*** | 188.8 ± 1.4*** | 1.2 ± 0.1*** | 46 |
| VHHW | 150 | 240 ± 3 | 258 ± 2 | 18 ± 1* | 83.6 ± 1.7 | 197.6 ± 0.9*** | 1.6 ± 0.1** | 29 |
| VHBK | 150 | 234 ± 2 | 256 ± 3 | 22 ± 2 | 84.2 ± 1.8 | 226.8 ± 0.0** | 2.0 ± 0.0 | 13 |
| VHSW | 150 | 230 ± 2 | 252 ± 1 | 22 ± 2 | 81.6 ± 1.1 | 220.8 ± 0.9** | 1.9 ± 0.1 | 17 |

Values are expressed as mean ± S.E.M. ($n = 5$).
Statistically significant from control group: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
VHHW: *Ventilago harmandiana* heart wood, VHBK: *Ventilago harmandiana* bark, VHSW: *Ventilago harmandiana* stem wood.

Table 5
Inhibitory activity of test extracts from *Ventilago harmandiana* and aspirin on writhing response in mice

| Drug | Dose (mg/kg) | Number of writhes | Inhibition of writhing response (%) |
|---|---|---|---|
| Control | – | 32 ± 0.5 | – |
| Aspirin | 150 | 1 ± 0.5*** | 97 |
| VHHW | 150 | 2 ± 0.8*** | 94 |
| VHBK | 150 | 7 ± 0.5*** | 78 |
| VHSW | 150 | 9 ± 0.4*** | 72 |

Values are expressed as mean ± S.E.M. ($n = 5$).
Statistically significant from control group: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
VHHW: *Ventilago harmandiana* heart wood, VHBK: *Ventilago harmandiana* bark, VHSW: *Ventilago harmandiana* stem wood.

Table 6
Inhibitory activity of test extracts from *Ventilago harmandiana* and reference drugs on the tail-flick test in rats

| Drug | Dose (mg/kg) | $T_c$ (s) | $T_t$ (s) | Inhibition (%) |
|---|---|---|---|---|
| Control | – | 3.87 ± 0.19 | 3.80 ± 0.15 | – |
| Aspirin | 150 | 3.57 ± 0.12 | 5.36 ± 0.08* | 25 |
| Morphine | 5 | 3.06 ± 0.21 | 10.00 ± 0.00*** | 100 |
| VHHW | 150 | 3.06 ± 0.15 | 5.28 ± 0.15* | 23 |
| VHBK | 150 | 2.97 ± 0.62 | 4.66 ± 0.14* | 14 |
| VHTW | 150 | 3.67 ± 0.17 | 4.54 ± 0.08* | 12 |

Values are expressed as $R \pm$ S.E.M. ($n = 5$).
Statistically significant from control group: $^{*}P < 0.05$, $^{**}P < 0.05$, $^{***}P < 0.001$.
VHHW: *Ventilago harmandiana* heart wood, VHBK: *Ventilago harmandiana* bark, VHSW: *Ventilago harmandiana* stem wood.

adsorbed by the pellet greatly influences the wet weight of the granuloma whereas the dry weight correlates well with the amount of granulomatous tissue formed. Most NSAIDs, like aspirin, possess only slight inhibition on the granuloma formation without any influence on the thymus weight and the body weight gain of the animals. The steroidal drug, on the contrary, exhibits profound reduction of the granuloma and thymus weight as well as the body weight gain (Swingle and Shideman, 1972).

As shown in Table 4, all test extracts used in this study elicited significant inhibitory activity on the wet weight of granuloma. Among the three extracts, VHHW seemed to possess the strongest inhibition on the transudative formation. This suggests an inhibitory effect of the test extract on vascular permeability. When assessment was made on the dry weight of granuloma, which projects the effect of test substances on the proliferative phase of inflammation, it was found that only VHHW appeared to be slightly effective in inhibition of granuloma formation. The other two extracts did not show any influence on the granuloma weight. Aspirin did not elicit any effect, whereas prednisolone markedly reduced the dry granuloma weight. Prednisolone also significantly reduced the dry thymus weight and the body weight gain, whereas the test extracts, like aspirin, did not exert any effect. It is suggested that the test extracts did not possess any steroidal-like activity (Table 4).

In the analgesic test, test extracts especially VHHW, like aspirin, exerted pronounced inhibitory activity on the writhing response (Table 5), but unlike morphine, produced only a slight inhibition on the tail-flick response (Table 6). It is suggested that the test extracts possess an analgesic effect similar to aspirin, which is mediated via a peripheral mechanism.

In the antipyretic test, test extracts, especially VHHW, markedly decreased the rectal temperature of pyretic rats similarly to aspirin (Table 7). The antipyretic activity is commonly mentioned as one of the characteristics of NSAIDs resulting from their inhibitory effect on protaglandin biosynthesis in the central nervous system (Flower, 1974; Vane, 1987). The production of prostaglandins, particularly the most potent pyretic agent, $PGE_2$, appears to be a final pathway responsible for fever production induced by several pyrogens (Milton, 1982). It is therefore suggested that the antipyretic effect of test extracts occurs in a similar fashion as aspirin or other NSAIDs, by inhibition of prostaglandin biosynthesis in the central nervous system.

Among the extracts tested we found that the extract from the heart wood of *Ventilago harmandiana* possesses stronger anti-inflammatory, analgesic, and antipyretic activities than the extracts from the stem bark and stem wood.

Table 7
Antipyretic activity of test extracts from *Ventilago harmandiana* on yeast-induced pyrexia in rats

| Drug | Dose (mg/kg) | Rectal temperature (°C) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Initial | 18 h after yeast injection | Time after drug administration | | | |
| | | | | 30 min | 60 min | 90 min | 120 min |
| Control | – | 38.3 ± 0.2 | 39.3 +0.1 | 39.5 ± 0.1 | 39.5 ± 0.2 | 39.5 ± 0.1 | 39.4 ± 0.01 |
| Aspirin | 150 | 38.2 ± 0.2 | 39.4 ± 0.1 | 38.6 ± 0.1*** | 38.1 ± 0.1*** | 37.8 ± 0.2*** | 37.6 ± 0.2*** |
| VHHW | 150 | 38.2 ± 0.1 | 39.4 ± 0.1 | 37.1 ± 0.1*** | 36.3 ± 0.2*** | 36.2 ± 0.1*** | 36.2 ± 0.2*** |
| VHBK | 150 | 38.3 ± 0.1 | 39.5 ± 0.2 | 37.5 ± 0.2*** | 36.9 ± 0.3*** | 36.6 ± 0.2*** | 36.6 ± 0.1*** |
| VHSW | 150 | 38.1 ± 0.1 | 39.2 ± 0.1 | 38.3 ± 0.1*** | 37.7 ± 0.1*** | 37.6 ± 0.1*** | 37.7 ± 0.1*** |

Values are expressed as mean ± S.E.M. ($n = 5$).
Statistically significant from rectal temperature 18 h after yeast injection: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
VHHW: *Ventilago harmandiana* heart wood, VHBK: *Ventilago harmandiana* bark, VHSW: *Ventilago harmandiana* stem wood.

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# Antiulcer activity of *Utleria salicifolia* rhizome extract

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**Abstract**

The effect of 50% ethanolic extract of *Utleria salicifolia* (USE) was assessed in different acute and chronic gastric ulcer models in rats. USE, 50–200 mg/kg administered orally, twice daily for 5 days showed dose-dependent ulcer protective effect in pylorus ligation (14.48–51.03% protection, $P < 0.5$ to $P < 0.01$), aspirin (28.80–56.52% protection, $P < 0.5$ to $P < 0.05$), ethanol (13.22–60.74% protection, $P < 0.05$ to $P < 0.001$), cold-restraint stress (21.22–77.14% protection, $P < 0.05$ to $P < 0.001$), and acetic acid (20.0–84.37% protection, $P < 0.5$ to $P < 0.001$)-induced acute and chronic ulcers. USE also significantly ($P < 0.001$) reduced the ulcer incidence (50 and 10%) and severity (67.83 and 91.34% protection) of duodenal ulcer, induced by cysteamine. Besides USE offered protection (53.52 and 60.58%) against ethanol-induced depletion of gastric wall mucus. However, USE reduced the ulcer index with significant decrease in plasma corticosterone (25.53 and 39.52% protection, $P < 0.1$ and $P < 0.05$), lipid peroxidation (18.75 and 47.92% protection, $P < 0.01$ and $P < 0.001$), superoxide dismutase (15.80 and 26.61% protection, $P < 0.05$ and $P < 0.001$) and increased in catalase (28.42 and 71.0% protection, $P < 0.05$ and $P < 0.001$) activity, respectively. Preliminary phytochemical screening of the USE gave the positive test for steroids, alkaloids, terpenoids, saponins and tannins. The HPTLC studies in the toluene: ethyl acetate: formic acid and the densitometric scanning at 254 nm gave three major spots with area corresponding to 28.16, 17.17, and 13.79% at 0.69, 0.78, and 0.88 $R_f$ values, respectively. The results indicate that USE possesses antiulcer activity.
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*Keywords: Utleria salicifolia*; Ulcer; Mucin; Antioxidant

## 1. Introduction

*Utleria salicifolia* Bedd. Ex. Hook. F. (Periplocaceae) is a branched shrub endemic to South Western Ghats of peninsular India. The species is known from three localities in South Western Ghats viz. Anamalais (Kerala and Tamil Nadu), Nelliampathy and Marayaoor forests (Kerala) (Radhakrishnan et al., 1998). The ethnobotanical use of *Utleria salicifolia* was recorded from the Malasar, Kadar and Muthuvam tribes inhabiting these areas. These tribes call *Utleria salicifolia* as "Mahali kizhangu". 'Mahali' refers to the Hindu Goddess of wealth "Mahalekshmy' and 'Kizhangu' root tubers. The Malasar and Kadar tribes use the tuber chips boiled in water for the preparation of pickles which are said to be good for intestinal ailments like colic and bleeding due to ulcer (Radhakrishnan et al., 1998).

Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood (Rao et al., 2000). Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage is caused by physical, chemical and psychological factors that leads to gastric ulceration in human and experimental animals (Rao et al., 1999). Most of the available drugs are thought to act on the offensive factors which neutralize acid secretion like antacids, $H_2$ receptor blockers like ranitidine, famotidine, anticholinergics like pirenzepin, telezipine, proton pump blockers like omeprazole, lansoprazole, etc. which interfere with acid secretion. Recently the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration (Sairam et al., 2001). To the best of our knowledge there were no scientific

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reports available in support of its traditional claims. Therefore, present study was designed to demonstrate the effect of *Utleria salicifolia* extract (USE) on physical and chemical factors induced gastric ulceration in rats.

## 2. Materials and methods

### 2.1. Materials

*Utleria salicifolia* rhizomes, were collected from the Parambikulam forests of the Southern Western Ghat region of Palakkad district of Kerala, India, in June 2002. The plant samples was authenticated using voucher specimens (K. Radhakrishnan 19725 and 29029) deposited in herbarium of Tropical Botanic Garden and Research Institute (TBGRI). Voucher specimens of the collected plant samples was also deposited in the National Herbarium of NBRI, Lucknow.

### 2.2. Preparation of extract

Air-dried powdered rhizomes of *Utleria salicifolia* (1000 g) were powdered and exhaustively extracted by overnight maceration with 10 volumes of 50% ethanol and centrifugation at 10,000 revolutions per minute. The extract was separated by filtration and concentrated on rotary evaporator (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure to obtain 42 g of solid residue (yield 4.2%, w/w).

### 2.3. Phytochemical screening

Preliminary qualitative phytochemical screening (Anshurathi et al., 2003) of the rhizomes of *Utleria salicifolia* gave the positive test for steroids, alkaloids, terpenoids, saponins, and tannins. The high performance thin layer chromatography (HPTLC) studies of the 50% ethanolic extract of *Utleria salicifolia* (USE) were carried out on pre-coated silica gel plate (Merck 60 F 254) as the stationary phase and toluene:ethyl acetate:formic acid (5:5:1) as the mobile phase. The extract was spotted using a Camag Linomat IV spotter. These plates were observed at UV 254 and were scanned on TLC scanner III using CAT software.

### 2.4. Test animals

Sprague–Dawley rats (140–180 g) were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at $26\pm2\,^{\circ}C$ and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18–24 h before the experiment though water was allowed ad libitum. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

### 2.5. Experimental procedure

USE in doses of 50, 100, and 200 mg/kg and $H_2$ receptor blocker, ranitidine, in the dose of 50 mg/kg were administered orally twice daily at 10:00 and 16:00 h, respectively, for 5 days for acute and up to 5 or 10 days for chronic ulcer protective studies. Reduced glutathione (RG) 150 mg/kg was injected intraperitoneally twice: once before 20 h and another 1 h prior to subjecting the animals to cold-restraint stress and reported to exerts its antioxidant defense mechanism (Das and Banerjee, 1993). Control group of animals received suspension of 1% carboxymethyl cellulose in distilled water (10 ml/kg).

### 2.6. Aspirin (ASP)-induced ulcers

ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after 4 h (Goel et al., 1985). The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows (Sanyal et al., 1982): 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1–2 mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated according to the method of Sanyal et al. (1982).

### 2.7. Cold-restraint stress (CRS)-induced ulcers

Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of $4–6\,^{\circ}C$ (Gupta et al., 1985). Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described above.

### 2.8. Pylorus ligated (PL)-induced ulcers

Drugs were administered for a period of 5 days as described above and the rats were kept for 18 h fasting and

care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period (Shay et al., 1945). After 4 h, stomachs were dissected out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach as mentioned in aspirin induced ulcers.

### *2.9. Ethanol (EtOH)-induced ulcers*

The gastric ulcers were induced in rats by administrating 100% EtOH (1 ml/200 g, 1 h) (Hollander et al., 1985) and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (square millimeters per rat).

### *2.10. Acetic acid-induced chronic ulcer*

Induction of chronic gastric lesions was studied according to the methods of Okabe et al. (1971). A solution of 0.06 ml 50% acetic acid was instilled into the glass tube of 6 mm in diameter and allowed to remain 60 s on the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end under anesthesia. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. USE was given in the dose of 100 and 200 mg/kg on day 1, orally, twice daily, 4 h after the application of acetic acid and continued either up to 5 or 10 days after induction of ulcer. The animals were than sacrificed after 18 h of the last dose of drug either on sixth or eleventh day of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (square millimeters per rat) of ulcers.

### *2.11. Cysteamine-induced duodenal ulcers*

The method described by Szabo (1978) was followed. Duodenal ulcers were induced by administrations of two doses of cysteamine hydrochloride, 400 mg/kg, p.o. in 10% aqueous solution at an interval of 4 h. USE at dose levels of 100 and 200 mg/kg, ranitidine (50 mg/kg, p.o.) were administered 30 min before each dose of cysteamine hydrochloride. All the animals were sacrificed 24 h after the first dose of cysteamine and duodena were excised carefully and opened along the antimesentric side. The duodenal ulcers were scored for intensity, using a scale of 0–3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmular necrosis, and 3 = perforated or penetrated ulcer (into the pancreas or liver).

### *2.12. Determination of gastric wall mucus*

Gastric wall mucus was determined according to the method of Corne et al. (1974). The glandular segments from stomachs were removed, weighed and incubated in tubes containing 1% Alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The alcian blue binding extract was centrifuged at 3000 rpm for 10 min and the absorbency of supernatant was measured at 498 nm. The quantity of alcian blue extracted (gram per gram of glandular tissue) was then calculated.

### *2.13. Estimation of lipid peroxidation (LPO)*

The fundic part of the cold-restraint stress (CRS)-induced ulcer stomach was homogenized (5%) in ice-cold 0.9% NaCl with a Potter–Elvehjem glass homogenizer for 30 s. The homogenate was centrifuged at $800 \times g$ for 10 min and the supernatant was again centrifuged at $12{,}000 \times g$ for 15 min and the obtained mitochondrial fraction was used for the following estimations (Das and Banerjee, 1993). A volume of the homogenate (0.20 ml) was transferred to a vial and was mixed with 0.2 ml of a 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 ml of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.50 ml of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged at $1000 \times g$ for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water (Jamall and Smith, 1985). 1,1,3,3-Tetraethoxypropan was used as standard for calibration of the curve and is expressed as nanomoles per milligram protein.

### *2.14. Assay of antioxidant enzymes*

The fundic stomach was homogenized (5%) and mitochondrial fraction was prepared as described above. Decomposition of $H_2O_2$ in presence of catalase (CAT) was followed at 240 nm (Aebi, 1974). One unit (U) of catalase was defined as the amount of enzyme required to decompose 1 μmol of $H_2O_2$ per minute, at 25 °C and pH 7.0. Results are expressed as units of CAT activity per milligram of protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitrobluetetrazolium reaction system as described by Nishikimi et al. (1972) as adapted by Kakkar et al. (1984). One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25 ± 2 °C) and the results have been expressed as units of SOD activity per milligram of protein.

### 2.15. Estimation of plasma corticosterone (PC)

The animals were lightly anesthetized with ether and blood was collected from the supraordital plexus using the microcapillary technique in CRS-induced ulcer model. Three hundred microlitres of isooctane was added to 100 μl of plasma. After mixing and centrifugation, the isooctane was discarded. Six hundred microliters of chloroform was added to each tube and after extraction 400 μl of chloroform was transferred to another stoppered tube. To this 800 μl of acid–alcohol (50%) solution (2:1) was added. After 1 h, acid layer fluorescence was measured at 462 nm (excitation) and (emission) using a spectrofluorimeter and expressed as micrograms per deciliter (Glick et al., 1964).

### 2.16. Statistical analysis

All the data were presented as mean ± S.E.M. and analyzed by Wilcoxon Sum Rank Test (Padmanabha pillai et al., 1982) and unpaired Student's $t$ test for the possible significant interrelation between the various groups. A value of $P < 0.05$ was considered statistically significant.

## 3. Results

The preliminary HPTLC studies revealed that the solvent system toluene:ethyl acetate:formic acid (5:5:1) was ideal and gave the well-resolved peaks of the sample (Fig. 1). The spots of the chromatogram were visualized at 254 nm with a 400 k filter at 0.10, 0.20, 0.59, 0.69, 0.78, and 0.88 $R_f$. The densitometric scanning at 254 nm gave major three spots with area of 28.16, 17.17, 13.79% at 0.69, 0.78, and 0.88 $R_f$ values, respectively.

Effects of USE at doses of 50–200 mg/kg, twice a day for 5 days prevented the acute gastric ulcers in a dose related manner. The range of percent protection were PL 14.48–51.03% ($P < 0.01$), ASP 28.80–56.52% ($P < 0.05$), EtOH 13.22–60.74% ($P < 0.05$ to $P < 0.001$) and CRS 21.22–77.14% ($P < 0.05$ to $P < 0.001$), respectively. The

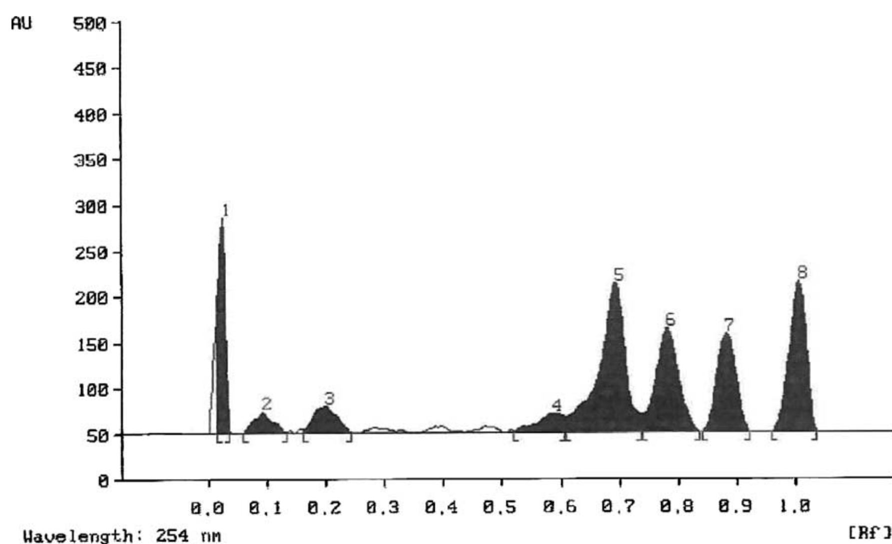


Fig. 1. HPTLC fingerprint profile of *Utleria salicifolia* extract.

Table 1
Effect of *Utleria salicifolia* extract (USE, twice daily for 5 days) on pylorus ligation (PL)-, aspirin (ASP)-, ethanol (EtOH)-, and cold-restraint stress (CRS)-induced gastric ulcers in rats

| Treatment (mg/kg) | Ulcer index | | | |
|---|---|---|---|---|
| | PL | ASP | EtOH (square millimeters per rat) | CRS |
| Control | 14.5 ± 2.5 | 18.4 ± 4.3 | 24.2 ± 5.5 | 24.5 ± 3.8 |
| USE 50 | 12.4 ± 2.6 | 13.1 ± 2.1 | 21.0 ± 5.1 | 19.3 ± 1.4 |
| USE 100 | 9.1 ± 2.1 | 10.2 ± 1.6 | 11.5 ± 4.2 | 14.6 ± 2.2* |
| USE 200 | 7.1 ± 1.2* | 8.0 ± 1.3* | 9.5 ± 2.5* | 5.6 ± 1.1*** |
| Ranitidine 50 | 5.1 ± 1.3** | 7.6 ± 1.5* | 10.3 ± 2.8* | 4.9 ± 1.0*** |

Values are mean ± S.E.M. for six rats.
* $P < 0.05$ compared to respective control group.
** $P < 0.01$ compared to respective control group.
*** $P < 0.001$ compared to respective control group.

Table 2
Effect of *Utleria salicifolia* extract (USE, twice daily for 5 days) on ethanol (EtOH)-induced gastric ulcers and gastric wall mucus in rats

| Treatment and dose (mg/kg) | Ulcer index (square millimeters per rat) | Percent protection | Gastric wall mucus (gram per gram wet glandular tissue) |
|---|---|---|---|
| Control | 0.0 ± 0.0 | – | 268.7 ± 15.1 |
| EtOH | 24.1 ± 5.1 | – | 171.2 ± 12.9† |
| USE 100 | 11.2 ± 4.2 | 53.52 | 207.4 ± 13.8 |
| USE 200 | 9.5 ± 2.7** | 60.58 | 277.3 ± 14.6** |
| Ranitidine 50 | 10.1 ± 2.7* | 58.09 | 213.8 ± 10.1* |

Values are mean ± S.E.M. for six rats.
† $P < 0.001$ compared to respective control group.
* $P < 0.05$ compared to respective EtOH group.
** $P < 0.001$ compared to respective EtOH group.

Table 3
Effect of *Utleria salicifolia* extract (USE, twice daily for 5 and 10 days) on 50% acetic acid-induced chronic ulcers in rats

| Treatment and dose (mg/kg) | Acetic acid-induced chronic ulcers | | | |
|---|---|---|---|---|
| | Five days treated | | Ten days treated | |
| | Ulcer index | Percent incidence of perforations | Ulcer index | Percent incidence of perforations |
| Control | 22.5 ± 2.2 | 70.5 | 12.8 ± 1.5 | 35.3 |
| USE 100 | 18.0 ± 1.5 | 48.9 | 7.5 ± 1.1* | 5.1 |
| USE 200 | 12.1 ± 1.3** | 26.7 | 2.0 ± 0.4*** | 0.0 |

Values are mean ± S.E.M. for six rats.
* $P < 0.05$ compared to respective control group.
** $P < 0.01$ compared to respective control group.
*** $P < 0.001$ compared to respective control group.

percent protection of ranitidine ranged from 57.44–80.0% ($P < 0.05$ to $P < 0.001$), respectively, in various gastric ulcer models (Table 1). Secretion of mucus and bicarbonate by surface epithelial constitute a mucus–bicarbonate barrier, which is regarded as first line of defense against potential ulcerogens. The gastric wall mucus was significantly ($P < 0.001$) enhanced by USE and is regarded as a first line of defence against EtOH-induced gastric ulcers showing cytoprotective property (Table 2). In chronic ulcers induced by 50% acetic acid, USE reduced ulcer index significantly with decreased perforations after 5 and 10 days treatment (Table 3).

Table 4
Effect of *Utleria salicifolia* extract (USE) on cysteamine-induced duodenal ulcers in rats

| Treatment and dose (mg/kg) | Ulcer incidence | | Ulcer score | |
|---|---|---|---|---|
| | No | Percent | Total lesion area (mm$^2$) | Percent protection |
| Control | 8/10 | 80 | 4.85 ± 0.32 | – |
| USE 100 | 5/10 | 50 | 1.56 ± 0.30* | 67.83 |
| USE 200 | 1/10 | 10 | 0.42 ± 0.11* | 90.34 |
| Ranitidine 50 | 2/10 | 20 | 1.05 ± 0.15* | 78.35 |

Values are mean ± S.E.M. for 10 rats.
* $P < 0.001$ compared to respective control group.

Table 5
Effect of *Utleria salicifolia* extract (USE, twice daily for 5 days) on plasma corticosterone (PC), lipid peroxidation (LPO), catalase (CAT), and superoxide dismutase (SOD) activities in cold-restraint stress (CRS)-induced ulcers

| Treatment and dose (mg/kg) | Ulcer index | PC | LPO | CAT | SOD |
|---|---|---|---|---|---|
| Control | 0.0 ± 0.0 | 21.7 ± 3.3 | 0.35 ± 0.01 | 33.1 ± 2.0 | 98.9 ± 10.0 |
| CRS | 24.0 ± 3.5†† | 37.2 ± 4.1† | 0.48 ± 0.02†† | 18.3 ± 1.5†† | 215.3 ± 11.3†† |
| USE 100 | 14.5 ± 2.0* | 27.7 ± 2.6 | 0.39 ± 0.01** | 23.5 ± 1.3* | 181.3 ± 5.7* |
| USE 200 | 5.6 ± 1.3*** | 22.5 ± 2.3* | 0.25 ± 0.01*** | 31.3 ± 1.9*** | 158.0 ± 4.9*** |
| Reduced glutathione 150 | 4.5 ± 2.0*** | 21.7 ± 1.1** | 0.28 ± 0.02*** | 31.1 ± 1.4*** | 149.1 ± 5.1*** |

Values are mean ± S.E.M. for six rats.
† $P < 0.05$ compared to respective control group.
†† $P < 0.001$ compared to respective control group.
* $P < 0.05$ compared to respective CRS group.
** $P < 0.01$ compared to respective CRS group.
*** $P < 0.001$ compared to respective CRS group.

Cysteamine produced duodenal ulcers in 80% of the control rats. Usually two ulcers were produced close to the pylorus, the larger on the anterior and the smaller on the posterior wall of the duodenum. They were elongated extending longitudinally down to the duodenum. Treatment with USE (100 and 200 mg/kg) produced a significant ($P < 0.001$) and dose-dependent reduction in the severity and incidence of cysteamine induced duodenal ulcers. However, the $H_2$ receptor blocker ranitidine (50 mg/kg) also produces a significant protective effect (Table 4).

A summary table (Table 5) is included to indicate the severity of ulcer index as well as enzyme activities. While studying the role played by the reactive oxygen species on CRS-induced gastric damage, lipid peroxidation, and SOD were increased significantly of the ulcerated stomachs ($P < 0.001$). Pretreatment with USE and a general antioxidant, reduced glutathione, significantly reduced the ulcer index, LPO, SOD levels and increased in CAT activity in comparison to the CRS ulcers ($P < 0.05$ to $P < 0.01$). Henke (1979) stated that the central nervous system played an important role in stress ulceration and regulation of plasma corticosterone. USE almost completely protected gastric ulceration by scavenging the free radicals that involved in the endocrinological plasma corticosterone.

## 4. Discussion and conclusion

The present study showed that the ethanolic extract of *Utleria salicifolia* possess gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by various physical and chemical agents. Pylorus ligation-induced ulcers are due to autodigestion of the gastric mucosa and break down of the gastric mucosal barrier (Sairam et al., 2002). Synthetic NSAIDs like aspirin cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of $H^+$ ions (Rao et al., 2000). The incidence of ethanol-induced ulcers is predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products (Oates and Hakkinen, 1988); and reactive oxygen species (Mizui et al., 1987) resulting in the damage of rat gastric mucosa (Peskar et al., 1986). Ethanol-induced depletion of gastric wall mucus has been prevented by USE. It implies that a concomitant increase in prostaglandins (Pihan et al., 1986); or sulfhydryl compounds (Szabo et al., 1981); contribute to protect the stomach from ethanol injury. A copious amount of gastric mucus is secreted during superficial mucosal damage and provides a favorable microenvironment in repair by restitution. Therefore, it is conceivable that the observed gastric ulcer protection of USE, provides a general evidence for the close relationship between these factors.

Stress plays an important role in etiopathology of gastro-duodenal ulceration. Increase in gastric motility, vagal over activity (Cho et al., 1976); mast cell degranulation (Cho and Ogle, 1979); decreased gastric mucosal blood flow (Hase and Moss, 1973); and decreased prostaglandin synthesis (Rao et al., 1999); are involved in genesis of stress induced ulcers. Complex neurochemical mechanisms are involved in the organism's biological response to noxious stimuli like stress. The pathologic alterations occur with the changes in the synthesis, actions and degradation of hormones, neurotransmitters and neuromodulators. The central nervous system plays an important role in stress ulceration and regulation of plasma corticosterone (Henke, 1979). As etiopathogenesis of these ulcer models are different, mechanism of USE should then include number of predisposing factors. On the other hand, the mucosal protection induced by non-prostanoid compounds was perhaps mediated through the mobilization of endogenous prostaglandins (Konturek et al., 1987).

Gastric ulcer is often a chronic disease and it may persist for 10–20 years characterized by repeated episodes of healing and re-exacerbations. Acetic acid-induced ulcer better resembles clinical ulcers in location, chronicity and severity and servers as the most reliable model to study healing process (Okabe and Pfeiffer, 1972). USE significantly healed the penetrating ulcers induced by acetic acid after 5 and 10 days treatment.

Free radicals affect lipids by initiating peroxidation. Superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^\bullet$) are important ROS causing tissue damage (Fridovich, 1986); and lipid peroxide level is an indicator for the generation of ROS in the tissue. The experimental data stated that the cold-restraint stress aggravated the ulcer severity, lipid peroxidation, and plasma corticosterone as compared to unstressed rats. The higher lipid peroxidation and SOD levels indicated increased production of $O_2^-$ within the tissue as elevated $O_2^-$ level was thought to increase the concentration of cellular radical level. These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins (Halliwell and Gutteridge, 1985). This effect was significantly reversed by prior administration of USE providing a close relationship between free radical scavenging activity and the involvement of endocrinological (plasma corticosterone) responses. The more work is required for the clear understanding of the mechanism of action with chemically identified active principles. However, in the present study the plant shows a potent antiulcer activity, which justifies the ethnomedical claims.

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# Immunoadjuvant potential of *Asparagus racemosus* aqueous extract in experimental system

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**Abstract**

The immunoadjuvant potential of *Asparagus racemosus* (Willd.) Family (Liliaceae) aqueous root extract was evaluated in experimental animals immunized with diphtheria, tetanus, pertussis (DTP) vaccine. Immunostimulation was evaluated using serological and hematological parameters. Oral administration of test material at 100 mg/kg per day dose for 15 days resulted significant increase ($P = 0.0052$) in antibody titers to *Bordtella pertussis* as compared to untreated (control) animals. Immunized animals (treated and untreated) were challenged with *B. pertussis* 18323 strain and the animals were observed for 14 days. Results indicate that the treated animals did show significant increase in antibody titers as compared to untreated animals after challenge ($P = 0.002$). Immunoprotection against intra-cerebral challenge of live *B. pertussis* cells was evaluated based on degree of sickness, paralysis and subsequent death. Reduced mortality accompanied with overall improved health status was observed in treated animals after intra-cerebral challenge of *B. pertussis* indicating development of protective immune response. Present study indicates applications of test material as potential immunoadjuvant that also offers direct therapeutic benefits resulting in less morbidity and mortality.
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*Keywords:* Vaccine adjuvant; Immunomodulator; Immunostimulant; Mouse protection test; Ayurveda; *Shatavari*; *Asparagus racemosus*

## 1. Introduction

Newer vaccines include highly purified subunit antigens that are weakly immunogenic. Vaccine formulations often require adjuvants for increased immunological efficiency and better vaccination schedules (Ruszala et al., 1988; Vogel, 2000; Jennings, 1995). Currently used adjuvants include aluminum hydroxide, aluminum phosphate, calcium phosphate, water-in-oil emulsions, products from bacteria and liposomes. Often there is compromise on level of adjuvanicity and acceptable level of safety. Other adjuvants such as mono-phosphoryl lipid A, ISCOMS, QS-21—a purified saponin from bark of *Quillaja saponaria* Molina and Syntex Adjuvant formulation (SAF) are under development as better and safer adjuvants (Gupta and Edgar, 1993; Vogel, 2000). Plant based immunomodulators are being considered as one option (Rivera et al., 2003; Hu et al., 2003; Lamm et al., 2001; Lavelle et al., 2002; Bostelmann et al., 2002).

Previously we have reported various immunomodulators of Ayurvedic origin. Our initial studies on extracts and formulations prepared from Ayurvedic medicinal plants including *Withania somnifera*, *Tinospora cordifolia* and *Asparagus racemosus*, demonstrated significant immunostimulatory activity particularly at humoral level in experimental systems with or without induced immunosuppression (Patwardhan et al., 1990; Ziauddin et al., 1996; Agarwal et al., 1999; Patwardhan, 2000).

Present study attempts to extend the reported immunopotentiating activity of botanical immunomodulators for their possible applications in immunotherapeutics and immunochemical industry. We report here immunoadjuvant potential of *Asparagus racemosus* in an animal model using diphtheria, tetanus, pertussis (DTP) vaccine with a challenge system of pertussis.

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## 2. Experimental procedures

### 2.1. Test material

The roots of *Asparagus racemosus* obtained from reliable sources were correctly identified and authenticated by routine pharmacognostic procedures. A voucher sample was retained and deposited at Botany Division, Agarkar Research Institute, Pune. Coarsely powdered plant material (250 g) was successively extracted as water decoction (2.5 l × 2) on 2 consequent days following a modified traditional method using an open stainless steel vessel. Pooled aqueous extract was dried at 60 °C and stored in airtight containers for further use. The extraction yield was 75% w/w. Test material was prepared as fresh suspensions in vehicle—2% sterile CMC for oral administration.

### 2.2. Chromatographic characterization of the extract

HPTLC characterization of the extract was done on the basis of literature reports that *Asparagus racemosus* contains steroidal saponins as one of major phytoconstituent. The presence of saponins in the extract was confirmed during preliminary phytochemical screening of the extract. The aqueous extract was characterized by HPTLC (F 254—Merck silica gel plates) using reported TLC method for steroidal saponins (Wagner, 1988). Chromatographic separation was carried out using solvent system chloroform:methanol:water (6.4:5:1, v/v) and plates were scanned at 225 and 365 nm (Camag Multiwavelength scanner III, version IV). The spots were developed with anisaldehdye sulfuric acid and scanned at visible range (wavelength 300–800 nm) (Stahl, 1964). The fingerprint developed by this method was used as tool for authentification and standardization of extract.

### 2.3. Experimental animals and immunization

Swiss albino mice of either sex weighing 14–16 g obtained from Serum Institute of India, Pune, India, were randomly distributed in total eight groups consisting four main groups and four replica groups ($N = 8$). Groups were maintained in replica in anticipation of the mortality associated with intra-cerebral challenge. For the practical purpose, data of all the surviving animals from each set of groups (main and replica) was used for analysis. All the animals were housed in uniform environment of temperature (18–21 °C), humidity and light and under 12 h day–night cycles. Animals in all groups were vaccinated with DTP adsorbed vaccine (Serum Institute of India; Batch number E-30166). All animals received 0.5 ml of 1:25 diluted standard dose of vaccine, intraperitoneally on day 0.

### 2.4. Challenge to vaccinated animals

The challenge dose was prepared as per standard WHO protocol (WHO Technical Series, 1990). The *B. pertussis* cells grown on Bordet–Gengou medium for less than 24 h were suspended in a diluent and cell density was adjusted to 100–1000 times median lethal dose ($LD_{50}$) in 0.03 ml of diluent.

### 2.5. Serology and hematology

The antibody response to D and T components of vaccine was evaluated by single radial immunodiffusion technique (tetanus and diphtheria components—data not reported) while pertussis component was evaluated by micro-slide agglutination technique. This paper reports results of pertussis component only. The technique has advantage over tube agglutination technique, being faster and requiring smaller volumes of reactants (Talwar, 1983). Level of antibody was expressed as titer, which is defined as the highest dilution of the test serum that gives a visible detectable reaction with specific antigen, i.e. MVP pool of *B. pertussis* vaccine containing 1, 2, and 3 agglutinogens at 180 IU/ml. This pool was prepared at Serum Institute of India production division that contains four different strains of *B. pertussis*. Four strains of *B. pertussis* are namely strain 134 containing agglutinogens 1 and 3, strain 509 containing 1 and 2, strain 6229 containing 1, 2 and 3 and strain 25525 containing 1, 2 and 3. Thus, total suspension contains agglutinogens 1, 2 and 3, which was used as antigenic substance in slide agglutination for anti-pertussis sera titre determination. The titers were expressed as $\log_2$ values. Appropriate controls were kept during antibody assay to avoid misinterpretation by autoagglutinating cells if any. Blood was collected in anticoagulant for hematological parameter studies. The cytological examination was carried out using Leishman stained smears. The cell counts (red cells, total and differential white cells and platelets) and estimation of hemoglobin was carried out as per standard protocol (Talwar, 1983).

### 2.6. Kendrick test

Immunostimulation potential of test material was evaluated in Kendrick test model. This test is based on an immunization and challenge procedure. It is also known as mouse protection test or intra-cerebral challenge test. It is one of the mandatory requirements for determination of potency of pertussis vaccines. In this study, we have chosen a single optimal dose based on experience of multi dose potency tests routinely performed at the vaccine manufacturing industry. The study design has been planned to check the immunopotentiating activity of extract under study. Challenged animals were observed from day 14 to day 28 for sickness, paralysis and subsequent death, which is generally seen after challenge (Kendrick et al., 1947). Development of protective immunity against intra-cerebral challenge with live *B. pertussis* cells in treated animals was evaluated using mortality rate and devised scoring system for degree of sickness in animals.

### 2.7. Scoring system

Scoring system was devised in order to grade the morbidity observed after challenge. The animals were scored as Score 0: for normal animals, no paralysis; Score 1: for mild paralysis (swollen head, impaired movement, less food intake, mild weight loss (1–5%)); Score 2: for severe paralysis (swollen head, no movement, no food intake, severe weight loss (40–50%)) and Score 3: for death. Protective immunity developed to intra-cerebral challenge with live *B. pertussis* cells in treated animals was evaluated using above scores and mortality rate.

### 2.8. Study design

Total duration of this study was of 28 days. On day 0, animals in all groups (I, IA, II, IIA, III, IIIA, IV and IVA) received DPT vaccine (Table 1). From day 1 to day 15, animals in treatment groups (II, IIA, IV and IVA) received test material (100 mg/kg per day) while control groups (I, IA, III and IIIA) received vehicle 2% CMC per oral route. On day 14, blood was collected for serology from groups I and II while animals in groups III, IIIA, IV and IVA received intra-cerebral challenge with *B. pertusis* strain (18323). Morbidity (sickness and paralysis) and mortality was recorded among challenged animals from day 14 to day 28 and animals dying within 3 days of challenge inoculation were not considered for the analysis (Kendrick et al., 1947). Animals were bled for hematology and serology on day 28. Weight of animals were recorded daily during the study period. Scores were analyzed statistically using Mann–Whitney test for independent values. Comparisons were made between groups I and II, III and IV to evaluate immunopotentiation and development of protective immunity by test material, respectively.

Data was analyzed using ANOVA (single factor at $\alpha = 0.05$) of Microsoft Excel 97.

## 3. Results and discussion

*Shatavari* is traditionally used in Ayurved as crude roots powder or its aqueous decoction (Charak Samhita, 1949). HPTLC analysis of extract confirmed presence of steroidal saponins. Apart from saponins it contains alkaloids, proteins, starch, tannin and mucilage. The extractive values and type of saponins varies with geographical distribution of species (Kanitkar et al., 1969; Subramanian and Nair, 1968). The main objective of the study was to observe the effect of oral administration of *Shatavari* extract on immune response to DTP vaccine. Being exploratory in nature, the study is on single batch of vaccine and employs preliminary parameters for evaluation. We modified Kendrick test model to simultaneously study immunostimulation, immunoprotection and adjuvant activity in a single experimental set up.

### 3.1. Immunostimulation (serology)

Oral administration of test material at 100 mg/kg per day dose for 15 days resulted significant increase ($P = 0.0052$) in antibody titers to *B. pertussis* as compared to untreated (control) animals. Immunized animals (treated and untreated) were challenged with *B. pertussis* 18323 strain and the animals were observed for 14 days. Results indicate that the treated animals did show significant increase in antibody titers as compared to untreated animals after challenge ($P = 0.002$) supporting our claim of immunostimulation (Table 1).

### 3.2. Immunoprotection studies

To study if this immunostimulation leads to immunoprotection or not, we observed morbidity and mortality associated with the challenge. Based on experience of multi dose potency test that is routinely performed at the vaccine manufacturing industry, we devised a scheme to grade and score

Table 1
Effect of test material on DPT immunized animals

| Group[a] | $Log_2$ average pertussis antibody titers | Hb (concentration in g%) | WBC ($N \times 10^3$ per µl) | Polymorph count (%) | Lymphocyte count (%) | Total no. of deaths ($D$) | Percent mortality |
|---|---|---|---|---|---|---|---|
| I ($N = 8$) | 6.14 ± 0.38[b,c] | NA | NA | NA | NA | NA | NA |
| IA ($N = 8$) | 5.56 ± 0.55 | 11.4 ± 2.69 | 10066.67 ± 750.76 | 7.66 ± 2.16 | 92.33 ± 2.16 | NA | NA |
| II ($N = 8$) | 7.33 ± 0.23[d] | NA | NA | NA | NA | NA | NA |
| IIA ($N = 8$) | 7.33 ± 0.52 | 12.75 ± 1.19 | 10100 ± 660.78 | 5.75 ± 1.70 | 94 ± 2.16 | NA | NA |
| III and III A ($N = 14$) | 7.00 ± 0.58[e] | 11.47 ± 1.97 | 3800 ± 329 | 10 ± 4.92 | 89.88 ± 5.13 | 7 | 50 |
| IV and IVA ($N = 12$) | 8.80 ± 0.84[f,g] | 10.56 ± 1.96 | 4012 ± 430 | 8.25 ± 2.86 | 90.50 ± 2.92 | 3 | 25 |

Values represent mean ± standard deviation. $N$: number of animals. NA: not applicable.

[a] Group I and IA: vaccine; group II and IIA: vaccine+test material; group III and IIIA: vaccine+challenge; group IV and IVA: vaccine+challenge+test material.

[b] $P = 0.0052$ (comparison of I with II).

[c] $P = 0.04$ (comparison of I with IA).

[d] $P =$ NS (comparison of II with IIA).

[e] $P = 0.0001$ (comparison of III with I).

[f] $P =$ NS (comparison of IV with II).

[g] $P = 0.002$ (comparison of IV with III).

the morbidity associated with challenge. There was significant reduction in morbidity score in animals receiving treatment as compared to untreated animals ($P = 0.03$). Less morbidity score indicates fewer incidences and lesser severity of paralysis that results in reduced mortality in treatment groups. The mortality incidence also confirmed the same as reduced mortality was seen in treatment group (25%) as compared to control (50%). These trends suggest possible immunopotentiation coupled with immunoprotection potential of test material, which improved resistance to challenge in animals.

### 3.3. Adjuvant activity

Adjuvants are used to enhance the immune response to a particular antigen of interest. This enhancement results in improving titers, sustained response duration and avidity. Pertussis antibody titers declined with duration (from day 14 to day 28) in untreated groups ($P = 0.04$). No such decline in antibody titers was observed in treated groups till the end of study (Table 1).

### 3.4. Hematology

No significant effect on Hb, polymorphs, lymphocyte and WBC counts was observed at end of study period (Table 1).

Thus, *Asparagus racemosus* aqueous extract exhibited three important characteristics in this experimental setup—immunostimulation, immunoprotection and adjuvant activity.

Many researchers have reported possible immunostimulation activity with steroidal saponins, however possible correlation of immunostimulation and saponin content in *Asparagus racemosus* remains to be established (Dhuley, 1997; Rege et al., 1999; Thatte and Dahanukar, 1988).

## 4. Conclusion

Our study indicates that *Shatavari* aqueous extract may have valuable applications in immunochemical industry to obtain more efficient and sustained immunostimulation resulting in increased yields of immune sera production and to improve immunogenecity of weak and or low dose antigens. Further studies on pharmacodynamics including antibody profiles, cytokine induction and regulation of immune response in terms of Th1 and Th2 needs to be undertaken to explore therapeutic and industrial applications.

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# The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats

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**Abstract**

Effects of three different varieties (Koimidori, Powerful-Reishi, and Hyakunari) of bitter melon (*Momordica charantia*) and those of methanol fraction extract of Koimidori variety on serum and liver triglycerides were studied in rats. Feeding of diets containing either bitter melon or various fractions isolated by organic solvents caused no adverse effects on food intake or growth of rats. When the effect of three different varieties of bitter melon was compared, the Koimidori variety was found to be the most effective in lowering hepatic triglyceride levels as compared to the other two varieties, suggesting a variety-dependent difference in their activity. Furthermore, the active component(s) responsible for the liver triglyceride lowering activity of Koimidori variety was assumed to be concentrated in the methanol fraction, but not in other fractions such as the *n*-hexane, the acetone, or the residual fraction. The triglyceride lowering activity was furthermore confirmed by the dose-dependent reduction of hepatic triglyceride, resulting the lowest level in rats fed 3.0% supplementation. In these experiments, the effects on serum lipids were marginal. The results of the present and previous studies clearly show that bitter melon, especially Koimidori variety, exhibits a potent liver triglyceride-lowering activity.
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*Keywords:* Bitter melon; Triglyceride-lowering effect; Rat

## 1. Introduction

Bitter melon (*Momordica charantia*; Cucurbitaceae family) is a vegetable indigenous to subtropical and tropical regions of South America and Asia. This vegetable is also cultivated in the southern part of Kyushu, Japan, due to its subtropical climate. Fruit and seeds of bitter melon are traditionally used as a medicinal herb and/or vegetable for treatment of diabetes in Southeast Asian countries (Karunanayake et al., 1984; Platel and Srinivasan, 1997). The empirical use of bitter melon as a medicinal herb for treatment of diabetes was confirmed experimentally by recent observations that bitter melon itself or fractions extracted with water from this vegetable, exhibits a potent hypoglycemic activity in normoglycemic and streptozotocin-induced diabetic rats as well as in human subjects with diabetes mellitus type II (Leatherdale et al., 1981; Bailey et al., 1985; Welihinda et al., 1986; Ali et al., 1993). The mechanism of this action still remains uncertain.

The westernization of dietary habits observed in Japan in the recent past has caused an increase in the incidence of several types of life style-related diseases such as diabetes, fatty liver, and hyperlipidemia. Consequently, there is an increased interest in finding a naturally occurring, plant-based, compound—a functional food ingredient, which could prevent and/or ameliorate such conditions.

We previously reported the beneficial effects of bitter melon on other factors such as lipid parameters as well. Serum and liver lipids, especially triglyceride concentration was significantly lowered in rats fed diets containing 1% bitter melon powder compared to those fed diets with no bitter melon (Jayasooriya et al., 2000). This result suggests that bitter melon contains components which improve and/or ameliorate lipid disorders such as hyperlipidemia and fatty liver. Since no previous attempts have been made to examine the effect of dietary bitter melon on serum and liver

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lipids, we focused on finding the active component(s) of bitter melon which can modulate serum and liver triglyceride levels in rats.

## 2. Materials and methods

### 2.1. Plant materials and extraction procedure

Three different varieties of bitter melon, Koimidori, Powerful-Reishi, and Hyakunari were used in the first experiment. Koimidori was developed and cultivated in Miyazaki Agricultural Experiment Station (Miyazaki, Japan); Powerful-Reishi was developed at Miyazaki Biotechnology Shubyou Center (Miyazaki, Japan) and cultivated by a local farmer at Miyazaki; Hyakunari was developed at Kurume Shubyou Engei in Kurume (Fukuoka, Japan) and cultivated by a local farmer at Miyazaki. Bitter melon powder of these varieties were prepared according to the method described elsewhere (Jayasooriya et al., 2000). In brief, unripe bitter melons were washed thoroughly in water and seeds were removed. The remaining edible portion was cut into small pieces, freeze-dried and powdered.

In experiment 2, we further carried out fractionation of Koimidori powder by organic solvents to isolate the active components. The freeze-dried powder of Koimidori variety was mixed with 10 vol of *n*-hexane for 1 h at continuous stirring, room temperature, followed by centrifugation. The supernatant was collected by decantation and the resulting precipitate was mixed again with 10 vol of *n*-hexane for another 1 h with continuous stirring. After centrifugation, the supernatants were combined, and evaporated to dryness using a rotary evaporator in vacuo. The fraction obtained was designated as a hexane extraction. This was followed by two acetone and methanol extractions using the same conditions as described for the hexane extraction, and designated as acetone and methanol fraction, respectively. The resulting precipitate was designated as the residual fraction. These four fractions were kept under a fume hood until the odor of organic solvents had dissipated, and thereafter used for dietary test materials. We obtained 0.8 g of the *n*-hexane fraction, 2.2 g of the acetone fraction, 15.1 g of the methanol fraction, and the residual fraction of 75.1 g out of 100 g of powdered bitter melon. Total recovery was 93.2%. Methanol fraction obtained in experiment 2 was used to analyze the dose–response effect of triglyceride metabolism in experiment 3.

Samples of plant materials (Lot no.: 11002–11004) used in these experiments were stored in the Agricultural Museum, Faculty of Agriculture, University of Miyazaki, Japan.

### 2.2. Animals and diets

Male Sprague–Dawley rats (weighing 195–210 g in experiment 1 and 145–155 g in experiments 2 and 3, respectively) obtained from Kyudo Co. (Kumamoto, Japan) were given a pellet-stoked chow (Type CE-2, Clea Japan, Tokyo, Japan) and were acclimated for several days in a temperature-controlled room (22–24 °C, light on 07:00–19:00 h). They were then divided into groups with equal mean weight as shown in Table 1. The control diet was prepared according to the recommendations of the American Institute of Nutrition (1977) and contained (by % by weight): casein, 20; corn oil, 5; vitamin mixture (AIN 76), 1.0; mineral mixture (AIN 76), 3.5; D,L-methionine, 0.3; choline bitartrate, 0.2; cellulose, 5; corn starch, 15; and sucrose to 100%. In experiment 1, three varieties of bitter melon powder were added to the control diet at 3.0% at the expense of sucrose. In experiment 2, bitter melon (Koimidori) powder 3.0%, hexane extraction 0.1%, acetone extraction 0.2%, and methanol extraction 1.0% (all of which were fractionated from Koimidori used in experiment 1) were added at the expense of sucrose. The residual fraction was added at 3.0% at the expense of cellulose. In experiment 3, the same methanol fraction as used in the experiment 2 was added at levels of 0.5, 1.0, and 3.0% at the expense of sucrose. In all experiments, the rats had free access to the diets and water for 14 days. Food intake and body weight were recorded every other day. Animal experiments were conducted according to internationally accepted principles of laboratory animal use, endorsed by University of Miyazaki.

### 2.3. Analytical procedures

The animals were killed by decapitation, and blood was collected. Livers were excised, rinsed in ice-cold saline, dried on filter paper, and weighed following the removal of extrahepatic tissues. The serum and livers were stored at −80 °C until analyses were performed. Lipids in serum and liver were extracted and purified according to Folch et al. (1957). Triglyceride, cholesterol and phospholipid were measured by the methods described elsewhere (Fukuda et al., 1995). Cholesterol ester was calculated as the difference between total and free cholesterol.

### 2.4. Statistics

Data were analyzed by one-way analysis of variance, and the statistical significance of the difference of the means was evaluated at the level of $P < 0.05$ (Duncan, 1955).

## 3. Results

### 3.1. Food intake, growth, weight of liver and adipose tissues

Neither feeding with three different varieties of dietary bitter melon at 3.0% of the diet nor the feeding with the various fractions isolated by organic solvents, influenced food intake and body weight gain during the experimental period in these three experiments, as shown in Tables 1–3. Relative liver weight, when compared to that of control rats, was

Table 1
Effects of three different varieties of *Momordica charantia* on growth parameters, serum glucose and lipid parameters of serum and liver in rats fed cholesterol-free diet (experiment 1)

| | Control | Bitter melon | | |
|---|---|---|---|---|
| | | Koimodori | Powerful-Reishi | Hyakunari |
| Growth parameters | | | | |
| Initial body weight (g) | 203 ± 2 | 204 ± 4 | 204 ± 3 | 204 ± 3 |
| Final body weight (g) | 321 ± 4 | 319 ± 8 | 327 ± 9 | 325 ± 8 |
| Food intake (g per day) | 25.6 ± 0.5 | 24.4 ± 0.5 | 26.4 ± 1.0 | 25.2 ± 0.8 |
| Liver weight (g/100 g BW) | 5.52 ± 0.08 a | 4.97 ± 0.05 b | 5.29 ± 0.13 ab | 5.26 ± 0.16 ab |
| Adipose tissue weight | | | | |
| Retroperitoneal (g/100 g BW) | 1.66 ± 0.15 | 1.51 ± 0.25 | 1.47 ± 0.13 | 1.47 ± 0.16 |
| Epididymal (g/100 g BW) | 1.26 ± 0.08 | 1.29 ± 0.15 | 1.30 ± 0.07 | 1.35 ± 0.12 |
| Serum parameters (mg/dl) | | | | |
| Triglyceride | 471 ± 37 ab | 359 ± 47 a | 500 ± 48 b | 567 ± 25 b |
| Cholesterol | | | | |
| Total | 108 ± 9 | 105 ± 7 | 104 ± 10 | 105 ± 4 |
| Free | 42.0 ± 2.8 | 44.9 ± 5.1 | 43.9 ± 6.1 | 43.7 ± 4.2 |
| Ester (%) | 60.7 ± 1.7 | 57.9 ± 2.7 | 58.1 ± 3.1 | 58.1 ± 3.1 |
| Phospholipid | 263 ± 12 | 247 ± 8 | 292 ± 25 | 289 ± 7 |
| Liver parameters (mg/g liver) | | | | |
| Triglyceride | 47.1 ± 3.7 a | 24.3 ± 4.3 b | 35.8 ± 4.9 ab | 30.8 ± 2.6 b |
| Cholesterol | | | | |
| Total | 2.30 ± 0.10 | 2.10 ± 0.10 | 2.20 ± 0.10 | 2.00 ± 0.10 |
| Free | 1.33 ± 0.03 | 1.41 ± 0.04 | 1.37 ± 0.03 | 1.34 ± 0.05 |
| Ester (%) | 40.4 ± 3.2 | 32.0 ± 3.1 | 35.8 ± 3.3 | 31.8 ± 3.8 |
| Phospholipid | 24.3 ± 0.9 | 25.5 ± 0.7 | 25.2 ± 0.8 | 27.6 ± 0.7 |

The values are means ± S.E. for seven rats per group.
Values not sharing common letters (a, b) are significantly different at $P < 0.05$.

Table 2
Effects of different extracts of Koimidori variety of bitter melon on serum glucose, serum and liver lipid parameters in rats fed the cholesterol-free diet (experiment 2)

| | Bitter melon | Extracts | | | |
|---|---|---|---|---|---|
| | | Hexane | Acetone | Methanol | Residue |
| Growth parameters | | | | | |
| Initial body weight (g) | 150 ± 5 | 150 ± 4 | 152 ± 3 | 149 ± 5 | 148 ± 5 |
| Final body weight (g) | 282 ± 11 | 271 ± 4 | 280 ± 5 | 274 ± 80 | 285 ± 11 |
| Food intake (g per day) | 23.0 ± 1.0 | 22.6 ± 0.55 | 23.4 ± 0.55 | 24.8 ± 0.65 | 24.1 ± 1.0 |
| Liver weight (g/100 g BW) | 5.50 ± 0.08 a | 5.89 ± 0.10 bc | 6.08 ± 0.18 b | 5.65 ± 0.13 ac | 5.81 ± 0.6 ab |
| Adipose tissue weight | | | | | |
| Retroperitoneal (g/100 g BW) | 1.23 ± 0.13 | 1.15 ± 0.11 | 1.22 ± 0.07 | 1.26 ± 0.06 | 1.37 ± 0.1 |
| Epididymal (g/100 g BW) | 1.24 ± 0.10 | 1.25 ± 0.08 | 1.22 ± 0.04 | 1.22 ± 0.04 | 1.24 ± 0.1 |
| Serum parameters (mg/dl) | | | | | |
| Triglyceride | 411 ± 55 | 536 ± 42 | 428 ± 56 | 383 ± 34 | 509 ± 97 |
| Cholesterol | | | | | |
| Total | 116 ± 5 a | 114 ± 4 a | 94 ± 5 b | 108 ± 7 ab | 118 ± 6 a |
| Free | 31.7 ± 2.1 ab | 29.0 ± 1.2 ab | 26.5 ± 2.1 ab | 25.0 ± 2.0 a | 32.1 ± 2.2 b |
| Ester (%) | 73.0 ± 2.1 ab | 74.5 ± 1.2 ab | 71.9 ± 1.4 a | 76.7 ± 1.1 b | 72.8 ± 1.2 ab |
| Phospholipid | 292 ± 22 | 284 ± 13 | 238 ± 8 | 251 ± 15 | 288 ± 19 |
| Liver parameters (mg/g liver) | | | | | |
| Triglyceride | 36.2 ± 9.1 a | 68.9 ± 11.8 ab | 97.8 ± 15.6 b | 39.1 ± 6.5 a | 60.3 ± 9.8 a |
| Cholesterol | | | | | |
| Total | 2.23 ± 0.07 a | 3.05 ± 0.12 bc | 3.35 ± 0.25 b | 2.52 ± 0.17 a | 2.70 ± 0.17 ac |
| Free | 1.59 ± 0.07 | 1.85 ± 0.14 | 1.86 ± 0.13 | 1.57 ± 0.12 | 1.53 ± 0.04 |
| Ester (%) | 28.8 ± 2.9 a | 38.8 ± 4.7 ab | 42.8 ± 5.4 b | 37.5 ± 3.8 ab | 42.1 ± 3.9 b |
| Phospholipid | 25.7 ± 0.6 | 24.7 ± 0.8 | 25.9 ± 0.9 | 27.1 ± 0.4 | 26.0 ± 0.6 |

The values are means ± S.E. for seven rats per group.
Values not sharing common letters (a, b) are significantly different at $P < 0.05$.

Table 3
Effects of increasing amounts of the methanol extract of Koimidori variety of bitter melon on serum glucose, serum and liver lipid parameters in rats fed the cholesterol-free diet (experiment 3)

| | Control | Extract of methanol | | |
|---|---|---|---|---|
| | | 0.5% | 1.0% | 3.0% |
| Growth parameters | | | | |
| Initial body weight (g) | 151 ± 2 | 150 ± 2 | 146 ± 3 | 153 ± 3 |
| Final body weight (g) | 281 ± 5 | 283 ± 5 | 286 ± 8 | 285 ± 6 |
| Food intake (g per day) | 23.6 ± 0.4 | 25.5 ± 0.6 | 25.9 ± 0.9 | 24.5 ± 0.5 |
| Liver weight (g/100 g BW) | 6.13 ± 0.25 | 5.88 ± 0.21 | 6.10 ± 0.1 | 5.99 ± 0.1 |
| Adipose tissue weight | | | | |
| Retroperitoneal (g/100 g BW) | 1.31 ± 0.09 | 1.37 ± 0.09 | 1.26 ± 0.16 | 1.02 ± 0.11 |
| Epididymal (g/100 g BW) | 1.13 ± 0.03 | 1.27 ± 0.06 | 1.17 ± 0.12 | 1.06 ± 0.07 |
| Serum parameters (mg/dl) | | | | |
| Triglyceride | 400 ± 79 | 508 ± 43 | 460 ± 67 | 378 ± 45 |
| Cholesterol | | | | |
| Total | 112 ± 6 | 106 ± 6 | 124 ± 18 | 102 ± 7 |
| Free | 30.7 ± 1.7 | 27.2 ± 2.1 | 30.9 ± 2.2 | 26.0 ± 1.6 |
| Ester (%) | 71.9 ± 2.3 | 74.2 ± 2.0 | 73.2 ± 2.9 | 73.3 ± 2.7 |
| Phospholipid | 255 ± 11 | 252 ± 8 | 255 ± 24 | 269 ± 12 |
| Liver parameters (mg/g liver) | | | | |
| Triglyceride | 63.7 ± 7.9 a | 51.8 ± 6.7 a | 36.0 ± 5.0 b | 25.3 ± 3.0 b |
| Cholesterol | | | | |
| Total | 3.12 ± 0.21 a | 2.86 ± 0.15 a | 2.41 ± 0.08 b | 2.09 ± 0.06 b |
| Free | 1.58 ± 0.07 | 1.59 ± 0.07 | 1.39 ± 0.06 | 1.44 ± 0.07 |
| Ester (%) | 47.5 ± 5.4 a | 44.5 ± 3.9 a | 41.5 ± 3.9 ab | 31.1 ± 2.9 b |
| Phospholipid | 24.3 ± 0.6 | 28.4 ± 0.8 | 24.1 ± 0.7 | 25.1 ± 0.8 |

The values are means ± S.E. for seven rats per group. Values not sharing common letters (a, b) are significantly different at $P < 0.05$.

significantly reduced in rats fed diets containing Koimidori at 3.0%, but not of the other two varieties (experiment 1). No enlargement of the liver was noted in rats fed diets containing up to 3.0% of the methanol fraction as compared to those of control rats (experiment 3). However, this parameter significantly increased in rats fed the acetone fraction while no such effect was noted in rats fed diets containing fractions extracted by hexane or methanol as well as in the animals fed diets containing the residual fraction. The weight of retroperitoneal adipose tissue, but not of epididymal adipose tissue, tended to decrease with increasing amounts of dietary methanol fraction (up to 3.0% level), although no significant difference was noted between the groups (experiment 3).

### 3.2. Serum lipids

The effects of the serum lipid parameters were marginal for the three different varieties of bitter melon powder as well as of the fractions extracted from Koimidori variety, except for the triglycerides in experiment 1. Thus, at the dietary levels of 3.0%, the concentration of serum triglyceride was lowest for the Koimidori group, and highest for Hyakunari group, Powerful-Reishi group being intermediate. The level of serum triglyceride observed in rats fed diets containing Hyakunari was significantly higher than in those fed control diets free of bitter melon. No effect of these varieties on the concentration of other lipid parameters such as cholesterol and phospholipid was noted in these three experiments.

### 3.3. Liver lipids

The effects of the three varieties of bitter melon on liver triglyceride concentration were very clear. Koimidori was most effective in lowering hepatic triglyceride concentration, and to a lesser extent by Hyakunari and Powerful-Reishi, respectively. This effect was statistically significant in Koimidori and Hyakunari. The reduction in triglycerides, when expressed as a percentage compared to the control, were 48.4% for Koimidori, 34.6% for Hyakunari and 24.8% for Powerful-Reishi.

We compared the effect of bitter melon (Koimidori variety) and the four organic solvent isolated fractions from this bitter melon on liver triglyceride concentration (Table 2) and found that the concentration was lowest in the methanol fraction fed group and highest in the acetone fraction fed group. The liver triglyceride levels in rats fed Koimidori alone was comparable to the levels observed in the rats fed the methanol fraction whereas the triglyceride levels in the rats fed the acetone fraction was significantly higher.

We further examined the dose-dependent effect of this fraction on liver triglyceride at dietary levels up to 3.0%, as shown in Table 3. Clearly, there was a dose-dependent reduction in the concentration of liver triglyceride; the extents of reduction were 18.6, 43.5, and 60.3%, for 0.5, 1.0, and 3.0% dietary levels, respectively. The reduction was already statistically significant at 1.0% compared to the control group. Interestingly, we found a dose-dependent reduction in the concentration of total liver cholesterol. The extent of

reductions was 8.7, 22.8, and 33.0% for the dietary levels of 0.5, 1.0, and 3.0%, respectively, with the difference being statistically significant at 1.0 and 3.0%. However, the effects of these fractions on serum triglyceride levels were marginal. On the other hand, the free cholesterol levels were not different between the groups, whereas the percentages of cholesterol ester decreased in a dose-dependent manner in rats fed diets containing increasing amounts of the methanol fraction (the difference between control and 3.0% group was statistically significant). The level of methanol fraction up to 3.0% in the diet had no effect on liver phospholipid concentration.

## 4. Discussion

Bitter melon had been used empirically in oriental countries as a traditional herb and/or vegetable that lowers blood glucose for a long time (Karunanayake et al., 1984; Platel and Srinivasan, 1997). Research of bitter melon has therefore centered on the question of whether this ingredient evidently exhibits a hypoglycemic effect in normal and diabetic rats as well as in human subjects with type II diabetes mellitus (Leatherdale et al., 1981; Bailey et al., 1985; Welihinda et al., 1986; Ali et al., 1993). Following these experiments, the mechanism underlying the hypoglycemic action of bitter melon still remains unclear, but it has been suggested that this action is exerted through an inhibitory effect on digestive enzymes and on absorption of glucose (Meir and Yaniv, 1985), increased utilization of glucose by the liver (Welihinda and Karunanayake, 1986; Shibib et al., 1993), or an insulin secretagogue action (Higashino et al., 1992).

We have previously reported that freeze-dried bitter melon powder prepared from Olympia variety causes a marked reduction in the concentration of serum glucose in the absence of cholesterol, but not in the presence of cholesterol in the diet (Jayasooriya et al., 2000). Further, we found for the first time a serum and liver triglyceride-lowering effects of bitter melon (Olympia variety) in rats (Jayasooriya et al., 2000).

As no additional information, except for our previous observations, is available regarding the effects of bitter melon on lipid parameters, especially on triglyceride levels (Jayasooriya et al., 2000), we therefore focus on finding the active component(s) of bitter melon that exhibits a conspicuous liver triglyceride-lowering potential.

In this experiment, we showed that the methanol fraction isolated from bitter melon, especially from Koimidori variety, contains some favorable component(s) which modify triglyceride metabolism without any detrimental side effects on growth parameters of rats (Tables 2 and 3). In the first series of experiments, we compared the efficacy of the triglyceride-lowering activity between three different varieties of bitter melon, which were cultivated by a local farmer in Miyazaki, Japan. As shown in Table 1, Koimidori at the dietary level of 3.0% was the most effective in lowering hepatic triglyceride levels followed by Hyakunari and Powerful-Reishi at lesser extents. This result is consistent with previous observations using the Olympia variety bitter melon (Jayasooriya et al., 2000), suggesting that there are some differences in the ability to lower the concentration of hepatic triglycerides, probably due to different concentrations of the active components in these bitter melon varieties.

Since we have found that Koimidori is the most effective bitter melon variety with respect to its potential to lower hepatic triglyceride concentration, the second experiment was performed to find out the active component(s) by fractionation of Koimidori powder by organic solvents, such as *n*-hexane, acetone, and methanol. The effects of the four fractions on serum and liver triglyceride-lowering activity were compared with that of Koimidori powder itself. The results clearly showed that liver triglyceride concentrations in rats fed diets containing the methanol fraction at 1.0% level is similar to those fed un-fractionated Koimidori at 3.0% whereas the two other fractions extracted by *n*-hexane and acetone, or the residual fraction, increased hepatic triglyceride levels. Thus, the potent active component(s) of Koimidori lowering liver triglyceride concentrations is found to be concentrated in the methanol fraction of this bitter melon.

In the third experiment, we furthermore examined how much of methanol fraction is required for a marked reduction in the concentration of liver triglyceride, and found it to be less than 1.0% supplementation. On the other hand, the effect of dietary methanol fraction on serum triglyceride concentration was marginal, even when there was a marked reduction of liver triglyceride. The reason for this remains uncertain. The liver triglyceride lowering activity of the methanol fraction at this relatively low dietary level suggests, that the active component(s) of the methanol fraction is absorbed from intestinal tract, incorporated into hepatic tissues, and influenced the metabolism of triglyceride therein.

Another intriguing observation in this experiment is that methanol fraction is able to lower liver cholesterol concentration in a dose-dependent manner. As free cholesterol remains unchanged, these effects are in part attributed to a decreased accumulation of cholesterol ester. This suggests that a component(s) in the methanol fraction of Koimidori is able to lower the concentration of triglyceride as well as that of cholesterol in the liver. The mechanism responsible for the observed reduction in the concentration of liver cholesterol ester remains uncertain. Clarification of this issue awaits further studies.

Regarding the cholesterol parameters, Platel et al. (1993) observed a significant reduction in the concentration of serum cholesterol in normal rats, but not in streptozotocin induced diabetic rats (Platel and Srinivasan, 1995), when fed diets containing 0.5% bitter melon. Hepatic cholesterol was not measured in their experiments. Further, they did not suggest a mechanism responsible for the observed reduction in the serum cholesterol concentrations. In the present studies, we observed no difference in serum cholesterol levels. The cause of the observed differences between

our results and their results might have caused by different concentration of active component(s) between these bitter melons, since we observed bitter melon variety-dependent different effect on liver triglyceride level (Table 1).

Bitter melon is known to contain several active components; one of these components is called charantin, a plant sterol glycoside, which is believed to be in part responsible for the hypoglycemic effect (Wong et al., 1985), and the other is momordin, a oleanolic acid glycoside, which is responsible for antitumor activity (Lee et al., 1998). Plant sterols themselves are known to exert a potent hypocholesterolemic effect through inhibition of cholesterol absorption in the intestinal tract (Moghadasian and Frohlich, 1999), but they show only minor triglyceride lowering activity. In addition, this effect is seen in rats fed diets enriched with cholesterol in combination with relatively large amounts of plant sterols (Ling and Jones, 1995). Accordingly, charantin is not likely to be responsible for the observed reduction in the concentration of triglyceride in the liver. At present, no information is available with regard to a link between the active component(s) reported previously and hepatic triglyceride-lowering activity. Further studies to reveal those active component(s) are being carried out in our laboratory.

In summary, the results of the present study, together with those of a preceding experiment (Jayasooriya et al., 2000), indicate that bitter melon is a useful vegetable that relieves and/or ameliorates life style-related diseases, such as fatty liver, hypertriglyceridemia and diabetes, with no undesirable effects for instance growth retardation detected in rats.

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# Vasodilator activity of *Michelia figo* Spreng. (Magnoliaceae) by in vitro functional study

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**Abstract**

The methanolic extract of leaves of *Michelia figo* Spreng. (Magnoliaceae), as well as several purified fractions, showed a concentration-dependent vasorelaxing effect on aortic rings endothelium-deprived and pre-contracted by norepinephrine (NE). For further pharmacological investigation on the mechanism of action, the fraction S4 was selected, since it showed the best vasodilator properties.

The pharmacological effect was not produced through the stimulation of cyclooxygenase, adenyl cyclase, or guanylyl cyclase, since selective inhibitors did not prevent the fraction S4-induced effects. Moreover, the vasorelaxing effect of the fraction was resistant to the block of nifedipine-sensitive $Ca^{2+}$ channels.

The fraction S4 ($10^{-4}$ g/ml) produced a shift towards the right of the concentration–contractile response curve to NE, in normal conditions, and the shift was more evident in $Ca^{2+}$-free Tyrode solution, suggesting an action on intracellular $Ca^{2+}$-channels.

The vasodilator action of fraction S4 on NE pre-contracted rings was not prevented by cyclopiazonic acid (blocker of $Ca^{2+}$/ATPase), which excludes a role for mechanisms involving the storage of $Ca^{2+}$ in the sarcoplasmic reticulum.

The reduction of the contraction elicited by caffeine, an opener of ryanodine-sensitive receptors, suggests that the fraction S4 of *Michelia figo* leaves could produce the vasorelaxing response by the blockade of ryanodine-sensitive $Ca^{2+}$ channels of the sarcoplasmic reticulum.
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*Keywords:* *Michelia figo*; Magnoliaceae; Rat aorta; Ryanodine-sensitive $Ca^{2+}$ channels; Vasorelaxing response

## 1. Introduction

Species belonging to *Michelia* genus are arboreous plants, growing in temperate zone of oriental India, southern China, Malaysia, and Indonesia. The species more utilized is *Michelia champaca*: its cortex and seeds are used as febrifuge and tonic-aromatic, roots are employed as emmenagogue, leaves as astringent, gemmae in the hemorrhage treatment, and flowers and fruits are believed to possess curative properties in enteritis. Less known species, as *Michelia figo*, are used as ornamental plants and to obtain essences. *Michelia figo* is an evergreen medium shrub, commonly called banana shrub, because of the heavy, sweet fragrance banana scent of its purple flowers. The plant is also known, in the folk Indian medicine, as remedy against hypertension (Woianon, 1976).

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Aim of the present study was to evaluate the vascular effect of the methanolic extract of *Michelia figo* leaves and, if any, to characterize the pharmacodynamic profile.

## 2. Materials and methods

### 2.1. Phytochemistry procedure

The leaves of *Michelia figo* were collected in April in Horti Botanici in Lucca (LU, Italy). The fresh material (750 g) were extracted with methanol (5 l), for 3 days, at room temperature. After filtration and evaporation of the solvent under reduced pressure, a crude extract (79.4 g) was obtained. Then, the extract was purified from chlorophylls by treatment with a water/ethylacetate/ethanol/petroleum ether (10:4:7:7) solution. The polar phase was submitted to evaporation of the solvent and the residue (17.15 g) was chromathographed on a Sephadex LH-20 column, eluting first

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with methanol and then with water to obtain 123 fractions that have been combined into 14 homogeneous fractions by mean of TLC analyses (S1–S14). The crude methanolic extract and all the fractions were submitted to the preliminary pharmacological investigations. S4 (1.7 g) was submitted to the further study aimed to detect the mechanism of action.

### 2.2. Pharmacological procedure

Male Wistar rats ($250 \pm 20$ g) were used, according with the guidelines of the European Community Council Directive 86/609. Isolated aortic rings, deprived of endothelium layer, were prepared according with the method previously described (Baragatti et al., 2002).

After an equilibration period of 60 min, preparations were contracted by norepinephrine (NE, 3 μM) and only aortae showing an acethylcholine (Ach, 10 μM)-induced relaxation $\leq$10%, indicative of an effective removal of the endothelium, were utilized for experiments. After a wash-out and an equilibration period of 60 min, three different experimental protocols were applied.

#### 2.2.1. 1° Protocol

The aortic preparations were pre-contracted by a single concentration of NE (3 μM) and then threefold increasing concentrations of crude methanolic extract or of the several purified fractions of *Michelia figo* ($10^{-6}$ to $10^{-3}$ g/ml) were added cumulatively to the organ bath.

When required by the experimental protocol, the pre-treatment with indomethacin (1 μM), ODQ (1 μM), SQ 22,536 (1 μM), nifedipine (1 μM) or cyclopiazonic acid (20 μM) was performed 30 min before the administration of NE 3 μM.

#### 2.2.2. 2° Protocol

The aortic rings were contracted with increasing cumulative concentrations of NE (0.1 nM–0.1 mM). Preparations were washed and after 30 min of equilibration a new cumulative curve with NE was performed; preliminary experiments had showed that the first concentration–response curve was almost identical to the second curve. Then, the pre-treatment with the fraction S4 ($10^{-4}$ g/ml) was carried out in the equilibration period. This experimental protocol was performed either in standard Tyrode solution or in $Ca^{2+}$-free Tyrode resolution.

#### 2.2.3. 3° Protocol

The aortic preparations were contracted by a single maximal concentration of caffeine (5 mM). After washing with Tyrode solution, the rings were incubated with the fraction S4 ($10^{-4}$ g/ml), 30 min before of a new addition of caffeine (5 mM).

### 2.3. Drugs

Norepinephrine bitartrate, cyclopiazonic acid, ODQ (1*H*-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one) and indomethacin from Sigma; caffeine, SQ 22,536 (9-(tetrahydro-2-furanyl)-9*H*-purin-6-amine) and nifedipine from RBI.

Indomethacin, ODQ and nifedipine were dissolved ($10^{-3}$ M) in absolute ethanol and further diluted in bidistilled water, cyclopiazonic acid was dissolved ($10^{-3}$ M) in dimethylsulfoxide (DMSO) and further diluted in bidistilled water, while all the other drugs were dissolved in bidistilled water. All solutions were freshly prepared on the day of the experiment.

## 3. Data analysis

### 3.1. 1° Protocol

The vasorelaxing efficacy and the potency of the fraction S4 were expressed, respectively, as $E_{max}$ = maximal vasorelaxing response, evaluated as percent relaxation of the NE-induced contraction, and $pIC_{50} = -\log_{10} IC_{50}$, calculated as concentration (g of extract/ml of organ bath) required to produce a half-reduction of the NE-induced contracting tone.

### 3.2. 2° Protocol

When cumulative curves with NE were performed, all the levels of response of the second curve were expressed as percent contraction of the maximal response of the first curve. The effect of fraction S4 of *Michelia figo* was expressed as efficacy and potency of NE after the incubation period. $E'_{max}$ = maximal contracting response was evaluated as percent of the maximal contraction of the first curve, and $pEC_{50} = -\log_{10} EC_{50}$, was calculated as molar concentration required to produce a half-effect in the NE concentration–response curves.

### 3.3. 3° Protocol

When caffeine was used as contracturant agent, changes of the caffeine-induced contraction obtained in the presence of the fraction ($10^{-4}$ g/ml), expressed as percent of the control caffeine-induced contraction (obtained in the absence of *Michelia figo*), were evaluated.

All analyses were performed by sigmoidal non-linear regression of concentration–response curves (computer program: Graph Pad Prism® 3.00).

All data are expressed as means ± S.E.M. of six experiments. The significance of differences was evaluated by means of ANOVA and Student's *t*-test for unpaired data. $P \leq 0.05$ was considered significant.

## 4. Results

In aortic rings pre-contracted by NE 3 μM, the methanolic extract of *Michelia figo* produced a concentration-dependent

Fig. 1. Concentration–response curves by fraction S4 of *Michelia figo* on NE-pre-contracted rat aortae, (■) in the absence, or in the presence of (●) indomethacin (1 μM), (▲) ODQ (1 μM), or (▼) SQ 22,536 (1 μM). The responses are expressed as percent of the maximal NE-induced contraction. In the abscissa, the g/ml concentrations of the fraction S4 of *Michelia figo* are expressed as logarithms. Vertical lines represent the S.E.M.

vasorelaxing response, and among the different fractions, the fraction S4 was the most active ($pIC_{50} = 4.03 \pm 0.03$ and $E_{max} = 97.3 \pm 0.8\%$, Fig. 1), thereafter only this one underwent to further pharmacological tests.

The pre-treatment with indomethacin (a selective aspecific inhibitor of cyclooxygenase, 1 μM) did not significantly influence the action of the fraction S4; moreover, neither SQ 22,536 (a inhibitor of adenyl cyclase, 1 μM) or ODQ (a guanylyl cyclase inhibitor, 1 μM) produced a significant change of the fraction S4 response (Fig. 1). The vasorelaxing response of the fraction S4 was not prevented by the pre-treatment of aortic rings with nifedipine (1 μM): the concentration–response curve was not significantly shifted to the right ($pIC_{50} = 4.30 \pm 0.03$, $E_{max} = 98.7 \pm 1.3\%$, data not shown).

Moreover, the fraction S4 ($10^{-4}$ g/ml) shifted significantly the cumulative concentration–response curve NE-induced (0.1 nM–0.1 mM) in normal conditions ($pEC_{50} = 8.23 \pm 0.05$, $E'_{max} = 100\%$ for the first curve; $pEC_{50} = 5.57 \pm 0.05$ and $E'_{max} = 79.6 \pm 3.7\%$ for the second curve), but more markedly in $Ca^{2+}$-free Tyrode solution ($pEC_{50} = 7.70 \pm 0.05$ and $E'_{max} = 100\%$ for the first curve; $pEC_{50} = 5.10 \pm 0.38$ and $E'_{max} = 24.4 \pm 4.7\%$ for the second curve; Figs. 2 and 3) was observed. Cyclopiazonic acid (20 μM), a blocker of $Ca^{2+}$/ATPase of the sarcoplasmic reticulum, did not prevent the vasorelaxing effect of the fraction S4 ($pIC_{50} = 4.33 \pm 0.04$, $E_{max} = 94.6 \pm 5.4\%$, Fig. 4). The fraction S4 ($10^{-4}$ g/ml) caused a marked inhibition of the contraction induced by caffeine (an opener of ryanodine-sensitive $Ca^{2+}$ channels), which resulted $30.6 \pm 1.8\%$ of the control caffeine-induced contraction (data not shown).

Fig. 2. Concentration–response curves with NE, in standard Tyrode solution, (■) in the absence or (▲) in the presence of fraction S4 of *Michelia figo* ($10^{-4}$ g/ml). In the abscissa, the molar concentrations of the NE are expressed as logarithms. Vertical lines represent the S.E.M.

Fig. 3. Concentration–response curves with NE, in free $Ca^{2+}$-Tyrode solution, (■) in the absence or (▲) in the presence of fraction S4 of *Michelia figo* ($10^{-4}$ g/ml). In the abscissa, the molar concentrations of the NE are expressed as logarithms. Vertical lines represent the S.E.M.

Fig. 4. Concentration–response curves by fraction S4 of *Michelia figo* on NE-pre-contracted rat aortae, (■) in the absence, or (▲) in the presence of cyclopiazonic acid (20 μM). The responses are expressed as percent of the maximal NE-induced contraction. In the abscissa, the g/ml concentrations of the fraction S4 of *Michelia figo* are expressed as logarithms. Vertical lines represent the S.E.M.

## 5. Discussion

In the present study, the fraction S4 of the methanolic extract of leaves of *Michelia figo* showed endothelium-independent vasodilator properties (Fig. 1).

The *Michelia figo*-induced response was not caused by cyclooxygenase, adenyl cyclase, or guanylyl cyclase pathways, since neither indomethacin (1 μM), nor SQ 22,536 (1 μM), nor ODQ (1 μM) antagonized the effect of the plant extract (Fig. 1).

*Michelia figo*-induced vasorelaxing effect was not affected by the block of the nifedipine-sensitive $Ca^{2+}$ channels; moreover the shift by the fraction S4 ($10^{-4}$ g/ml) of the cumulative concentration–response curve for NE, both in the presence (Fig. 2) and in the absence of extracellular $Ca^{2+}$ (Fig. 3) suggested a mechanism of action linked to a stimulation of the intracellular $Ca^{2+}$ uptake or intracellular $Ca^{2+}$ release mechanisms from the sarcoplasmic reticulum.

The accumulation of $Ca^{2+}$ into the stores of the sarcoplasmic reticulum is accomplished by a $Ca^{2+}$/ATPase (Suzuki et al., 1992), and two major pathways for $Ca^{2+}$ release from endoplasmic reticulum are recognized: one is operated by $IP_3$ (the $IP_3$ receptor), while the other is ryanodine-sensitive (Maissner, 1994; Ferris and Snyder, 1992).

Cyclopiazonic acid, a blocker of $Ca^{2+}$/ATPase, did not modify the concentration–response curve of *Michelia figo*, suggesting a role of the ryanodine-sensitive or of the $IP_3$-sensitive $Ca^{2+}$ channels in the vasorelaxing property of the fraction S4.

This hypothesis was supported by the demonstration that the fraction produced an inhibitory effect on contraction induced by caffeine, an activator of ryanodine receptors (Shima and Blaustein, 1992). It may be concluded that the vasorelaxing effects of *Michelia figo* leaves extract are elicited by active principle(s) contained in the fraction S4, through a mechanism of action which probably involves the block of ryanodine-sensitive $Ca^{2+}$ channels, localized on sarcoplasmic reticulum.

The direct vasorelaxing action detected could be at least in part responsible of the ethno-pharmacological interest growing up from the application of the drug in the Indian folk medicine.

Attempts to isolate the active principle(s) of *Michelia figo* leaves are actually in progress.

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# *Salix caprea* inhibits skin carcinogenesis in murine skin: inhibition of oxidative stress, ornithine decarboxylase activity and DNA synthesis

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**Abstract**

Chemoprevention of free radical-mediated diseases including cancer by natural products is an emerging discipline due to its wider applicability and acceptance. The present study deals with the chemopreventive effect of *Salix caprea* against phorbol ester-induced oxidative stress and tumor promotion in murine skin. In the present investigation, it was observed that a single application of 12-*O*-tetradecanoyl-13-phorbol acetate (TPA) (20 nmol/0.2 ml acetone/animal) caused a significant ($P < 0.05$) depletion of cutaneous antioxidants viz., glutathione, glutathione reductase, glutathione peroxidase, catalase and phase II drug metabolizing enzymes viz., glutathione-*S*-transferase, quinone reductase. An increase in the hydrogen peroxide generation and protein oxidation (measured in terms of protein carbonyl content) was also observed with a single application of TPA. However, the pretreatment of animals with different doses of *Salix caprea* (0.5, 1.0 and 1.5 mg/kg/0.2 ml acetone) caused a significant recovery in the TPA-mediated depletion in antioxidant levels. The pretreatment of animals with *Salix caprea* was observed to inhibit the TPA-mediated depletion in phase II enzymes. It was also observed that *Salix caprea* reversed the TPA-mediated depletion in the activity of phase II enzymes that is an important characteristic of cancer chemopreventive agents. Phorbol esters are known to induce the tumor promotion by increasing rate of DNA synthesis, ornithine decarboxylase activity (ODC), and xanthine oxidase activity. In the present investigation, it was observed that the pretreatment of animals with *Salix caprea* caused a significant ($P < 0.05$) depletion in the TPA-induced DNA synthesis, ODC and xanthine oxidase activity in mice skin. *Salix caprea* significantly reduced the tumor promotion in mice skin when tested in two-stage chemical carcinogenesis model. It was observed to inhibit significantly ($P < 0.05$) the 7,12-dimethyl benz[*a*] anthracene (DMBA)-initiated phorbol ester promoted skin carcinogenesis. It was concluded from the results that *Salix caprea* is an effective antioxidant and chemopreventive agent against phorbol ester-induced tumor promotion.
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*Keywords:* Chemoprevention; Oxidative stress; *Salix caprea*; Ornithine decarboxylase; Phase II enzymes

## 1. Introduction

Oxidative stress and inflammation have been reported to be closely associated with tumor promotion stage of carcinogenesis (Jang and Pezzuto, 1998). Many known inflammatory agents such as phorbol esters, e.g. 12-*O*-tetradecanoyl-13-phorbol acetate (TPA), are also potent tumor promoters and have been reported to act through the generation of reactive oxygen species (ROS) (Boutwell, s1974; Blumberg, 1980, 1981; Cerruti, 1985; Repine et al., 1974). The generation of ROS and with its subsequent accumulation induces oxidative stress at cellular level. Free radicals have been shown to be capable of damaging many cellular components such as DNA, proteins and lipids (Halliwell and Arouma, 1991; Liggins and Furth, 1997; Reznick and Packer, 1994; Stadtman, 1990). Proteins can be damaged by oxygen radicals leading to a loss of enzymatic activity and the conversion of amino acids to carbonyl derivatives (Cakatay et al., 2000). The common feature of most of the tumor promoters is that these induce oxidative stress, increase ornithine decarboxylase (ODC) activity and enhance rate of DNA synthesis. Many anti-inflammatory agents synthetic as well as natural are reported to possess antitumor property (Rowlands, 1985). Most of the antitumor agents have been shown to act through the inhibition of oxidative stress, ODC activity and DNA synthesis (Sartorelli and Johns, 1975; Colvin et al., 1976; Tong and Ludlum, 1980; Iqbal and Athar, 1998). Recently, we have shown that some common anti-inflammatory medicinal plants such as *Mentha spicata*, *Tephrosia purpurea*, *Cheiranthus cheiri*, *Lawsonia alba* and *Myrica nagi* act as potent antioxidants as well as antitumor agents (Saleem et al., 2001a,b; Ahmed

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et al., 2000; Alam et al., 2000). *Salix caprea* L. (Family: Salicaceae, common name: goat willow) an ornamental plant, has been reported to possess strong anti-inflammatory property and is used as astringent, antiseptic, eye tonic, antipyretic, analgesic and cardiotonic in Indian System of Medicine (Bhattacharjee, 1998; Phodke, 1990; Hollman, 1991; Kallman, 1994; Tunon et al., 1995; Chopra et al., 1996). Phytochemical investigations of *Salix caprea* have revealed the presence of many potent anti-oxidants such as luteolin, dihydrokaempferol and quercetin as its principle constituents along with (+)-catechin and isorhamnetin as minor constituents (Malterud et al., 1985; Mehrotra, 1991; Nasudari et al., 1972; Park et al., 1999; Rastogi and Mehrotra, 1991; Thapliyal and Bahugana, 1993). Further, *Salix caprea* has been reported to exhibit a strong anti-oxidant activity in many in vitro systems (Rohnert et al., 1998). Taking in consideration, its anti-inflammatory and anti-oxidant potential, it was speculated if *Salix caprea* could act as antioxidant in vivo and prevent tumorigenesis. The aim of present investigation was to show that *Salix caprea* inhibit the phorbol ester-induced oxidative stress, ODC activity and DNA synthesis. *Salix caprea* was also tested for its antitumor promotion potential by two-stage chemical carcinogenesis protocol in Swiss albino mice skin.

## 2. Chemicals

Reduced glutathione (GSH), oxidized glutathione (GSSG), nicotinamide adeninedinucleotide phosphate reduced (NADPH), flavine adeninedinucleotide (FAD), nitroblue tetrazolium (NBT), xanthine oxidase, xanthine, dinitrophenyl hydrazine (DNPH), bovine serum albumin (BSA), calf thymus DNA, 1,2-dithio-bis-nitrobenzoicacid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), glutathione reductase, horseradish peroxidase, phenol red, 7,12-dimethyl benz[*a*] anthracene (DMBA), croton oil and 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), were obtained from Sigma Chemicals Co. (St. Louis, MO). [$^{3}$H] thymidine and DL[$^{14}$C] ornithine were purchased from Amersham corporation (Little Chalfort, UK). All other chemicals were of the highest purity commercially available.

## 3. Plant material

The flowers of *Salix caprea* were collected from Kashmir valley, India. The identity of plants was verified by Professor Mohammed Iqbal, Medicinal Plant Division, Department of Environmental Botany, Hamdard University, New Delhi. The voucher (No. Sc-JK 1/JH) was deposited in the Department of Medical Elementology and Toxicology, Hamdard University, New Delhi. The plant material was dried in air and then milled to a fine powder of 1-mm mesh size as described by Antonio and Souza Britto (1998).

## 4. Preparation of extract

The extraction procedure was exactly the same as described earlier (Saleem et al., 1999). Briefly, powdered plant material (200 g) was repeatedly extracted in 4000 ml round bottom flask with 2000 ml solvents of increasing polarity starting with petroleum ether, benzene, ethyl acetate, acetone, methanol and double distilled water. The reflux time for each solvent was 4 h. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotatory evaporator (Buchi Rotavapor). For *Salix caprea* flowers the residues yield for each solvent was 9, 6, 10, 2.0, 2.5 and 4 g. The acetone soluble fraction of *Salix caprea* was used for all in vivo studies. All the extracts were stored at 4 °C.

## 5. Animals

Eight weeks old adult female Swiss albino mice (20–25 g) were obtained from the Central Animal House Facility of Hamdard University, New Delhi and were housed in a ventilated room at $22 \pm 4$ °C under a 12-h light/dark cycle. The mice were allowed to acclimatize for 1 week before the study and had free access to standard laboratory feed (Hindustan Lever Ltd., Bombay, India) and water ad libitum. The dorsal skin of the mice was shaved with an electric clipper (Oster A2) followed by the application of hair removing cream (Anne French, Geoffrey Manners, Bombay, India) at least 2 days before treatment. Only mice showing no signs of hair re-growth were used for experiments.

## 6. Treatment of animals

*Salix caprea* extract was applied on dorsal side of mice skin in a manner to prevent spill over and complete dryness of skin was ensured. A gap of 1 h was maintained between the drying of skin (after *Salix caprea* pretreatment) and TPA or croton oil application to ensure complete absorption of test material as well as tumor promoter in the tissue. This precaution reduces the chances of interaction between the test material and tumor promoter before reaching to the target site, hence increases bio-availability of both substances.

## 7. Experimental protocol

To study the effect of pretreatment of animals with *Salix caprea* on TPA-mediated cutaneous oxidative stress, 36 female mice were randomly allocated to six groups of six mice in each. The animals of group 1 received a topical application of acetone (0.2 ml/animal) and served as controls. Groups 3–5 received a single topical application of *Salix caprea* at a dose level of 0.5, 1.0 and 1.5 mg/kg body weight, respectively in acetone. The animals belonging to group 6 received

the highest dose (1.5 mg/kg) of plant extract treatment only. One hour after the treatment of *Salix caprea* the animals of groups 2–5 received a single topical application of TPA (20 nmol/0.2 ml acetone/animal). All the animals were sacrificed by cervical dislocation 12 h after TPA treatment and skin was removed quickly and processed for sub-cellular fractionation.

Thirty-six female mice were randomly allocated to six groups of six mice in each to study the effect of pretreatment of animals with *Salix caprea* on TPA-mediated induction of cutaneous ODC activity. The animals of group 1 received topical application of acetone (0.2 ml/animal) and served as controls. The animals of groups 3–5 received single topical application of *Salix caprea* at the dose level of 0.5, 1.0 and 1.5 mg/kg body weight, respectively, in acetone. The animals belonging to group 6 received the highest dose (1.5 mg/kg) of plant extract treatment alone. One hour after the treatment of *Salix caprea*, the animals of groups 2–5 received a single topical application of TPA (20 nmol/animal/0.2 ml acetone). All these mice were sacrificed 6 h after TPA treatment by cervical dislocation. The skin was quickly removed and processed for sub-cellular fractionation.

For studying the effect of pretreatment of animals with *Salix caprea* on TPA-mediated [$^3$H] thymidine incorporation in cutaneous DNA ([$^3$H] thymidine incorporation in DNA is a measure of rate of DNA synthesis), the experimental protocol was exactly identical to that described for ODC activity. One hour after the last treatment of *Salix caprea* or acetone, the animals of groups 2–5 received single topical application of TPA (20 nmol/animal/0.2 ml acetone). 18 h after the treatment with TPA or acetone, the animals of all groups were given [$^3$H] thymidine (20 μCi/animal/0.2 ml saline) as an i.p. injection and were sacrificed after 2 h by cervical dislocation. Their skin tissues were quickly removed, cleaned free of extraneous material and homogenized in cool distilled water for its further processing and separation of DNA.

### 7.1. Treatment of animals for tumor studies

For tumorigenesis studies 4–6 weeks old mice were shaved on dorsal skin with electric clippers 2 days prior to the beginning of the experiment. The animals in the resting phase of hair cycle were used only. One hundred animals were grouped in five groups of 20 animals each. Skin tumors were induced chemically by standard two-stage initiation-promotion protocol. The initiation was accomplished with a single topical application of 25 μg DMBA/animal/0.2 ml acetone in groups 1–4. Ten days after initiation, all of the mice were treated twice weekly with topical application of croton oil (phorbol ester) (0.5%/0.2 ml acetone/animal, v/v) for 25 weeks. Group 1 was considered as control group. For anti-tumor promotion studies, different doses of *Salix caprea* (0.5, 1.0 and 1.5 mg/kg body weight in acetone) were applied topically 60 min prior to each application of croton oil (phorbol ester) in groups 2–4, respectively. Animals belonging to group 5 were treated with only highest dose of *Salix caprea* after initiating with a single application of DMBA. Treatment was continued for 25 weeks. During this period the tumor yield and incidence were recorded weekly. The criteria for the diagnosis of various tumors were the same as described by O'Connel et al. (1986). The data are expressed percent mice with tumors and number of tumors per mouse and are plotted as a function of weeks on test.

### 7.2. Tissue preparation

After the desired time period, control and treated animals were killed by cervical dislocation. The animals were immediately dissected to remove their skin, which was washed in ice cold saline and extraneous materials were removed. All subsequent operations were carried out on ice at temperature not above 4 °C. The tissues were further processed for sub-cellular fractionation. The post-mitochondrial supernatant and cytosolic fraction were prepared by the method as described by Iqbal and Athar (1998).

### 7.3. Estimation of glutathione reduced (GSH), glutathione-S-transferase (GST), glutathione reductase, glutathione peroxidase, catalase and hydrogen peroxide

The reduced glutathione (GSH), glutathione-*S*-transferase (GST), glutathione reductase, glutathione peroxidase, catalase and hydrogen peroxide in skin were determined by the methods as described earlier (Saleem et al., 1999).

### 7.4. Assay for xanthine oxidase

The cutaneous xanthine oxidase activity was estimated by the modified method of Stripe and Della Corte (1969) as described by Rezazadeh and Athar (1998). The post-mitochondrial supernatant (PMS) (10% w/v) was diluted with phosphate buffer (0.1 M, pH 7.4) in 0.2:1 ratio and incubated at 37 °C for 5 min. After incubation, reaction was started by adding 0.1 ml xanthine (1 mM) to the PMS/buffer mixture. The total reaction mixture was incubated at 37 °C for 20 min. The reaction was terminated by the addition of 0.5 ml ice cold perchloric acid (10% v/v). The final reaction mixture was kept at room temperature. After 10 min, 2.5 ml of water was added to the precipitated reaction mixture, which was then centrifuged at 4000 rpm for 10 min. The clear supernatant was read at 290 nm and results were expressed as μg uric acid formed per mg protein.

### 7.5. Quinone reductase activity

The quinone reductase activity was measured by the method of Benson et al. (1980). The assay mixture consisted of 0.1 ml cytosolic fraction (10%), 0.7 ml of BSA (0.1%), 0.02 ml Tween-20 (1%), 0.1 ml of FAD (150 μM), 0.02 ml of NADPH (0.2 mM), 0.05 ml of 2,6-DCIP (0.29%) and 2 ml of Tris–HCl buffer (25 mM pH 7.4) with a final volume of 3 ml and the optical density was read at 600 nm

for 3 min. The enzyme activity was calculated as nmol 2,6-dichloro indophenol (DCIP) reduced/min/mg protein.

### 7.6. Protein carbonyl estimation

Protein carbonyl was estimated by the method of Levine et al. (1990). An aliquot of 1 ml (10% w/v) of cytosolic fraction (105,000 × *g*) treated with an equal volume of 2,4-dinitrophenylhydrazine (0.1%) in 2N HCl and incubated for 1 h at room temperature. This mixture was treated with 0.5 ml of TCA (10% w/v) and after centrifugation, the precipitate was extracted three times with ethanol/ethyl acetate (1/1 v/v). The protein sample was dissolved with 2 ml solution of guanidine hydrochloride 8 M and EDTA 13 mM in Tris–HCl 133 mM (pH 7.2). The optical density was read at 365 nm. The results were expressed as moles of dinitrophenyl hydrazine (DNPH) incorporated/100 mg protein using a molar extinction coefficient of 21 $mM^{-1} cm^{-1}$.

### 7.7. Assay for ornithine decarboxylase activity

Ornithine decarboxylase (ODC) activity was determined using 0.4 ml epidermal (100,000 × *g*) supernatant fraction/assay tube by measuring the release of $^{14}CO_2$ from the DL [$^{14}$C] ornithine by the method of O'Brian et al. (1975) as described earlier (Saleem et al., 2000). The skin tissues were homogenized in Tris–HCl buffer (50 mM, pH 7.5) containing EDTA (0.1 mM), pyridoxal 5-phosphate (0.1 mM), PMSF (0.1 mM), 2-mercaptoethanol (1.0 mM), dithiothreitol (0.1 mM) and Tween-80 (0.1%) at 4 °C using a polytron homogenizer (Kinematica AGPT 3000). In brief, the reaction mixture contained 0.4 ml of epidermal 100,000 × *g* supernatant and 95 μl cofactor mixture containing pyridoxal 5-phosphate (0.32 mM), EDTA (0.4 mM), dithiothreitol (4.0 mM), ornithine (0.4 mM), brij35 (0.02%) and $^{14}$C ornithine (0.05 μCi) in a total volume of 0.495 ml. After adding the buffer and co-factor mixture to blank and other test tube, the tubes were covered immediately with a rubber cork containing 0.2 ml ethanolamine and methoxyethanol mixture in the central well and kept in water bath at 37 °C. After 1 h of incubation, the enzyme activity was arrested by injecting 1.0 ml of citric acid solution (2.0 M). Finally, the solution contained in the central well is transferred to a vial containing 2 ml of ethanol and 5 ml of toluene-based scintillation fluid, followed by counting of radioactivity in a liquid scintillation counter (LKB-Wallace-1410). ODC activity was expressed as pmol $^{14}CO_2$ released/h/mg protein.

### 7.8. Quantitation of epidermal DNA synthesis

The isolation of cutaneous DNA and incorporation of [$^{14}$C] thymidine in DNA was done by the method employed by Smart et al. (1986) as described earlier (Saleem et al., 2000). The skin tissues were quickly excised from the animal, cleaned free of extraneous material and homogenate (20%) was prepared in ice cold water. The precipitate thus obtained was washed with cold trichloroacetic acid (5%) and incubated with cold perchloric acid (10%) at 4 °C for 24 h. After the incubation it was centrifuged and the precipitate was washed with cold perchloric acid (5%). Then the precipitate was dissolved in warm perchloric acid (5%) followed by incubation in boiling water bath for 30 min and filtered through a Whatman 50 paper. The filtrate was used for [$^3$H] thymidine counting in liquid scintillation counter (LKB-Wallace-1410) by adding the scintillation fluid. The amount of DNA in the filtrate was estimated by diphenylamine method of Giles and Mayers (1965). The amount of [$^3$H] thymidine incorporated was expressed as dpm/μg DNA.

### 7.9. Protein estimation

The protein concentration in all samples was determined by the method of Lowrey et al. (1951).

### 7.10. Histopathological study

The histopathological studies were performed as per the standard method described by Luna (1968).

### 7.11. Statistical analysis

The level of significance between different groups was based on Dunnet's *t*-test followed by analysis of variance.

## 8. Result

Treatment of mouse skin with TPA caused overproduction of cellular oxidants and modulation of anti-oxidant defense system. As observed during the study, a single application of TPA alone (20 nmol/0.2 ml acetone/animal) to mouse skin led to modulation of several parameters of oxidative stress, relative to control animals receiving acetone only. Treatment of animals with TPA alone resulted in a 67% depletion of cutaneous glutathione and a 40% decrease in the activity of glutathione reductase as compared to acetone treated control (Table 1). However, pretreatment of animals with *Salix caprea* (at doses 0.5, 1.0 and 1.5 mg/kg body weight) prior to the application of TPA resulted in a significant partial recovery of reduced glutathione and glutathione reductase ranging from 17 to 50% and 16 to 56%, respectively as compared with TPA treated control animals (Table 1). Treatment with TPA alone resulted in the reduced activity of cutaneous anti-oxidant enzymes, i.e., glutathione peroxidase and catalase to the levels 53 and 54% of the acetone treated control, respectively. The recovery of anti-oxidant enzymes with the pretreatment of animals with different doses of *Salix caprea* ranged from 18 to 40% and 12 to 29% as compared with TPA control. The TPA treatment alone caused a significant depletion of 39–67% of the acetone treated control in the levels of phase II enzyme, i.e., quinone

Table 1
Effect of pretreatment with *Salix caprea* on TPA-mediated cutaneous modulation in glutathione level and on the activity of glutathione-*S*-transferase glutathione peroxidase, glutathione reductase catalase and quinone reductase in mice

| Treatment | Reduced glutathione (nmol GSH/g tissue) | Glutathione-*S*-transferase (nmol CDNB conjugate formed/min/mg protein) | Glutathione peroxidase (nmol NADPH oxidized/min/mg protein) | Catalase (nmol $H_2O_2$ consumed/min/mg protein) | Quinone reductase (2,4-DCIP/min/mg protein) | Glutathione reductase (nmol NADPH oxidized/min/mg protein) |
|---|---|---|---|---|---|---|
| Acetone (0.2 ml) | $3.0 \pm 0.3$ | $200.0 \pm 8.0$ | $23.0 \pm 1.5$ | $300.0 \pm 15.0$ | $90.0 \pm 4.0$ | $45.0 \pm 3.0$ |
| TPA (20 nmol/0.2 ml acetone) | $1.0 \pm 0.05^{**}$ | $120.0 \pm 7.0^{*}$ | $11.0 \pm 1.0^{**}$ | $140.0 \pm 8.0^{**}$ | $55.0 \pm 7.0^{*}$ | $15.0 \pm 1.0^{**}$ |
| *Salix caprea* (0.5 mg/kg) + TPA (20 nmol/0.2 ml acetone) | $1.5 \pm 0.08^{++}$ | $145.0 \pm 8.0^{+}$ | $15.0 \pm 1.0^{+}$ | $175.0 \pm 9.0^{++}$ | $70.0 \pm 4.0^{+}$ | $22.0 \pm 1.0^{+}$ |
| *Salix caprea* (1.0 mg/kg) + TPA (20 nmol/0.2 ml acetone) | $2.0 \pm 0.1^{++}$ | $160.0 \pm 9.0^{+}$ | $17.0 \pm 1.5^{++}$ | $195.0 \pm 11.0^{++}$ | $80.0 \pm 7.0^{+}$ | $28.0 \pm 1.5^{++}$ |
| *Salix caprea* (1.5 mg/kg) + TPA (20 nmol/0.2 ml acetone) | $2.5 \pm 0.2^{++}$ | $175.0 \pm 7.0^{+}$ | $20.0 \pm 1.5^{++}$ | $225.0 \pm 5.0^{++}$ | $85.0 \pm 5.0^{++}$ | $40.0 \pm 2.0^{++}$ |
| *Salix caprea* (1.5 mg/kg) only | $3.5 \pm 0.2$ | $210.0 \pm 11.0$ | $28.0 \pm 1.5$ | $320.0 \pm 30.0$ | $97.0 \pm 6.0$ | $48.0 \pm 2.0$ |

Each value represents mean $\pm$ S.E., $n = 6$.
$^{*}P < 0.05$ and $^{**}P < 0.001$ represent when compared with the corresponding value for acetone treated control. $^{+}P < 0.05$ and $^{++}P < 0.001$ represent when compared with TPA treated control.

Table 2
Effect of pretreatment with *Salix caprea* on TPA-mediated modulation in cutaneous protein carbonyl incorporation, hydrogen peroxide generation and on the activity of xanthine oxidase in mice

| Treatment | Hydrogen peroxide (nmol $H_2O_2$/g tissue) | Protein carbonyl (2,6-DNPH incorporated/0.1 g protein) | Xanthine oxidase (μg uric acid formed/min/mg protein) |
|---|---|---|---|
| Acetone (0.2 ml) | 325.0 ± 9.0 | 275.0 ± 14.0 | 40.0 ± 4.0 |
| TPA (20 nmol/0.2 ml acetone) | 450.0 ± 11.0$^{**}$ | 308.0 ± 11.0$^{**}$ | 75.0 ± 5.0$^{**}$ |
| *Salix caprea* (0.5 mg/kg) + TPA (20 nmol/0.2 ml acetone) | 410.0 ± 12.0$^{+}$ | 345.0 ± 12.0$^{+}$ | 60.0 ± 2.0$^{+}$ |
| *Salix caprea* (1.0 mg/kg) + TPA (20 nmol/0.2 ml acetone) | 390.0 ± 8.0$^{++}$ | 335.0 ± 10.0$^{+}$ | 50.0 ± 7.0$^{+}$ |
| *Salix caprea* (1.5 mg/kg) + TPA (20 nmol/0.2 ml acetone) | 350.0 ± 11.0$^{++}$ | 300.0 ± 9.0$^{++}$ | 40.0 ± 5.0$^{++}$ |
| *Salix caprae* (1.5 mg/kg) only | 320.0 ± 11.0 | 270.0 ± 10.0 | 38.0 ± 3.0 |

Each value represents mean ± S.E., $n = 6$.
$^{**}P < 0.001$ represents when compared with the corresponding value for acetone treated control. $^{+}P < 0.05$ and $^{++}P < 0.001$ represent when compared with TPA treated control.

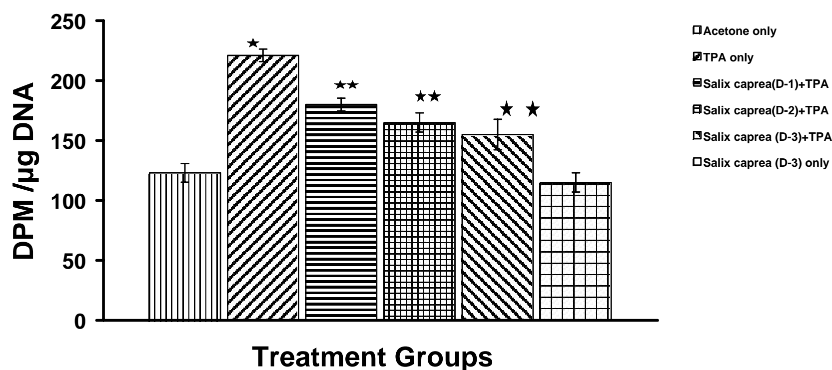


Fig. 1. Effect of pretreatment of animals with *Salix caprea* extract on TPA-mediated enhancement in cutaneous [$^3$H] thymidine incorporation. Dose regimen and treatment protocol are described in the text. Each value represents mean ± S.E. of six animals. (*) Significant ($P < 0.05$), when compared with acetone treated control group. (**) Significant ($P < 0.05$), when compared with TPA treated control group. Doses 1–3 (D-1 D-2 and D-3) represents application of 0.5, 1.0 and 1.5 mg *Salix caprea* extract/kg body weight.

reductase and glutathione-*S*-transferase, however, the pretreatment of *Salix caprea*, caused a recovery of 16–33% in quinone reductase and 10–17% in glutathione-*S*-transferase activity as compared with TPA treated control (Table 1).

The effect of pretreatment of animals with *Salix caprea* on TPA-mediated increase in the activity of xanthine oxidase and rate of protein oxidation (measured in terms of protein carbonyl incorporation) is shown in Table 2. Treatment of TPA resulted in the enhanced levels of hydrogen peroxide and protein carbonyl incorporation with a simultaneous increased activity of xanthine oxidase as much as 38, 38, and 87%, respectively as that of acetone treated control. However, pretreatment with *Salix caprea* resulted in the significant recovery ranging from 12 to 31% and 13 to 29% of hydrogen peroxide level and protein carbonyl incorporation, respectively, as compared with TPA treated control (Table 2). The level of xanthine oxidase reached to the normal values at the highest dose of *Salix caprea* (Table 2).

Treatment of TPA alone caused a significant increase in the rate of cutaneous DNA synthesis marked by an enhanced incorporation of thymidine uptake in the DNA. As shown in Fig. 1, TPA treatment alone caused a 1.8-fold enhancement in the incorporation of [$^3$H] thymidine in cutaneous DNA. However, the animals pretreated with *Salix caprea* exhibited an inhibition of 34–54% dose dependently on TPA-mediated enhancement in the [$^3$H] thymidine incorporation of cutaneous DNA (Fig. 1).

The effect of pretreatment of animals with *Salix caprea* on TPA-mediated induction of cutaneous ODC activity is shown in Fig. 2. Treatment with TPA resulted 5.5-fold increase in cutaneous ODC activity as compared to acetone treated control animals. The pretreatment of animals with *Salix caprea* resulted in a significant inhibition of TPA-mediated induction of cutaneous ODC activity in a dose dependent manner as shown in Fig. 2. With the pretreatment of animals with *Salix caprea*, the activity of TPA induced cutaneous ODC was partially restored and only a 2.0-fold increase was observed at higher dose as compared with TPA treated control.

The efficacy of *Salix caprea* as a antipromoting agent was assessed by two-stage carcinogenesis model. The effect of pretreatment of animals with *Salix caprea* on croton oil (phorbol ester)-mediated tumor promotion in DMBA-initiated mice is shown in Figs. 3 and 4. As observed, *Salix caprea* inhibited a dose dependent skin tumorigenesis. This inhibition was evident when tumor data were considered as the percentage of mice with tumors (Fig. 3) and the number of tumors per mouse (Fig. 4). After the 7 weeks of ex-

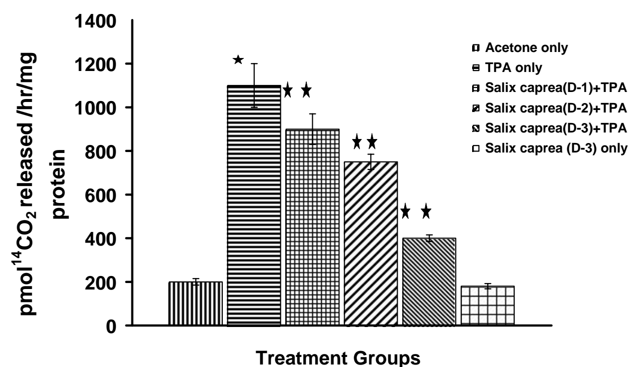


Fig. 2. Effect of pretreatment of animals with *Salix caprea* extract on TPA-mediated induction of cutaneous ODC activity. Dose regimen and treatment protocol are described in text. Each value represent mean ± S.E. of six animals. (*) Significant ($P < 0.05$), when compared with acetone treated control group. (**) Significant ($P < 0.05$), when compared with TPA treated control group. Doses 1–3 (D-1, D-2 and D-3) represents application of 0.5, 1.0 and 1.5 mg *Salix caprea* extract/kg body weight.

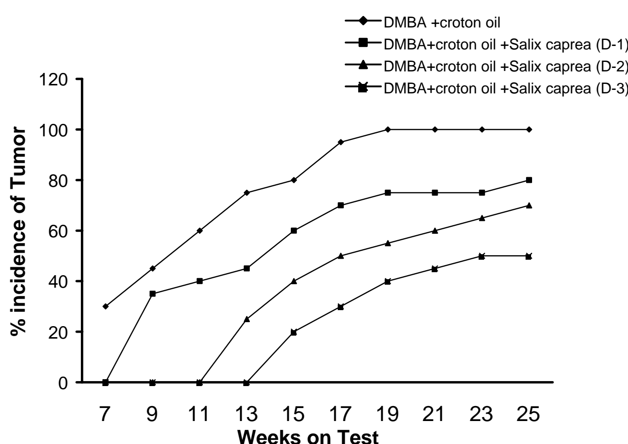


Fig. 3. Effect of pretreatment of *Salix caprea* on percent incidence of tumor in DMBA-initiated and phorbol ester (croton oil) promoted mice skin.

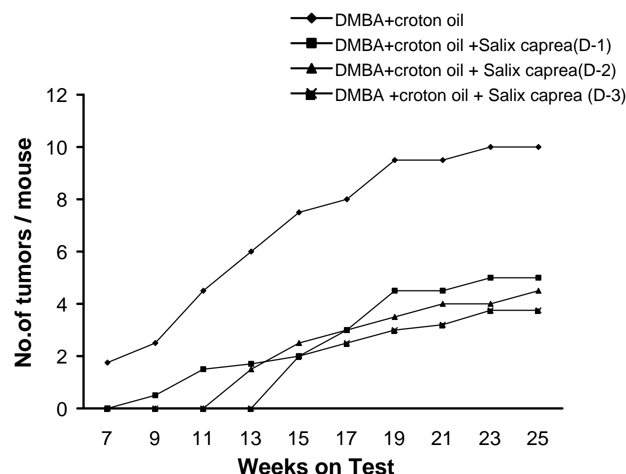


Fig. 4. Effect of pretreatment of *Salix caprea* on average number of tumors/animal in DMBA-initiated and phorbol ester (croton oil) promoted mice skin. Doses 1–3 (D-1 D-2 and D-3) represents application of 0.5, 1.0 and 1.5 mg *Salix caprea* extract/kg body weight.

periment, the occurrence of skin papilloma was noted in the DMBA-initiated croton oil (phorbol ester) promoted animals (control group). It was observed that the pretreatment with *Salix caprea* on DMBA-initiated croton oil (phorbol ester) promoted animals caused a significant increase in the tumor latency period. In such animals, the occurrence of papilloma was noted from 8.0 to 13 weeks as compared with control group. At the termination of the experiment at 25 week, control group exhibited a 100% tumor incidence. The pretreatment of animals with *Salix caprea* observed to cause a reduction in tumor incidence and number of tumors per mouse ranging from 20 to 50% and 50 to 63%, respectively as compared to the croton oil (phorbol ester) treated control group. Mice treated with either DMBA, or *Salix caprea* or croton oil yielded no tumors (data not shown).

Histopatholgical examination (Fig. 5) revealed that tumors encountered in this study were squamous cell papillomas and keratoacanthomas. Squamous cell papillomas were mostly pedunculated with a well-defined and vascularized fibrotic inner core around which squamous epithelial cells were arranged in an arboreal pattern. A few papillomas were broad based and hence designated as flat. In keratoacanthomas, the hair shafts of the superficial follicles were replaced by whorled horny masses of keratin. In case of animals pretreated with *Salix caprea*, the tumors were nodule like with very small size (at higher dose) indicating the potential of *Salix caprea* to reduce tumor size.

## 9. Discussion

The tumor promoting potential of croton oil has been related with the presence of TPA, a phorbol ester present in it as a major constituent (Perwaiz and Sultana, 1998; Xiaoguang et al., 1998). Mechanisms by which phorbol ester acts directly on the plasma membrane and triggers a host of biochemical and cellular responses have been extensively studied (Slaga, 1984; Castagna et al., 1982). The treatment with TPA has been reported to induce a variety of changes in murine skin, including dark basal keratinocytes and sustained epidermal hyperplasia, reactive oxygen species formation in epidermis, elevated epidermal cycloxygenase, lipoxygenase activities and elevated epidermal ODC activity leading to increase in polyamine biosynthesis (Kozumbo et al., 1983; Katiyar et al., 1992; Pegg et al., 1995; Rezazadeh and Athar, 1998).

The central finding in the present study is that *Salix caprea* suppresses the TPA-mediated induction in cutaneous ODC activity, [$^3$H] thymidine incorporation in DNA, oxidative stress and also inhibit phorbol ester promoted skin tumorigenesis in mice. TPA has been reported to act by the generation of free radicals during tumor promotion. Our results are parallel to the previous reports that showed the depletion of reduced glutathione and inhibition in the activities of glutathione reductase, glutathione peroxidase and catalase by the treatment of TPA (Perwaiz and Sultana, 1998; Jang

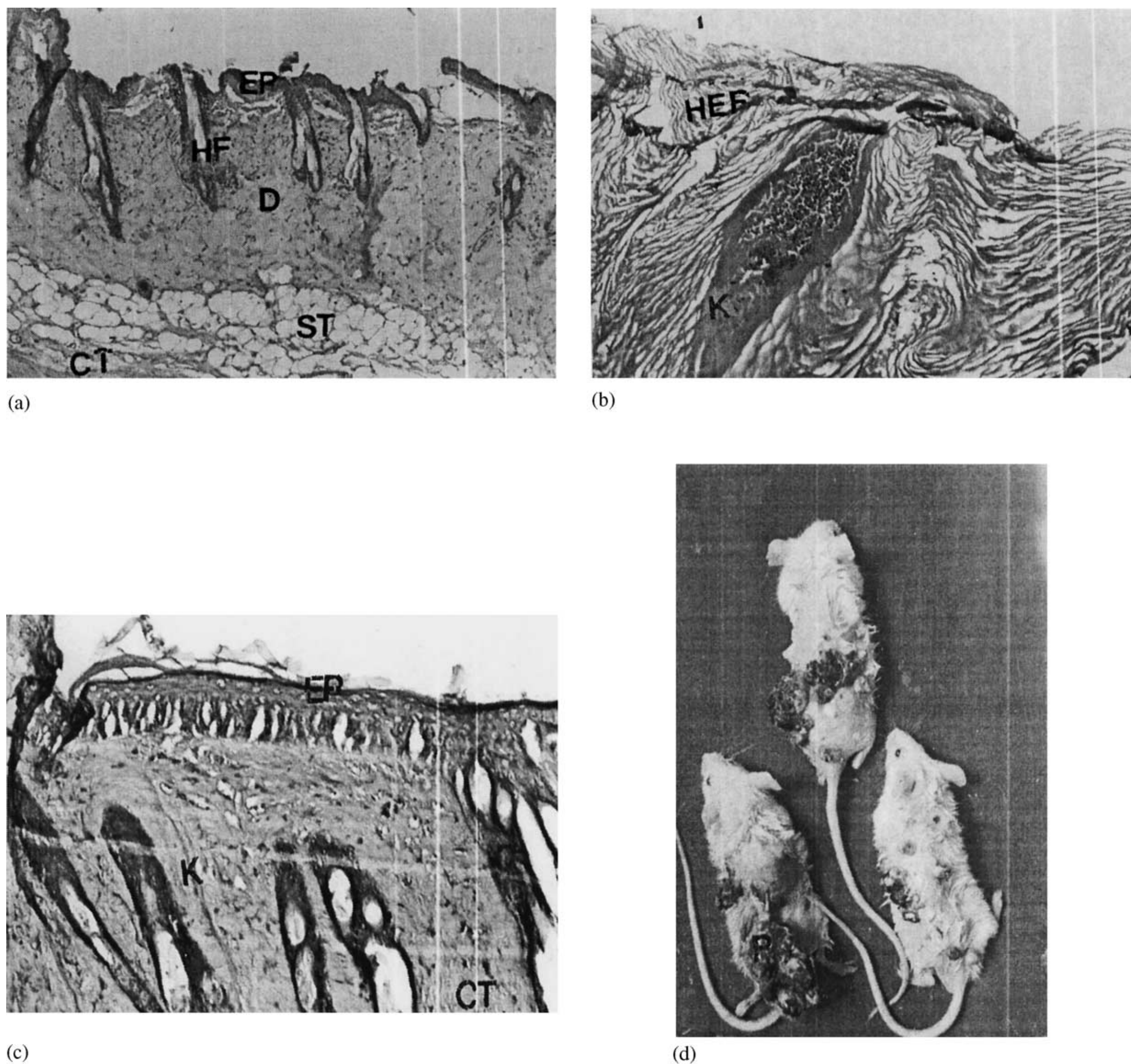


Fig. 5. Effect of pretreatment of animals with *Salix caprea* extract on DMBA and TPA-mediated skin carcinogenesis in mice. (a) Histopathology of normal skin (H&E 100×), (b) histopathology of control DMBA + croton oil (H&E 100×), (c) histopathology of DMBA + *Salix caprea* (1.5 mg/kg) + croton oil (H&E 100×), (d) photograph of tumor-bearing mice. EP: epidermis; HF: hair follicle; D: dermis; ST: subcutaneous tissue; CT: connective tissue; K: keratoacanthoma.

and Pezzuto, 1998; Perchellet et al., 1985). In the present study, it was observed that the pretreatment of *Salix caprea*, caused a significant reversal in all TPA-mediated modulations in cutaneous antioxidant levels of mice thus indicated the antioxidant potential of *Salix caprea*. Free radicals damage cellular macromolecules such as proteins, thus altering their structure and function. The inactivation of enzymes by free radicals and the accumulation of oxidized proteins may play a critical role in the alteration of cellular function and cell death (Cakatay et al., 2000). Some of proteins may be essential growth regulatory proteins that lose their function when damaged by free radicals (Ciolino and Levine, 1997; Cerruti, 1988). In the present investigation, *Salix caprea* has been shown to reduce the TPA-induced protein oxidation. This further suggests the antioxidant role of *Salix caprea*. Superoxide radicals and hydrogen peroxide have been reported to induce cell proliferation (Cunningham and Lockesh, 1983; Slaga, 1984). Most of the tumor promoters including TPA have been reported to increase the activity of xanthine oxidase thus increase superoxide radical generation and enhance hydrogen peroxide generation (Fischer et al., 1998; Slaga, 1984). In the present study, the pretreatment of *Salix caprea* was observed to reverse the TPA-mediated induction in xanthine oxidase activity and hydrogen peroxide content. This suggests the anti-tumor promoting potential of *Salix caprea*. The phase II enzymes such as glutathione-*S*-transferase and quinone reductase have been reported to play an important role in the prevention of carcinogenesis (Wallig et al., 1998). *Salix caprea* has been observed to restore level of phase II enzymes that was depleted by TPA-treatment in the present investigation, hence strengthen its anti-tumor role. ODC is a rate limiting enzyme of polyamine

biosynthesis and the activity of ODC has been reported to increase at the time of acceleration of cell proliferation and development (Megosh et al., 1995). Most of the inhibitors of ODC induction and blockers/or inhibitors of DNA synthesis tested to date also protect against tumor promotion (Katiyar et al., 1992; Wei et al., 1998; Peter et al., 1992). A sharp decrease in TPA-mediated induction in ODC activity and decline in the enhancement of [$^3$H] thymidine incorporation with the pretreatment of *Salix caprea* further suggests the anti-tumor promoting potential of *Salix caprea*. The present study also exhibits the delay in onset of tumor formation with the animals pretreated with *Salix caprea* in DMBA-initiated/croton oil-promoted mice skin which further suggests the anti-tumor promoting potential of *Salix caprea*.

The anti-tumor activity of plants is related with the presence of many antioxidant compounds particularly phenolic compounds as their main constituents (Inada et al., 1997; Pezzuto, 1993; Stoner and Mukhtar, 1995). The anti-oxidant and anti-tumor property of *Salix caprea* may be related with the potent antioxidants present in it. Some of the major constituents of *Salix caprea* such as luteolin, dihydrokaempferol and quercetin have been reported to be effective against many types of tumors in various animal model systems (Mbwambo et al., 1996; Middleton, 1996).

The major proposal for the action and efficacy of *Salix caprea* seems to be its effectiveness to intercept the free radicals and protect cellular macromolecules from oxidant damage. Other possible mechanism may be its effectiveness in inhibiting the ODC activity and maintaining the activity of phase II enzymes after toxicant exposure.

In summary the data of this study suggest that *Salix caprea* facilitates a number of responses relevant to cancer chemoprevention and inhibit phorbol ester induced tumor promotion in mouse skin.

## Acknowledgements

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# Pharmacokinetic and pharmacodynamic studies on interaction of "Trikatu" with diclofenac sodium

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**Abstract**

"Trikatu"—an Ayurvedic formulation comprising of a 1:1:1 ratio of dried fruits of *Piper nigrum*, *Piper longum* and dried rhizomes of *Zingiber officinale* is widely used to enhance the bioavailability of drugs, like vasicine, indomethacin, etc. The enhanced biological response might lead to alteration of therapeutic regimens of commonly prescribed drugs. The present work was aimed to study the effect of concomitant administration of Trikatu on the pharmacokinetics and pharmacodynamics of diclofenac sodium, a frequently prescribed non-steroidal anti-inflammatory drug, having a poor oral bioavailability ($54 \pm 2\%$). The effect of Trikatu on the bioavailability profile of diclofenac sodium was studied in rabbits. It was observed that Trikatu significantly decreased the serum levels of diclofenac sodium. The pharmacodynamic study was carried out to evaluate the effect of Trikatu on the anti-inflammatory activity of diclofenac sodium using carragenin-induced rat paw edema model. It was observed that the mean percent edema inhibition shown by the combination of Trikatu and diclofenac was similar to that shown by Trikatu alone but significantly less than that shown by diclofenac alone. Thus, the experimental findings indicated that Trikatu pretreatment might decrease the bioavailability of certain drugs probably through a drug–herb interaction thereby adversely affecting the therapeutic efficacy of these drugs.
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*Keywords:* Trikatu; Diclofenac sodium; Pharmacokinetics; Anti-inflammatory activity; Herbal interaction

## 1. Introduction

In the traditional Indian system of medicine (Ayurveda), a mixture of the dried fruits of *Piper nigrum* Linn. (Piperaceae), *Piper longum* Linn. (Piperaceae) and the dried rhizomes of *Zingiber officinale* Roscoe. (Zingiberaceae) in 1:1:1 proportion is commonly known as "Trikatu" (Dash and Junius, 1987). Out of the 370 compound formulations listed in the Handbook of Domestic Medicine and Common Ayurvedic Remedies, 210 contain either Trikatu or its individual components (Annamalai and Manavalan, 1990). Trikatu has gained importance in the traditional system of medicine due to its chief alkaloidal constituent, viz. piperine. Literature has revealed a number of pharmacological properties of piperine, one of them being its anti-inflammatory activity (Lee et al., 1984).

Apart from its indigenous uses, Trikatu has also gained importance in modern medicine due to piperine (Atal et al., 1975). Documented reports indicate the importance of piperine (one of the most important constituent of Trikatu) in enhancing the bioavailability of drugs, like phenytoin (Bano et al., 1987), theophylline (Bano et al., 1991), vasicine (Zutshi and Kaul, 1982), oxyphenylbutazone (Mujumdar et al., 1999) and rifampicin (Zutshi et al., 1984). Trikatu by itself has been shown to enhance the bioavailability of drugs, like indomethacin (Karan et al., 1999), vasicine (Zutshi and Kaul, 1982), etc. Improved oral bioavailability of poorly absorbed drugs can help in altering the therapeutic dosages of such drugs or even routes of drug administration. However, some of the experimental findings also indicate the ability of Trikatu to decrease the bioavailability of drugs, like rifampicin (Dahanukar et al., 1982) and isoniazid (Karan et al., 1998).

Considering the importance of Trikatu as an adjuvant in modern medicine, specially in altering the bioavailability of drugs, the present work was undertaken to study the implications of concomitantly administered Trikatu on the bioavailability profile of a modern drug. Amongst the various modern drugs available, diclofenac sodium, a non-steroidal anti-inflammatory drug having a poor oral

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bioavailability (54 ± 2%) (Benet et al., 1996) but frequently used in clinical medicine to treat inflammatory and postoperative conditions, was selected for the study.

## 2. Methodology

### 2.1. Materials

Diclofenac sodium was obtained from Mac Laboratories Ltd., Mumbai. Its identity and purity was confirmed by UV and IR spectral data.

Trikatu was formulated by mixing an equal amount of *Piper nigrum*, *Piper longum* dried fruit powder and *Zingiber officinale* dried rhizome powder (60#).

Calcium carragenin analytical grade was obtained from Merck (I) Ltd.

The experimental work was divided into two sections, viz. pharmacokinetic studies and pharmacodynamic studies.

### 2.2. Pharmacokinetic studies

#### 2.2.1. Preparation of suspensions

A suspension of diclofenac sodium of concentration 10 mg/ml, and a suspension of a combination of diclofenac sodium (10 mg/ml) and Trikatu (60#) (100 mg/ml) were prepared. Sodium CMC (1%, w/v) + gum acacia (1%, w/v) were used as suspending agents. The vehicle [sodium CMC (1%, w/v) + gum acacia (1%, w/v) in distilled water] was used as negative control.

#### 2.2.2. Experimental design

Male Albino rabbits of New Zealand strain weighing 2–2.5 kg were randomly selected for the pharmacokinetic study. The rabbits were divided into three groups and each group comprised of three animals. The first group was maintained as negative control, the second group was administered orally with diclofenac sodium (25 mg/kg) and the third group was administered orally with diclofenac sodium (25 mg/kg) + Trikatu (500 mg/kg). The animals were fasted 16 h prior to the study with water ad libitum. Blood samples were withdrawn from the ear vein at different time intervals, viz. 0, 0.5, 1, 2, 4 and 6 h. Serum was separated and stored at −20 °C till the analysis was carried out. After a lag period of 10 days, the animals were crossed over and the experiment was repeated.

#### 2.2.3. Analysis of serum samples

The serum concentration of diclofenac sodium was determined by HPTLC method developed and validated in our laboratory (Lala et al., 2002).

The pharmacokinetic profile of diclofenac sodium alone and in combination with Trikatu was determined and is outlined in Table 1. The results of the concentration of diclofenac sodium obtained when given alone and in combination with Trikatu were plotted against time (hours) as shown in Fig. 1.

The data obtained were subjected to one-way ANOVA and Student's "*t*"-test to determine the level of significance and a $P$ value of <0.05 was considered as statistically significant.

### 2.3. Pharmacodynamic studies

The pharmacodynamic study was taken up to evaluate the anti-inflammatory activity of diclofenac sodium with and without Trikatu, using carragenin-induced rat hind paw edema model (Winter et al., 1962).

#### 2.3.1. Preparation of test compounds

A suspension of diclofenac sodium (6.25 mg/ml), Trikatu (125 mg/ml) and a combination of diclofenac sodium (6.25 mg/ml) + Trikatu (125 mg/ml) was prepared in distilled water using sodium CMC (1%, w/v) + gum acacia (1%, w/v) as vehicle.

#### 2.3.2. Phlogistic agent

Calcium carragenin was used as a phlogistic agent. A concentration of 1% (w/v) carragenin was prepared in distilled water.

#### 2.3.3. Experimental design

Albino rats, Wistar strain of both sexes weighing between 150 and 200 g were used for the evaluation of the anti-inflammatory activity. The rats were grouped randomly into four groups of five each. Each group was administered the following test drugs orally:

- Group 1: diclofenac sodium (25 mg/kg)
- Group 2: Trikatu (500 mg/kg)
- Group 3: diclofenac sodium (25 mg/kg) + Trikatu (500 mg/kg)
- Group 4: negative control treated with sodium CMC (1%, w/v) + gum acacia (1%, w/v)

Table 1
Comparative data of serum concentration and AUC of diclofenac sodium when given alone and after combination with Trikatu

| Treatment (dose and route) | Serum concentration of diclofenac (μg/ml) at different time intervals | | | | | AUC (μg/ml h) |
|---|---|---|---|---|---|---|
| | 0.5 h | 1 h | 2 h | 4 h | 6 h | 0–6 h |
| Diclofenac sodium (25 mg/kg, p.o.) | 19.67 ± 2.05 | 17.12 ± 1.69 | 12.67 ± 3.44 | 9.18 ± 0.69 | 2.32 ± 0.20 | 62.36 |
| Diclofenac sodium (25 mg/kg, p.o.) + Trikatu (500 mg/kg, p.o.) | **6.76 ± 1.17** | **5.31 ± 0.35** | **2.19 ± 0.90** | **0.77 ± 1.15** | **0.61 ± 0.10** | **12.80** |

Bold values indicate significant difference ($P < 0.05$) from diclofenac-treated animals.

Fig. 1. Serum concentration of diclofenac sodium at different time intervals following the administration of diclofenac sodium, with and without Trikatu.

The right hind paw of the animals were marked to a point on the skin over the lateral malleous and the initial paw volume was recorded. Fasted rats (16 h) were administered orally with diclofenac sodium (25 mg/kg), with and without Trikatu (500 mg/kg). After a period of half an hour, 0.1 ml of carragenin solution was injected subcutaneously in the plantar region of the right hind paw of each rat. Following the injection of carragenin the paw volumes of each rat were measured at different time intervals, viz. 0.5, 1, 1.5, 2, 2.5, 3 and 4 h with a plethysmograph by volume displacement method. After a lag period of 10 days the same animals were reused in a crossed over manner and the experiment was repeated.

The percent inhibition in edema formation at the third hour with different treatment considering the negative control group edema formation as 100% was calculated.

The data obtained were subjected to statistical analysis using one-way ANOVA and Student's "*t*"-test and a $P$ value of $<0.05$ was considered as statistically significant.

## 3. Results

### 3.1. Pharmacokinetic studies

Findings of the pharmacokinetic studies are shown in Table 1 and the serum concentration profile at different time intervals is depicted in Fig. 1. It is observed that concomitant administration of Trikatu and diclofenac sodium decreased significantly the bioavailability of diclofenac sodium. Fig. 1 clearly suggests that there was no alteration in the $T_{max}$. In other words, the onset of action of the drug, diclofenac remains unaltered but, the maximum concentration ($C_{max}$) decreased significantly.

### 3.2. Pharmacodynamic studies

The pharmacodynamic experimental findings demonstrated that the anti-inflammatory activity calculated as percent edema inhibition at the third hour elicited by the combination of diclofenac sodium and Trikatu (59.37%) was significantly lower as compared to diclofenac alone (74.42%). It is also observed that Trikatu by itself also induced a significant reduction in edema formation (62.85%) and the degree of inhibition is comparable with that of diclofenac sodium + Trikatu.

## 4. Discussion and conclusion

In the present investigation, it was observed that the administration of diclofenac with Trikatu to rabbits significantly decreased diclofenac serum levels as compared to diclofenac alone treated rabbits (Table 1 and Fig. 1).

These experimental findings explicitly convince that there is a possible interaction between diclofenac and the active components of the herbal formulation which has resulted in decreased serum diclofenac levels. Our experimental findings are in agreement with Dahanukar et al. (1982) and Karan et al. (1998) who have demonstrated that Trikatu decreased the bioavailability of rifampicin and isoniazid, respectively. It is likely that each individual component may elicit a differential effect on absorption, distribution, metabolism, excretion or transport of diclofenac. To site an example, piperine, one of the major components of the herbal formulation has shown to affect the metabolism of oxyphenylbutazone, pentobarbital and others, largely by inhibiting various forms of monooxygenase (hepatic microsomal enzyme) involved in the oxidation of aliphatic hydroxylation (Atal et al., 1989). Our findings are conflicting with that of Atal et al. (1989), and can be attributed to the larger opposing effects of other components of Trikatu especially on metabolizing enzymes. From the experimental findings (Fig. 1), it has been observed that in the initial half an hour, the absorption of the drug is declined significantly by Trikatu. Alternatively, one is tempted to predict

that Trikatu might affect or interfere with the transport or other processes, like absorption, distribution or excretion of the drug, as demonstrated in the case of phenylbutazone (Mujumdar et al., 1999).

Our pharmacokinetic findings are in agreement with our pharmacodynamic observation, i.e. the anti-inflammatory activity. Diclofenac alone treated rats showed a greater effect than its combination with Trikatu, which can be correlated well with the serum concentrations of diclofenac (see Table 1 and Fig. 1), where Trikatu decreased the serum levels of diclofenac to almost one-third in the initial half an hour. Such low levels of diclofenac may not be able to elicit significant anti-inflammatory activity and hence the activity elicited by the combination of diclofenac and Trikatu may be exclusively due to Trikatu itself.

Interestingly, Trikatu by itself showed a significant anti-inflammatory activity in carragenin-induced rat hind paw edema model. This may be attributed to the presence of piperine in the Trikatu mixture, as piperine has been reported to induce anti-inflammatory activity by Lee et al. (1984). Considering the anti-inflammatory activity of Trikatu per se, the combination of diclofenac and Trikatu should have elicited a greater degree of anti-inflammatory activity. Since the combination failed to show potentiation of diclofenac anti-inflammatory activity, we are compelled to attribute that the components of Trikatu are interacting with the drug, which might result in modifying both, the pharmacokinetic and the pharmacodynamic aspects of the drug and the herbal formulation.

In conclusion, it can be stated that a thorough understanding of herbal drug interactions with the synthetic drugs is of paramount importance, as these interactions may lead to alteration of therapeutic responses of these drug materials.

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# The herbal market of Thessaloniki (N Greece) and its relation to the ethnobotanical tradition

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**Abstract**

The results of a survey of the medicinal plants found in the herbal market of Thessaloniki, which comprises traditional shops, modern shops and open-air market stalls, are presented. A total number of 172 taxa, Pteridophyta and Spermatophyta, were found in 18 selected market spots. Information is provided on the origin, the plant parts used, the ways of drug preparation and the medicinal uses of the herbs found. The majority of them (133 taxa) are of Greek origin and are gathered from the wild (99). A remarkable number of herbs (93) found in the market of Thessaloniki are mentioned by Dioscurides whereas the comparison to the recent ethnobotanical information shows that the utilization of Dioscurides' plants remains uninterrupted. Thus it is suggested that the herb trade is still based on the Greek ethnobotanical tradition, dating from antiquity.
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*Keywords:* Medicinal plants; Herbal market; Greece; Thessaloniki; Dioscurides; Ethnobotany

## 1. Introduction

The use of herbal drugs in Greece has its roots in ancient times. Hippocrates (5th century B.C.) and Dioscurides (1st century A.D.) established medicine as a science using the healing properties of the different plant species. This knowledge survived until our days and an array of herb shops are found in the markets of the modern Greek cities.

Thessaloniki is the second largest city of Greece and one of the oldest in Europe, continuously inhabited for more than 2300 years. Because of its strategic position (a port lying between Asia and Europe), it has been populated—besides Greeks—by several ethnic communities from the beginning and throughout its history. Thus, Thessaloniki became a multicultural city and an important commercial center of the East Mediterranean. From 1500 up to early 20th century the commerce was mainly in the hands of the Greeks and the Jews and among the traded goods, medicinal plants possessed a noticeable place (cf. Svoronos, 1956; Vakalopoulos, 1983; Nehama, 2000).

Nowadays the trade of medicinal plants in Thessaloniki mainly takes place in three groups of market spots:

(i) Traditional shops, situated in the market lying in the city center; such shops exist in the same area at least since 15th century (Dimitriadis, 1983; Vakalopoulos, 1983). These shops are old, with stalls and shelves simply arranged. The herbs are not packaged but stored in large fabric bags or in bundles. They are weighed according to the customers demand and packed in plastic or paper bags. Except herbs, these shops often sell spices, food and other items, like baskets and matters for religious ceremonies. The traditional shops are usually a family business, which passes from parents to children. The owners are not professional healers, but traders, who are able, when asked, to recommend specific herbs to cure common ailments. Their major suppliers are professional collectors, while the owners themselves may collect some of the plants.

(ii) Modern shops, which have opened in the last decade following the worldwide trend towards the increased demand in health care and food natural products. These shops, scattered throughout the city, are carefully decorated and their products are industrially manufactured, attractively packaged and displayed on shelves. They also sell other products of natural origin, e.g. soaps, essential oils, perfumes, ointments and lotions. The shop owners often have a university degree, but no formal education concerning plants. Their knowledge on herbal drugs mostly derives from modern textbooks and the information provided by the company suppliers.

(iii) Stalls in open-air markets, which run in different places of Thessaloniki once a week, with bunches of herbs or

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sacks of crushed material. Stall keepers are themselves professional plant collectors, with a comparatively low educational level and an empirical experience in herbal medicine, based on tradition.

The purpose of this investigation is to trace the current trends in the market of the medicinal plants in Thessaloniki, addressing to the following questions: (i) Which are the herbs traded? (ii) Are they imported or domestic? (iii) If domestic, do they derive from the wild or from cultivation? Moreover, information on the plant uses, the plant parts used and drug preparations was collected. Considering the above results, the relation of the current market trends to the traditional ethnobotany is further discussed.

## 2. Materials and methods

During a preliminary survey the authors visited repeatedly the herbal market of Thessaloniki. In the course of these visits many stores were examined in order to select a number of market spots that could form a reliable and representative model for this research. Finally a total number of 18 market spots, six from each of the three groups (traditional shops, modern shops, open-air market stalls) was chosen. Herb traders and customers were interviewed and a questionnaire was filled in, asking information on: (i) the commercial names of the different herbs; (ii) their origin (imported or domestic); (iii) if domestic, their source (from the wild or from cultivation); (iv) the plant parts used; (v) the medicinal uses, and (vi) the way of drug preparation (Table 1).

All the medicinal herbs found in each spot were surveyed. Spices were included only if the traders mentioned any medicinal use. Products of plant metabolism such as resins or latex, if traded in their raw form, were also included.

The plant taxa were identified using Flora Hellenica (Strid and Tan, 1997) and Mountain Flora of Greece (Strid, 1986; Strid and Tan, 1991), for the taxa therein included, and Flora Europaea (Tutin et al., 1968–1980,1993). The taxa of the genus *Mentha* (Labiatae) were identified according to Kokkini (1983). For American, Asiatic or African herbs the scientific names corresponding to the commercial ones were given consulting major pharmacognostic publications (Trease and Evans, 1983; Phokas, 1984; Samuelsson, 1992; Bruneton, 1993) and reliable Internet sources.

In Table 1 information on the medicinal herbs reported in Dioscurides, derives from Codex Neapolitanus Graecus 1 of the National Library of Naples, which was recently reprinted by Genus Publications, Athens, Greece (1999). The comments on Dioscurides text mentioned by Kavadas (1956–1964) and Gennadios (1959) have also been taken into account. Recent ethnobotanical information concerning taxa used in Greek traditional medicine derives from Fragaki (1969), Tamarro and Xepapadakis (1986) and Vokou et al. (1993).

The diseases treated according to the traders' information are summarized in Table 1 as follows:

Cardiovascular diseases (CV); endocrinal diseases (E); ENT diseases (ENT); diseases of the gastrointestinal tract (GI); diseases of the haemopoietic system (H); diseases of the immunological system (I); infectious diseases (IN); metabolic diseases (M); neoplastic diseases (N); neuropsychiatric diseases (NP); ophthalmologic diseases (OP); diseases of the oral cavity (OR); diseases of the respiratory system (R); skin diseases (SK); systematic diseases (S); diseases of the urogenital system (UG); other (O).

## 3. Results and discussion

A total number of 172 plant taxa belonging to 72 plant families are recorded in all market spots (4 families of Pteridophyta and 68 families of Spermatophyta). The best-represented families in respect of the number of taxa found are Labiatae (27 taxa), Compositae (16) and Umbelliferae (10) (Table 1). It should be noted that different species are used similarly, under the same commercial and/or local name, e.g. "dyosmos," for three spearmint (carvone-like) scented *Mentha* taxa, or "tsai tou vounou," for five taxa of *Sideritis* (Greek mountain tea). On the other hand, different commercial and/or local names are attributed to the same species, e.g. valsamo, spathohorto and Ai Giannis for *Hypericum perforatum*, trigonella, tsimeni and moshositaro for *Trigonella foenum-graecum*. In a few cases, different plant parts of the same taxon have a different commercial name and are recommended for the same or different diseases. For example, the fruits and the leaves of *Senna alexandrina* (fylla alexandrias and sinnamiki, respectively) are both used against constipation; the petals of *Rosa* spp. (rodopetala) are used to cure several diseases, while the fruits (kynorodo) are additionally recommended for coronary diseases and influenza and are considered as aphrodisiac and stimulant.

The majority (131) of the taxa traded are of Greek origin, while 75 are imported. In particular, 97 taxa are exclusively provided from Greece, 41 are exclusively imported and 34 taxa are both Greek and imported. Most of the Greek taxa (80) are gathered exclusively from the wild, whereas 31 taxa are exclusively cultivated and 20 taxa are both wild and cultivated.

In most cases (67 herbs) the whole aerial part of the plants is sold, while the trade of leaves, fruits, roots, seeds or bark is recorded for others. Specific plant parts like styles (*Zea mays*) and stigmas (*Crocus sativus*) or products of plant metabolism such as resin (*Pistachia lentiscus* var. *chia*) and latex (*Ficus carica*) are recorded for one or two herbs.

Internal uses predominate over external ones and decoctions or infusions in water are the most usual drug preparations (99 and 98 records, respectively). Ingestion or chewing is recommended for 28 herbs (entire, pulverized or crushed), while 26, mainly aromatic, are recommended as seasonings and one (seeds of *Helianthus annuus*) as food

Table 1
Medicinal plants sold in the market of Thessaloniki

| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| Pteridophyta | | | | | |
| Adiantaceae | | | | | |
| *Adiantum capillus-veneris* L.[1,3], aerial parts | Adiantos, *polytrihi* *ΚΑΛΛΙΤΡΙΧΟΝ* (=*KALLITRIHON*) | GR (W) | D, Ig (pulverized), EA (Ws) | SK: 2 (hair loss), UG: 1 (renal disorders) | TR (1), MOD (1) |
| Aspleniaceae | | | | | |
| *Asplenium ceterach* L. (Syn.: *Ceterach officinarum* DC.)[1,3], aerial parts | *Skorpidi ΑCΠΛΗΝΟΝ* (=*ASPLINON*) | GR (W), IM | If | GI: 2 (gallstones, intestinal disorders), NP: 1 (neuralgia), UG: 5 (kidney stones, prostate) | TR (5), OM (1) |
| Equisetaceae | | | | | |
| *Equisetum* spp.[3], aerial parts | Equizeto, ippouris, *polykobi ΙΠΠΟΥΡΙC* (=*IPPOURIS*) | GR (W), IM | If, D, EA (Cm, P, Ws) | CV: 1 (hypertension), GI: 2 (constipation, gallstones), H: 1 (haemostatic), M: 1 (cholesterol), S: 5 (arthritis, rheumatisms), SK: 5 (hair loss, hair and nail tonic, sanative), UG: 8 (kidney stones, menstruation disorders, prostate, renal disorders), O: 3 (stimulant) | TR (6), MOD (4), OM (1) |
| Hypolepidaceae | | | | | |
| *Pteridium aquilinum* (L.) Kuhn, aerial parts | *Fteri ΠΤΕΡΙC* (=*PTERIS*) | GR (W) | If | GI: 1 (intestinal disorders), S: 1 (arthritis, rheumatisms), O: 1 (antibacterial activities) | TR (3) |
| Spermatophyta | | | | | |
| Agavaceae | | | | | |
| *Yucca filamentosa* L., root | Yucca | IM | D | CV: 1 (blood circulation stimulant), S: 1 (arthritis, rheumatisms), O: 1 (stimulant) | MOD (1) |
| Anacardiaceae | | | | | |
| *Pistacia lentiscus* L. var. *chia* DC., resin | *Mastiha Chiou ΜΑCΤΙΧΗ* (=*MASTIHI*) | GR (C) | Ig (pulverized) | E: 7 (diabetes), GI: 3 (stomach ulcer), M: 5 (cholesterol), UG: 1 (diuretic) | TR (5), MOD (1), OM (2) |
| Apocynaceae | | | | | |
| *Vinca minor* L., leaves | Vinca *ΚΛΗΜΑΤΙC* ? (=*KLIMATIS*) | IM | If, EA (P) | CV: 1 (coronal disease), ENT: 1 (tonsillitis), GI: 1 (diarrhoea), H: 1 (anaemia, haemostatic), R: 1 (bronchectasis), UG: 1 (gonorrhoea), O: 1 (blood purification, stimulant) | MOD (1) |
| Araceae | | | | | |
| *Acorus calamus* L., root | Acoros, *kalamoriza* *ΑΚΟΡΟΝ* (=*ACORON*) | IM | D | ENT: 1 (igmoritis), GI: 1 (bloating, dyspepsia, gastritis), M: 3 (uric acid), S: 1 (arthritis, rheumatisms), UG: 1 (diuretic, prostate) | TR (2), MOD (1) |
| Araliaceae | | | | | |
| *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., aerial parts | Eleftherococcos | IM | If | CV: 2 (hypertension), M: 2 (cholesterol), NP: 2 (brain stimulant, calmative), O: 2 (stimulant) | TR (1), MOD (1) |
| *Hedera helix* L.[1,3], leaves | *Kissos ΚΙCCΟC* (=*KISSOS*) | GR (W) | If | ENT: 1 (laryngitis), GI: 2 (gallstones), IN: 1 (whooping cough), NP: 1 (neuralgia), R: 2 (bronchitis), S: 1 (rheumatisms), SK: 4 (cellulites) | TR (4), MOD (1) |

Table 1 (*Continued*)

| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| *Panax ginseng* C.A. Meyer, pulverized root | Ginseng | IM | D, Ig | E: 3 (diabetes), M: 3 (cholesterol, triglycerides), N: 2 (anti-cancer capacities), NP: 3 (brain stimulant, calmative, depression), O: 8 (aphrodisiac, stimulant) | TR (4), MOD (4) |
| Betulaceae | | | | | |
| *Betula pendula* Roth, leaves | *Symida* | GR (W), IM | If, D | M: 2 (urea, uric acid), S: 1 (arthritis, rheumatisms), SK: 3 (cellulites), UG: 4 (diuretic, renal disorders) | TR (1), MOD (2) |
| Bignoniaceae | | | | | |
| *Stereospermum suaveolens* DC., bark | Lapaho, iperoxo | IM | D, EA (Ws) | CV: 2 (blood circulation stimulant, hypertension), H: 2 (anaemia), M: 2 (obesity), NP: 2 (calmative, neuralgia), SK: 2 (dermatitis), O: 4 (stimulant) | TR (1), MOD (4) |
| Boraginaceae | | | | | |
| *Borago officinalis* L.[1], aerial parts | *Borantza* | GR (W), IM | If, D | CV: 1 (heart stimulant), GI: 1 (gall disorders), R: 1 (bronchitis, common cold), S: 2 (arthritis, rheumatisms), UG: 1 (kidney stones), O: 1 (blood purification) | TR (1), MOD (1) |
| *Symphytum ottomanum* Friv., root | Symphyto CYMΦYTON (=*SYMPHYTON*) | GR (W) | D, EA (Cm) | CV: 1 (haemorrhoids), GI: 1 (diarrhoea, duodenal ulcer), IN: 1 (influenza), R: 1 (asthma, common cold) | MOD (1) |
| Cannabaceae | | | | | |
| *Humulus lupulus* L., inflorescences | *Lykiskos* | GR (W) | If, D | E: 7 (diabetes), GI: 1 (colitis), M: 1 (cholesterol), NP: 5 (calmative), UG: 1 (diuretic), O: 2 (aphrodisiac, blood purification) | TR (4), MOD (2), OM (2) |
| Caprifoliaceae | | | | | |
| *Sambucus nigra* L.[3], inflorescences, fruits | Zampoukos, *kouphoxylia AKTH* (=*AKTI*) | GR (W, C) | If, EA (Cm, Ws) | CV: 1 (haemorrhoids), ENT: 1 (pharyngitis), GI: 1 (constipation)[a], I: 1 (allergy), IN: 1 (influenza), R: 14 (asthma, bronchitis, common cold), S: 4 (arthritis, rheumatisms), SK: 1 (antiseptic), UG: 6 (diuretic, infections of the vagina), O: 1 (antipyretic) | TR (6), MOD (4), OM (3) |
| Caryophyllaceae | | | | | |
| *Saponaria officinalis* L., root, aerial parts | Saponaria, *halvadoriza*[b], *tsoueni CTPOYΘION* (=*STROUTHION*) | GR (W), IM | D, EA (Cm, P, Ws) | GI: 3 (constipation, gall disorders, gallstones), H: 1 (haemostatic), R: 1 (common cold), S: 2 (arthritis, rheumatisms), SK: 3 (eczema, hair loss, herpes), UG: 1 (kidney stones), O: 1 (antipyretic, stimulant) | TR (2), MOD (1), OM (1) |
| Compositae | | | | | |
| *Achillea millefolium* L.[3], aerial parts | Ahillea, *ahilleas AXIΛΛIOC* (=*AHILLIOS*) | GR (W) | If, D, EA (Cm, Ws) | CV: 4 (haemorrhoids, hypertension), E: 1 (diabetes), GI: 7 (colitis, constipation, gallstones, gastritis, intestinal disorders, liver disorders), IN: 1 (influenza), S: 6 (arthritis, rheumatisms), SK: 5 (greasiness), UG: 4 (diuretic, dysmenorrhoea, menstruation disorders), O: 6 (antipyretic, appetizer, blood purification, stimulant) | TR (6), MOD (5), OM (3) |
| *Arctium lappa* L., aerial parts | *Lapa*, *kollitsida APKION H ΠPOCΩΠIΔA* (=*ARKION I PROSOPIDA*) | GR (W) | If | E: 1 (diabetes), GI: 1 (dyspepsia), S: 1 (rheumatisms), O: 2 (aphrodisiac, blood purification) | TR (1), MOD (1) |

| | | | | | |
|---|---|---|---|---|---|
| *Arnica montana* L., root, aerial parts | Arnica | IM | If, EA (Cm, Ws) | S: 1 (arthritis, rheumatisms), SK: 1 (sanative) | MOD (1) |
| *Artemisia absinthium* L.[1,3], aerial parts | *Apsithia ΑΨΙΝΘΙΟΝ* (=*APSINTHION*) | GR (W) | If, D, EA (Ws) | E: 9 (diabetes), GI: 3 (diarrhoea, intestinal parasites), H: 2 (anaemia), M: 5 (obesity), NP: 3 (calmative, depression, epilepsy), SK: 2 (antiseptic), UG: 4 (dysmenorrhoea, menstruation disorders), O: 4 (antipyretic, appetizer, stimulant) | TR (5), MOD (5), OM (1) |
| *Calendula officinalis* L., inflorescences | Calendoula | GR (C), IM | D, If, EA (Cm, Ws) | CV: 2 (phlebitis), GI: 3 (jaundice, liver disorders, stomach ulcer), SK: 3 (eczema, pimples), UG: 5 (dysmenorrhoea), O:1 (antifungal activities, blood purification) | TR (4), MOD (3), OM (1) |
| *Chamomilla recutita* (L.) Rauschert[1,3] (Syn.: *Matricaria chamomilla* L. pro parte), inflorescences | *Hamomilo*, *hamomili ΧΑΜΑΙΜΗΛΟΝ* (=*HAMEMILON*) | GR (W, C), IM | If, D, EA (Cm, Ws) | GI: 8 (antiemetic, bloating, diarrhoea, dyspepsia, gall disorders, jaundice, spasmolytic), I: 1 (allergy), IN: 2 (influenza), M: 2 (obesity), NP: 12 (calmative), OP: 2 (eye inflammation), R: 6 (common cold), S: 5 (arthritis, rheumatisms), SK: 9 (antiseptic, eczema, hair tonic, sanative), UG: 2 (infections of the vagina, menstruation disorders), O: 5 (antipyretic, appetizer) | TR (6), MOD (6), OM (6) |
| *Cichorium intybus* L.[1,3], aerial parts | Kihorio, *agrio radiki ΑΓΡΙΑ CΕΡΙC*, *ΚΙΧΩΡΙΟΝ*, *ΠΙΚΡΙC* (=*AGRIA SERIS*, *KIHORION*, *PIKRIS*) | GR (W) | D, EA (Ws) | GI: 3 (liver disorders, spasmolytic), M: 1 (cholesterol), SK: 1 (antiseptic) | TR (2), MOD (1) |
| *Cnicus benedictus* L., aerial parts | Cnicos | GR (W) | If | GI: 1 (liver disorders), I: 1 (allergy), UG: 1 (diuretic) | TR (1), MOD (1) |
| *Cynara scolymus* L.[1], aerial parts | *Aginara ΚΥΝΑΡΑ* (=*KYNARA*) | GR (C) | If | CV: 1 (arteriosclerosis), E: 1 (diabetes), GI: 5 (gall disorders, liver disorders), M: 3 (cholesterol), NP: 1 (serrical syndrome), UG: 2 (diuretic, nephritis), O: 1 (stimulant) | TR (3), MOD (2) |
| *Echinacea angustifolia* DC., root | Echinatsia | IM | D, EA (Cm, P, Ws) | ENT: 2 (ear inflammation, pharyngitis), N: 2 (anti-cancer capacities), R: 2 (common cold), SK: 2 (abscesses, antiseptic, eczema, gum wounds, herpes, psoriasis, sanative, stomatitis), O: 3 (antibacterial and antifungal activities, snakebite antidote, stimulant) | TR (1), MOD (1) |
| *Helianthus annuus* L., seeds | *Ilios* | GR (C) | F | M: 1 (cholesterol), UG: 1 (prostate), O: 1 (blood purification) | TR (2) |
| *Silybum marianum* (L.) Gaertner, seeds | Sylvio, *gaidouragatho CΙΛΥΒΟΝ* (=*SILYVON*) | GR (W) | D (with caution) | GI: 1 (gallstones), I: 1 (allergic cough), O: 1 (blood purification) | TR (1), MOD (1) |
| *Solidago virgaurea* L.[3], aerial parts | Solidaho, *hrysoverga* | GR (W) | If | CV: 1 (hypertension), I: 1 (allergic asthma), R: 2 (asthma, common cold), UG: 3 (renal disorders) | TR (1), MOD (2) |
| *Tanacetum parthenium* (L.) Schultz Bip.[2,3], aerial parts | Tanatseto | GR (W), IM | D, If | GI: 1 (ascitis, liver disorders spasmolytic), NP: 2 (depression, dizziness, headache, migraine), S: 2 (arthritis), UG: 3 (dysmenorrhoea, renal disorders), O: 2 (stimulant) | TR (2), MOD (1) |
| *Taraxacum* spp.[1], aerial parts | Taraxako, *radiki*, *pikralithra* | GR (W), IM | If, D, EA (Cm, P, Ws) | CV: 2 (arteriosclerosis, hypertension), E: 7 (diabetes), GI: 5 (constipation, gall and liver disorders), H: 1 (anaemia), M: 11 (cholesterol, triglycerides, pancreas disorders, uric acid), N: 3 (breast cancer), S: 1 (arthritis, rheumatisms), SK: 3 (boils, eczema, freckles, pimples), UG: 4 (kidney stones, prostate), O: 5 (blood purification, stimulant) | TR (6), MOD (4), OM (3) |

Table 1 (*Continued*)



| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| *Tussilago farfara* L., aerial parts | *Vihio ΒΗΧΙΟΝ* (=*VIHION*) | GR (W) | D, If, EA (Ws) | CV: 1 (phlebitis), GI: 1 (spasmolytic), IN: 3 (influenza), R: 8 (asthma, bronchitis, common cold, pneumonia), SK: 1 (sanative) | TR (4), MOD (3), OM (1) |
| Cruciferae | | | | | |
| *Capsella bursa-pastoris* (L.) Medicus[3], aerial parts | Capsella *ΘΛΑCΠΙ* (=*THLASPI*) | GR (W) | D | NP: 1 (menopause emotional disorders), UG: 1 (menstruation disorders) | TR (1), MOD (1) |
| *Nasturtium officinale* R. Br., aerial parts | Nastourtio, *nerokardamo* | GR (W), IM | If, EA (Cm, Ws) | GI: 1 (liver disorders), H: 1 (anaemia), R: 1 (common cold), S: 1 (rheumatisms), SK: 2 (antiseptic, freckles), UG: 1 (diuretic, renal disorders), O: 2 (appetizer, stimulant) | TR (1), MOD (1) |
| *Sinapis alba* L.[1], seeds | *Sinaposporos ΝΑΠΥ*, *CΙΝΗΠΙ* (=*NAPY*, *SINIPI*) | GR (W, C), IM | Ch, D, EA (Fb, P—with caution) | CV: 2 (coronary disease, heart stimulant), GI: 3 (constipation, spasmolytic), NP: 1 (neuralgia), OR: 1 (toothache), R: 3 (bronchitis, common cold), S: 6 (arthritis, rheumatisms), SK: 1 (sanative), O: 2 (stimulant) | TR (6), MOD (2) |
| Cupressaceae | | | | | |
| *Juniperus oxycedrus* L.[2,3], fruits, bark | *Kedros*, *kedrokoukoutsa*[c] *ΑΡΚΕΥΘΙC ΜΙΚΡΑ* (=*ARKEFTHIS MIKRA*) | GR (W) | D | GI: 2 (gallstones, stomach ulcer), UG: 5 (kidney stones, prostate), O: 2 (stimulant) | TR (5), MOD (1), OM (1) |
| Ephedraceae | | | | | |
| *Ephedra foeminea* Forsskal[3], leaves | Ephedra | GR (W), IM | Ig, EA (P, Ws) | I: 1 (allergic fever and cough), IN: 1 (whooping cough), R: 1 (asthma, bronchitis), SK: 1 (eczema, itch) | MOD (1) |
| Ericaceae | | | | | |
| *Arbutus unedo* L.[1], leaves | *Koumaria ΚΟΜΑΡΟC* (=*KOMAROS*) | GR (W) | D, EA (Ws) | SK: 1 (antiseptic), UG: 4 (cystitis, haematuria, prostate) | TR (2), MOD (2) |
| *Vaccinium myrtillus* L., leaves, fruits | Myrtillos, myrtidion | GR (W) | If, Ig (pulverized leaves), EA (Cm, Ws) | E: 2 (diabetes), OP: 2 (cataract due to diabetes, eye irritation, myopia, optical acuteness), O: 1 (stimulant) | TR (1), MOD (2) |
| Fagaceae | | | | | |
| *Castanea sativa* Miller[3], leaves, inflorescences | *Castania ΔΙΟC ΒΑΛΑΝΟC*, *ΚΑCΤΑΝΟΝ* (=*DIOS VALANOS*, *KASTANON*) | GR (W, C) | If, D, EA (Cm, Ws) | GI: 1 (diarrhoea), OP: 1 (eye irritation), R: 2 (common cold), O: 1 (antipyretic, stimulant) | TR (1), MOD (1) |
| Gentianaceae | | | | | |
| *Centaurium erythraea* Rafn[1,3], aerial parts | Kentavrio, erythrea, *thermohorto ΛΕΠΤΟΝ ΚΕΝΤΑΥΡΕΙΟΝ*, *ΜΙΚΡΟΝ ΚΕΝΤΑΥΡΕΙΟΝ* (=*LEPTON KENTAVRION*, *MIKRON KENTAYRION*) | GR (W) | D, Ig (pulverized) | GI: 2 (constipation, spasmolytic), M: 1 (obesity), S: 1 (arthritis), UG: 1 (diuretic, dysmenorrhoea), O: 1 (antipyretic) | TR (1), MOD (1) |
| *Gentiana lutea* L., root | Gentiani, *agriokapnos ΓΕΝΤΙΑΝΗ* (=*GENTIANI*) | GR (W), IM | D | CV: 2 (blood circulation stimulant), GI: 2 (bloating, dyspepsia), UG: 1 (menstruation disorders), O: 4 (appetizer, stimulant) | TR (2), MOD (3) |

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|---|---|---|---|---|---|
| Ginkgoaceae | | | | | |
| *Ginkgo biloba* L., leaves, fruits | Ginko to dilovo | IM | If, Ig (pulverized—prescription required) | CV: 2 (arteriosclerosis, coronal disease, vinous insufficiency), ENT: 1 (ear disorders), NP: 1 (brain stimulant, depression, headache), UG: 1 (impotence) | MOD (2) |
| Graminae | | | | | |
| *Elymus repens* (L.) Gould[3], root | *Agriada* | GR (W) | If, D, EA (Ws) | GI: 1 (gallstones), NP: 1 (serrical syndrome), S: 5 (arthritis, rheumatisms), SK: 1 (hair tonic), UG: 12 (cystitis, kidney stones, prostate), O: 1 (stimulant) | TR (6), MOD (4), OM (2) |
| *Hordeum vulgare* L.[1], seeds | *Krithari apofliomeno ΚΡΙΘΗ* (=*KRITHI*) | GR (C) | D | M: 1 (cholesterol), UG: 2 (kidney stones) | TR (2) |
| *Zea mays* L.[1], styles of female flowers | *Kalampoki*, *mallia kalampokiou* | GR (C) | If | R: 2 (common cold), UG: 10 (diuretic, cystitis, incontinence, kidney stones, prostate) | TR (6), MOD (2), OM (2) |
| Guttiferae | | | | | |
| *Hypericum perforatum* L.[1,2,3], aerial parts | *Valsamo*, *spathohorto*, *Ai Giannis ΥΠΕΡΕΙΚΟΝ* (=*YPERIKON*) | GR (W) | If, EA (P) | GI: 14 (diarrhoea, dyspepsia, spasmolytic, stomach ulcer), NP: 4 (calmative, depression, menopause emotional disorders, neuralgia), R: 3 (bronchitis, common cold), SK: 9 (antiseptic, burns, sanative, skin tumours), UG: 4 (diuretic, incontinence), O: 2 (antipyretic) | TR (6), MOD (3), OM (6) |
| Hamamelidaceae | | | | | |
| *Hamamelis virginiana* L., leaves | Amamilis | IM | If, EA (Cm, P, Ws) | CV: 5 (haemorrhoids, phlebitis), H: 1 (haemostatic), SK: 1 (antiseptic) | TR (2), MOD (3) |
| Illiciaceae | | | | | |
| *Illicium verum* Hook fil., fruits | Asteroides, bantiana, asteroidis glykanissos | IM | D, If | CV: 2 (blood circulation stimulant), GI: 5 (bloating, dyspepsia, spasmolytic), NP: 2 (anti-convulsive, calmative) | TR (4), MOD (1) |
| Iridaceae | | | | | |
| *Crocus sativus* L., stigmas | *Krokos*, *zafora*, *safran ΚΡΟΚΟΣ* (=*KROKOS*) | GR (C) | S, If, EA (M) | GI: 1 (dyspepsia, liver disorders), N: 3 (anti-cancer capacities), NP: 1 (calmative), OR: 1 (teething pains), R: 2 (bronchitis, pneumonia), UG: 1 (dysmenorrhoea), O: 4 (aphrodisiac, stimulant) | TR (6), MOD (3) |
| Junglandaceae | | | | | |
| *Junglans regia* L.[1], leaves, green peel of the fruit, bark | *Karydia ΚΑΡΥΑ* (=*KARYA*) | GR (W, C) | D (leaves), EA (Cm, M, Ws), Ig (pulverized bark) | E: 3 (diabetes), GI: 1 (dyspepsia), M: 2 (cholesterol), OP: 2 (eyelid inflammation), S: 1 (arthritis, rheumatisms), SK: 5 (antiseptic, frost bites, hair tonic, herpes, sanative), O: 5 (antifungal activities, appetizer, blood purification) | TR (3), MOD (3) |
| Labiatae | | | | | |
| *Acinos suaveolens* (Sibth. & Sm.) G. Don fil., aerial parts | *Menta* | GR (W) | If, D, EA (Ws) | ENT: 1 (tonsillitis), GI: 10 (bloating, dyspepsia, gall disorders, spasmolytic), IN: 2 (influenza), NP: 7 (anti-convulsive, calmative, dizziness, headache), OR: 1 (gingivitis), R: 8 (bronchitis, common cold), S: 1 (rheumatisms), SK: 1 (antiseptic), UG: 3 (diuretic, dysmenorrhoea), O: 9 (antipyretic, aphrodisiac, blood purification, stimulant) | TR (6), MOD (1), OM (5) |

Table 1 (*Continued*)

| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| *Lavandula angustifolia* Miller subsp. *angustifolia*, leaves, inflorescences | *Levanta* | GR (C), IM | If, D, EA (Ws) | CV: 7 (coronary disease, heart stimulant, hypertension, hypotension), GI: 4 (spasmolytic), NP: 9 (anti-convulsive, calmative, dizziness, headache, migraine, neuralgia), SK: 2 (antiseptic, hair loss), O: 2 (stimulant) | TR (6), MOD (4), OM (2) |
| *Marrubium vulgare* L.[3], aerial parts | Marrouvio *ΠΡΑCΙΟΝ* (=*PRASION*) | GR (W) | D | E: 1 (diabetes), R: 2 (asthma, common cold), O: 1 (antipyretic) | TR (1), MOD (2) |
| *Melissa officinalis* L.[2,3], aerial parts | Melissa, *melissohorto* *ΜΕΛΙCCΟΦΥΛΛΟΝ* (=*MELISSOFYLLON*) | GR (W, C) | If, D | CV: 8 (blood circulation stimulant, heart stimulant, hypertension), ENT: 1 (ear aches), GI: 10 (bloating, dyspepsia, spasmolytic), M: 2 (cholesterol, uric acid), NP: 12 (brain stimulant, calmative, depression, dizziness, headache, migraine), R: 2 (common cold) | TR (6), MOD (6), OM (4) |
| *Mentha* × *piperita* L., aerial parts | *Menta* | GR (C) | If, D | GI: 1 (spasmolytic), R: 1 (common cold), UG: 1 (diuretic) | MOD (1) |
| *Mentha pulegium* L.[1], aerial parts | *Fliskouni ΛΗΧΟΝ* (=*GLIHON*) | GR (W) | D | CV: 2 (heart stimulant), GI: 1 (antiemetic), NP: 2 (anti-convulsive, calmative, dizziness, migraine) | TR (1), MOD (1) |
| *Mentha* spp.[d,1,2], aerial parts | *Dyosmos ΗΔΥΟCΜΟC*, *CΙCΥΜΒΡΙΟC* (=*IDYOSMOS*, *SISIMVRIOS*) | GR (W, C), IM | If, D, S | CV: 5 (hypertension), GI: 11 (antiemetic, bloating, diarrhoea, dyspepsia, spasmolytic), M: 11 (cholesterol), NP: 1 (calmative, headache), UG: 2 (renal colic), O: 3 (aphrodisiac, blood purification, stimulant) | TR (6), MOD (4), OM (5) |
| *Micromeria juliana* (L.) Bentham ex Reichenb.[1], aerial parts | Micromeria, yssopos, *kyparissaki ΥCCΩΠΟC* (=*YSSOPOS*) | GR (W) | If, D | E: 1 (diabetes), ENT: 1 (tonsillitis), GI: 2 (dyspepsia, spasmolytic, stomach ulcer), M: 1 (cholesterol), NP: 1 (calmative), R: 2 (bronchitis, common cold), UG: 12 (dysmenorrhoea, kidney stones, prostate) | TR (6), MOD (2), OM (4) |
| *Ocimum basilicum* L.[1], aerial parts | *Vassilikos ΩΚΙΜΟΝ* (=*OKIMON*) | GR (C), IM | If, D, S | GI: 3 (constipation, spasmolytic), NP: 13 (brain stimulant, calmative, depression, headache, migraine), R: 1 (bronchitis, common cold), UG: 1 (diuretic), O: 3 (antipyretic, appetizer, stimulant) | TR (5), MOD (5), OM (5) |
| *Origanum dictamnus* L.[1], aerial parts | *Diktamos*, *erontas* *ΔΙΚΤΑΜΝΟΝ* (=*DIKTAMNON*) | GR (W, C) | If, EA (Ws, Cm) | E: 1 (diabetes), GI: 13 (liver disorders, spasmolytic, stomach ulcer), M: 2 (cholesterol), NP: 3 (brain stimulant, headache), SK: 4 (antiseptic, sanative), UG: 5 (diuretic, dysmenorrhoea), O: 7 (antibacterial activities, aphrodisiac, stimulant) | TR (6), MOD (4), OM (4) |
| *Origanum majorana* L.[1], aerial parts | *Mantzourana* *CΑΜψΟΥΧΟΝ*, *ΑΜΑΡΑΚΟΝ* (=*SAMPSOUHON*, *AMARAKON*) | GR (C) | If, D | CV: 4 (hypertension, hypotension), GI: 13 (antiemetic, bloating, spasmolytic), IN: 1 (whooping cough), NP: 7 (calmative, dizziness, headache, migraine), R: 4 (asthma, common cold), UG: 1 (dysmenorrhoea), O: 2 (antipyretic) | TR (6), MOD (5), OM (5) |
| *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart (Syn.: *Origanum heracleoticum* auct. non L.)[1,2], aerial parts | *Rigani ΟΡΙΓΑΝΟC* *ΗΡΑΚΛΕΙΟΤΙΚΗ* (=*ORIGANOS IRAKLIOTIKI*) | GR (W, C), IM | Ch, If, D, EA (Fb, Ws), S | E: 1 (diabetes), GI: 6 (constipation, bloating, diarrhoea, dyspepsia, spasmolytic), M: 1 (cholesterol), NP: 1 (neuralgia), OR: 1 (toothache), R: 3 (asthma, common cold), S: 1 (rheumatisms), SK: 2 (antiseptic), UG: 1 (dysmenorrhoea), O: 2 (antipyretic, stimulant) | TR (4), MOD (4), OM (4) |
| *Origanum vulgare* L. subsp. *vulgare*, aerial parts | *Tsai dramas*, *pontiako tsai*, *kokkino tsai* | GR (W) | If | GI: 1 (bloating), NP: 1 (calmative), R: 2 (common cold), O: 2 (appetiser, stimulant) | OM (3) |

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| *Rosmarinus officinalis* L.[1], leaves | *Dendrolivano ΛΙΒΑΝΩΤΙC* ? (=*LIVANOTIS*) | GR (W, C), IM | If, EA (Fb, Ws), S | CV: 7 (blood circulation stimulant, hypertension), E: 1 (diabetes), GI: 5 (bloating, diarrhoea, dyspepsia, gallstones, liver disorders), IN: 1 (influenza), M: 1 (cholesterol), NP: 4 (calmative, depression, headache), OP: 1 (optical acuteness), S: 1 (rheumatisms), SK: 8 (hair loss), UG: 1 (diuretic), O: 7 (stimulant) | TR (5), MOD (5), OM (4) |
| *Salvia fruticosa* Miller[1] (Syn.: *Salvia triloba* L. fil.), aerial parts | *Faskomilo ΕΛΕΛΙCΦΑΚΟC* (=*ELELISFAKOS*) | GR (W) | If, D, EA (Fb, M, Ws) | CV: 8 (hypotension), E: 8 (diabetes), ENT: 5 (laryngitis, pharyngitis, tonsillitis), GI: 5 (constipation, diarrhoea, spasmolytic), H: 1 (anaemia), NP: 8 (brain stimulant, calmative, depression), R: 3 (common cold), S: 1 (arthritis), SK: 5 (hair loss, hair tonic, stomatitis), UG: 1 (dysmenorrhoea), O: 6 (stimulant) | TR (6), MOD (6), OM (5) |
| *Satureja thymbra* L.[1], aerial parts | *Throubi ΘΥΜΒΡΑ* (=*THYMVRA*) | GR (W) | D, If, S, EA (Fb) | E: 1 (diabetes), GI: 4 (diarrhoea, dyspepsia, spasmolytic), H: 1 (anaemia), IN: 1 (influenza), M: 1 (gout), NP: 1 (calmative), R: 2 (common cold), S: 2 (arthritis, rheumatisms), UG: 2 (kidney stones, renal colic), O: 5 (aphrodisiac, appetizer, stimulant) | TR (5), MOD (3), OM (2) |
| *Sideritis* spp.[e,1,3], aerial parts | *Tsai tou vounou* | GR (W, C) | If, D | GI: 1 (dyspepsia), H: 4 (anaemia), IN: 1 (influenza), NP: 1 (calmative), R: 11 (common cold), UG: 1 (diuretic), O: 5 (antipyretic, stimulant) | TR (6), MOD (5), OM (4) |
| *Teucrium chamaedrys* L.[3], aerial parts | Tefkrio hamedryo, hamedrys *ΧΑΜΑΙΔΡΥC* (=*HAMEDRYS*) | GR (W) | If (flowers), D, Ig (pulverized) | CV: 1 (hypertension), E: 1 (diabetes), GI: 2 (bloating, dyspepsia), R: 2 (bronchitis, common cold), UG: 1 (prostate, renal disorders), O: 1 (antipyretic, stimulant) | TR (1), MOD (1) |
| *Teucrium polium* L.[1,2,3], aerial parts | Yssopos, *lagokimithia ΠΟΛΙΟΝ ΤΟ ΟΡΕΙΝΟΝ* (=*POLION TO ORINON*) | GR (W) | D, EA (Cm) | CV: 3 (haemorrhoids, hypertension), E: 13 (diabetes), ENT: 1 (tonsillitis), GI: 3 (bloating, diarrhoea, dyspepsia), M: 2 (cholesterol), NP: 1 (calmative), R: 4 (asthma, bronchitis, common cold), S: 2 (arthritis, rheumatisms), SK: 2 (sanative), UG: 1 (dysmenorrhoea), O: 1 (blood purification, stimulant) | TR (6), MOD (4), OM (5) |
| *Thymus* spp.[f,1,2], aerial parts | *Thymari ΘΥΜΟC*[g], *ΕΡΠΥΛΛΟC*[h] (=*THYMOS*, *ERPYLLOS*) | GR (W) | If, D, S, EA (M, Ws) | GI: 1 (diarrhoea), IN: 2 (influenza, whooping cough), M: 1 (cholesterol), NP: 2 (brain stimulant, calmative), OR: 1 (teething pains), R: 8 (asthma, bronchitis, common cold, pneumonia), SK: 7 (antiseptic, hair loss, hair tonic), UG: 3 (diuretic, dysmenorrhoea), O: 7 (appetizer, stimulant) | TR (5), MOD (3), OM (4) |
| Lauraceae | | | | | |
| *Cinnamomum verum* Presl (Syn.: *Cinnamomum zeylanicum* Blume), bark | *Kanella* | IM | D, S | CV: 1 (heart stimulant), GI: 1 (antiemetic, diarrhoea, dyspepsia, intestinal parasites), R: 1 (common cold), UG: 1 (abortive, diuretic), O: 4 (aphrodisiac, stimulant) | TR (2), MOD (2), OM (1) |
| *Laurus nobilis* L.[1,3], leaves, fruits | *Dafni*[i], *dafnokoukoutsa*[j] *ΔΑΦΝΗ* (=*DAFNI*) | GR (W, C) | If, S, EA (Ws) | GI: 4 (bloating, diarrhoea, dyspepsia, enteritis, intestinal parasites, spasmolytic), NP: 1 (neuralgia), R: 3 (igmoritis), SK: 2 (hair loss, hair tonic), O: 1 (stimulant)[k] | TR (2), MOD (2), OM (2) |
| *Astragalus membranaceus* Moench, root | Astragalos | IM | D, Ig (pulverized) | CV: 1 (hypertension), GI: 1 (dyspepsia), R: 1 (common cold) | MOD (1) |
| *Ceratonia siliqua* L.[1], fruits | *Haroupia ΚΕΡΑΤΕΑ* (=*KERATEA*) | GR (W, C) | D, Ig | CV: 1 (blood circulation stimulant) | TR (1) |
| *Glycyrrhiza glabra* L., root | *Glykoriza ΓΛΥΚΥΡΙΖΑ* (=*GLYKYRIZA*) | GR (W, C), IM | D | E: 1 (diabetes), GI: 2 (constipation, spasmolytic), I: 1 (allergy), R: 2 (asthma, common cold, pneumonia), S: 1 (arthritis), O: 2 (stimulant) | TR (3), MOD (2) |

Table 1 (*Continued*)

| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| *Medicago sativa* L., aerial parts | *Midiki* | GR (W) | If, Ig (pulverized) | E: 1 (diabetes), GI: 1 (liver disorders, intestinal disorders), H: 1 (anaemia), S: 1 (arthritis), UG: 1 (infections of the genitalia) | MOD (1) |
| *Senna alexandrina* Miller (Syn.: *Cassia senna* L., *Cassia angustifolia* Vahl), fruits, leaves | *Fylla alexandrias*[l], *sinnamiki*[m] | IM | If[1] (prescription required), D[2], Ig (pulverized leaves) | GI: 15 (constipation) | TR (6), MOD (5), OM (4) |
| *Trifolium pratense* L., aerial parts | *Triphylli agrio* | GR (W) | If, EA (Cm) | CV: 1 (arteriosclerosis), E: 1 (diabetes), H: 1 (anaemia), IN: 1 (whooping cough), NP: 1 (anti-convulsive), R: 1 (bronchitis, common cold), SK: 1 (eczema, psoriasis), O: 1 (appetizer, blood purification, stimulant) | MOD (1) |
| *Trigonella foenum-graecum* L.[1], seeds | Trigonella, *tsimeni*, *moshositaro ΤΗΛΙC* (=*TILIS*) | GR (C), IM | D, Ig (pulverized) | E: 2 (diabetes), NP: 1 (calmative), O: 2 (appetizer, stimulant) | TR (3), MOD (1) |
| Liliaceae | | | | | |
| *Allium cepa* L.[1], bulbs | *Kremmydi ΚΡΟΜΜΥΟΝ* (=*KROMMYON*) | GR (C) | Ig, EA (Ws) | NP: 1 (brain stimulant), R: 1 (common cold), S: 1 (arthritis), SK: 1 (antiseptic), O: 1 (stimulant) | TR (2) |
| *Allium sativum* L.[1], bulbs, aerial parts | *Skordo CΚΟΡΔΟΝ* (=*SKORDON*) | GR (C), IM | Ig, EA (Ws) | CV: 4 (hypertension), N: 1 (anti-cancer capacities), SK: 1 (antiseptic), O: 1 (stimulant) | TR (4) |
| *Aloe vera* (L.) Burm. fil., latex | *Aloi ΑΛΟΗ* (=*ALOI*) | GR (W), IM | If, EA (Cm) | GI: 1 (constipation), R: 1 (common cold), S: 1 (arthritis, rheumatisms), SK: 1 (sanative) | TR (4), MOD (1) |
| *Ruscus aculeatus* L., aerial parts | *Lagomilia*, *argiomyrsini ΟΞΥΜΥΡCΙΝΗ*, *ΜΥΡCΙΝΗ ΑΓΡΙΑ* (=*OXYMYRSINI*, *MYRSINI AGRIA*) | GR (W) | If, D, EA (Cm, Ws) | CV: 1 (haemorrhoids), OP: 1 (retinitis), SK: 1 (itch, skin cracks), UG: 1 (menstruation disorders) | MOD (1) |
| *Smilax officinalis* Kunth, root | Smilax | IM | If | M: 1 (urea, uric acid), S: 1 (arthritis), UG: 1 (diuretic), O: 1 (antipyretic, blood purification) | MOD (1) |
| Linaceae | | | | | |
| *Linum usitatissimum* L.[1], seeds | *Linarosporos ΛΙΝΟΝ* (=*LINON*) | GR (C), IM | D, EA (P) | GI: 6 (constipation, gallstones, gastritis, jaundice, liver disorders), R: 14 (bronchitis, common cold, pneumonia), S: 1 (rheumatisms), SK: 2 (boils, pimples), UG: 1 (renal disorders) | TR (6), MOD (3), OM (5) |
| Loranthaceae | | | | | |
| *Viscum album* L., leaves, fruits | *Ixos*, *gi* | GR (W) | D, If (prescription required) | CV: 5 (hypertension, arteriosclerosis), NP: 2 (calmative, menopause emotional disorders) | TR (3), MOD (2) |
| Lythraceae | | | | | |
| *Lawsonia inermis* L., pulverized leaves | *Henna ΚΥΠΡΟC* (=*KYPROS*) | IM | EA (Ws with the water solution) | SK: 2 (hair tonic) | TR (1), MOD (1) |
| Malvaceae | | | | | |
| *Althaea officinalis* L.[3], root, flowers | *Althea ΑΛΘΑΙΑ* (=*ALTHEA*) | GR (W), IM | D, EA (Cm, Ws) | CV: 2 (coronal disease, hypertension), ENT: 2 (laryngitis, pharyngitis), GI: 3 (constipation, diarrhoea, enteritis, gastritis), R: 5 (bronchitis, common cold), SK: 5 (aphthae, gum wounds, pimples, stomatitis), UG: 3 (cystitis, kidney stones, nephritis), O: 1 (blood purification) | TR (4), MOD (2), OM (1) |

| | | | | | |
|---|---|---|---|---|---|
| *Hibiscus sabdariffa* L., flowers | *Iviskos* | IM | IF, D, EA (Ws) | E: 3 (diabetes), GI: 4 (constipation, dyspepsia), M: 6 (cholesterol, obesity, triglycerides), SK: 1 (hair tonic), O: 7 (blood purification, stimulant) | TR (5), MOD (4), OM (2) |
| *Malva sylvestris* L.[1,3], aerial parts | *Moloha ΜΑΛΑΧΗ* (=*MALAHI*) | GR (W) | If, D, EA (Cm, P) | ENT: 3 (laryngitis, pharyngitis), GI: 4 (diarrhoea, dyspepsia, gastritis, intestinal disorders), NP: 1 (epilepsy), R: 14 (asthma, bronchitis, common cold), SK: 1 (bruises, pimples), UG: 3 (cystitis, diuretic), O: 1 (sprains) | TR (6), MOD (3), OM (5) |
| Monimaceae | | | | | |
| *Peumus boldus* Molina, aerial parts | Boldo | IM | If | GI: 1 (dyspepsia, liver disorders), UG: 1 (diuretic), O: 1 (stimulant) | MOD (1) |
| Moraceae | | | | | |
| *Ficus carica* L.[1], latex | *Sykia* | GR (W, C) | EA (P) | SK: 1 (callus) | TR (1) |
| Myristicaceae | | | | | |
| *Myristica fragrans* Houtt., seeds | *Moshokarydo* | IM | D, S | GI: 5 (diarrhoea, dyspepsia, gallstones, spasmolytic), NP: 1 (calmative), UG: 1 (dysmenorrhoea), O: 1 (aphrodisiac, appetizer) | TR (3), MOD (1), OM (3) |
| Myrtaceae | | | | | |
| *Eucalyptus globulus* Labill.[1], leaves | *Efkalyptos* | GR (C) | Ih (If vapours), D, EA (Cm, Ws) | E: 1 (diabetes), ENT: 6 (higmoritis), IN: 4 (influenza), R: 12 (asthma, bronchitis, common cold), S: 1 (arthritis), SK: 5 (antiseptic), O: 1 (antipyretic) | TR (6), MOD (4), OM (3) |
| *Eugenia caryophyllata* Thunb. [Syn.: *Syzygium aromaticum* (L.) Merril & Perry], flowers | *Garyfalla*, *mosxokarfia* | IM | D, Ch, S | E: 2 (diabetes), GI: 2 (diarrhoea, dyspepsia, intestinal parasites), NP: 1 (neuralgia), OR: 2 (toothache), O: 1 (stimulant) | TR (4), OM (2) |
| Oleaceae | | | | | |
| *Fraxinus ornus* L.[3], leaves | Fraxinos, *fraxos*, *melios ΜΕΛΙΑ* (=*MELIA*) | GR (W) | If | GI: 1 (constipation), S: 1 (arthritis), UG: 2 (kidney stones, prostate) | TR (1), MOD (1) |
| *Olea europaea* L.[1], leaves | *Elia ΕΛΑΙΑ* (=*ELEA*) | GR (W, C) | If | CV: 2 (hypertension), E: 1 (diabetes), M: 1 (cholesterol), S: 1 (arthritis, rheumatisms), UG: 1 (diuretic), O: 1 (blood purification) | TR (2), MOD (1) |
| *Syringa vulgaris* L., inflorescences | *Pashalia* | GR (C) | If | GI: 1 (bloating), M: 1 (gout), S: 1 (rheumatisms), O: 1 (antipyretic) | OM (1) |
| Orchidaceae | | | | | |
| *Orchis* spp., pulverized bulbs | *Salepi ΟΡΧΙC* (=*ORHIS*) | GR (W), IM | D | R: 7 (common cold), O: 2 (stimulant) | TR (5), MOD (2) |
| Papaveraceae | | | | | |
| *Fumaria officinalis* L.[1], leaves | Foumaria, *kapnohorto ΚΑΠΝΟC* (=*KAPNOS*) | GR (W) | Ig (pulverized) | GI: 1 (gall disorders), O: 1 (blood purification) | MOD (1) |
| *Papaver somniferum* L.[1], seeds | *Paparouna ΜΗΚΩΝ ΗΜΕΡΟC Ο ΚΗΠΕΟC* (=*MIKON IMEROS O KIPEOS*) | IM | D | NP: 3 (calmative) | TR (2), MOD (1) |
| Passifloraceae | | | | | |
| *Passiflora caerulea* L., leaves, fruits | Passiflora, *rologia* | GR (C) | If, D | CV: 1 (hypertension), NP: 5 (anti-convulsive, calmative, neuralgia) | TR (3), MOD (1), OM (1) |
| *Passiflora incarnata* L., flowers, leaves | Passiflora | IM | If | CV: 2(hypertension), NP: 4 (anti-convulsive, calmative, neuralgia) | TR (2), MOD (2) |

Table 1 (*Continued*)

| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| Pedaliaceae | | | | | |
| *Harpagophytum procumbens* (Burch.) DC. ex Meisn., root | Arpagophyto | IM | If (prescription required) | E: 2 (diabetes), GI: 2 (gallstones, liver disorders), M: 2 (gout), NP: 2 (anti-convulsive, calmative, neuralgia), S: 4 (arthritis, back pains, rheumatisms), UG: 2 (renal disorders), O: 1 (stimulant) | TR (2), MOD (2) |
| Piperaceae | | | | | |
| *Piper nigrum* L., fruits | *Piperi mavro ΜΕΛΑΝ ΠΕΠΕΡΙ* (=*MELAN PEPERI*) | IM | S | G: 1 (dyspepsia), NP: 1 (calmative), R: 1 (common cold), O: 1 (aphrodisiac) | MOD (2) |
| Plantaginaceae | | | | | |
| *Plantago major* L.[1], aerial parts | Plantago, *pentanevro ΑΡΝΟΓΛΩCCON ΤΟ ΜΕΙΖΟΝ* (=*ARNOGLOSSON TO MIZON*) | GR (W) | If, D, EA (Cm, Ws) | CV: 1 (hypertension), E: 1 (diabetes), GI: 2 (constipation, gallstones), H: 3 (haemostatic), I: 1 (allergic rinitis), N: 1 (anti-cancer capacities), R: 2 (common cold), SK: 3 (sanative), UG: 5 (kidney stones, prostate), O: 2 (antipyretic, blood purification, sprains) | TR (4), MOD (3), OM (1) |
| Platanaceae | | | | | |
| *Platanus orientalis* L., fruits | *Platanos*, *platanomila ΠΛΑΤΑΝΟC* (=*PLATANOS*) | GR (W) | D, EA (Cm, P) | H: 1 (haemostatic), SK: 1 (abscesses, burns), UG: 4 (kidney stones, prostate) | TR (3), MOD (2) |
| Polygonaceae | | | | | |
| *Polygonum aviculare* L., aerial parts | *Polykobo ΠΟΛΥΓΟΝΟΝ* (=*POLYGONON*) | GR (W) | If, D, EA (Cm, P) | E: 2 (diabetes), GI: 3 (diarrhoea, spasmolytic), H: 1 (haemostatic), M: 1 (cholesterol), S: 2 (arthritis, rheumatisms), SK: 1 (sanative), UG: 9 (kidney stones, prostate) | TR (6), MOD (1), OM (2) |
| *Rheum palmatum* L., chopped or pulverized root, aerial parts | *Raventi* | GR (C), IM | If | GI: 5 (constipation), | TR (4), MOD (1) |
| *Rumex* spp.[1], aerial parts | *Lapatha*, *lapata ΚΗΠΕΥΤΙΚΟΝ ΛΑΠΑΘΟΝ* (=*KIPEFTIKON LAPATHON*) | GR (W, C) | D, Ig (pulverized) | GI: 1 (liver disorders), UG: 1 (diuretic), O: 2 (blood purification) | TR (2), MOD (1) |
| Primulaceae | | | | | |
| *Primula veris* L., aerial parts | Primoula | GR (W) | Ig (fresh or dried), EA (P) | GI: 1 (gall disorders), H: 1 (haemostatic) | MOD (1) |
| Punicaceae | | | | | |
| *Punica granatum* L.[1], peel | *Rodia ΡΟΙΑ* (=*RIA*) | GR (W, C) | D | GI: 4 (diarrhoea, intestinal parasites) | TR (2), MOD (1) |
| Ranunculaceae | | | | | |
| *Delphinium staphisagria* L.[1], fruits | *Papazoto CΤΑΦΙC ΑΓΡΙΑ* (=*STAPHIS AGRIA*) | IM | EA (Ws) | SK: 3 (hair loss, hair tonic, louse) | TR (2), MOD (1) |
| *Hydrastis canadensis* L., aerial parts | Ydrastis | IM | Ig (pulverized) | O: 1 (antibiotic) | MOD (1) |

| | | | | | |
|---|---|---|---|---|---|
| Rhamnaceae | | | | | |
| *Paliurus spina-christi* Miller, fruits | *Paliouri ΠΑΛΙΟΥΡΟC* (=*PALIOUROS*) | GR (W) | If | GI: 1 (diarrhoea), M: 1 (cholesterol), R: 1 (common cold), UG: 3 (kidney stones, prostate, urinary infection), O: 1 (blood purification) | TR (3), OM (1) |
| *Rhamnus purshiana* DC., bark | Ramnos, frangoula, kaskara | IM | D, Ig (pulverized) | CV: 1 (blood circulation, stimulant), GI: 3 (bloating, constipation, gall disorders), NP: 1 (dizziness, headache) | TR (1), MOD (2) |
| Rosaceae | | | | | |
| *Agrimonia eupatoria* L.[3], aerial parts | Agrimonio *ΑΡΓΕΜΩΝΗ* (=*ARGEMONI*) | GR (W), IM | If | ENT: 4 (laryngitis, pharyngitis, tonsillitis), GI: 5 (diarrhoea, gallstones, intestinal parasites), R: 3 (common cold), S: 4 (arthritis, rheumatisms), UG: 4 (diuretic) | TR (4), MOD (2), OM (1) |
| *Crataegus monogyna* Jacq.[3], leaves, flowers, fruits | Krategos | GR (W) | If, D | CV: 6 (arteriosclerosis, coronary disease, heart stimulant, hypertension), GI: 2 (diarrhoea), NP: 4 (calmative, menopause emotional disorders) | TR (4), MOD (2), OM (1) |
| *Cydonia oblonga* Miller[1,3], leaves, dried fruits, seeds | *Kydonia* | GR (C) | D | GI: 1 (diarrhoea), R: 1 (common cold) | TR (1), OM (1) |
| *Prunus avium* L.[1,3], fruit pedicels | *Kerasooures* | GR (C) | If | E: 1 (diabetes), M: 1 (obesity), UG: 6 (kidney stones, renal colic) | TR (5), MOD (2), OM (1) |
| *Prunus spinosa* L.[2,3], fruits, leaves | *Koromilia Afrikis*, *tsapournia ΑΓΡΙΟΝ ΚΟΚΚΥΜΗΛΟΝ* (=*AGRION KOKKYMILON*) | GR (W) | If, D (prescription required) | UG: 2 (diuretic, prostate) | TR (1), MOD (1) |
| *Rosa* spp.[1,2,3], fruits, petals | *Kynorodo*[n], *rodopetala*[o], *ΡΟΔΟΝ* (=*RODON*) | GR (W, C), IM | D, If, EA (Cm, Ws) | CV[n]: 2 (coronary disease), E: 2 (diabetes), ENT: 3 (tonsillitis), GI: 5 (constipation, enteritis), IN[n]: 3 (influenza), NP: 5 (calmative, dizziness, headache, migraine), OP: 3 (eye inflammation, eyelid irritation), R: 3 (common cold), SK: 2 (aphthae), UG: 5 (infections of the vagina, kidney stones, menstruation disorders), O[n]: 10 (aphrodisiac, stimulant) | TR (6), MOD (3), OM (3) |
| *Rubus* spp.[1,3], root, aerial parts | *Vatos ΒΑΤΟC* (=*VATOS*) | GR (W) | If, D | E: 7 (diabetes), ENT: 3 (pharyngitis), GI: 6 (diarrhoea), H: 2 (anaemia), NP: 1 (senility), UG: 2 (diuretic) | TR (3), MOD (3), OM (1) |
| *Spiraea japonica* L. fil., aerial parts | Spirea | IM | D, Ig (pulverized) | CV: 1 (heart stimulant), M: 1 (obesity), S: 1 (arthritis, rheumatisms), SK: 1 (cellulites), UG: 1 (diuretic, renal colic) | MOD (1) |
| Rubiaceae | | | | | |
| *Cinchona pubescens* Vahl (Syn.: *C. succirubra* Pavon ex Kotzsch), bark | Kina–kina | IM | D | IN: 1 (malaria), O: 1 (antipyretic, appetizer) | TR (1), MOD (1) |
| *Galium aparine* L.[1], aerial parts | *Kollitsida ΑΠΑΡΕΪΝΗ* (=*APAREINI*) | GR (W) | D, EA (Ws) | M: 3 (uric acid), SK: 2 (dandruffs), UG: 4 (cystitis, kidney stones), O: 2 (blood purification) | TR (2), MOD (2) |
| *Galium odoratum* (L.) Scop, aerial parts | Asperoula | GR (W) | D | GI: 1 (jaundice), NP: 1 (calmative, anti-convulsive), | TR (1) |
| *Pausinystalia johimbe* (K. Schum.) Pierre ex Beille, bark | Yohimb | IM | D | O: 3 (aphrodisiac, stimulant) | TR (2), MOD (1) |
| Rutaceae | | | | | |
| *Dictamnus albus* L.[3], aerial parts | *Agiovarvaro ΔΙΚΤΑΜΝΟΝ* ? (=*DIKTAMNON*) | IM | If | GI: 1 (spasmolytic), O: 1 (stimulant) | MOD (1) |

Table 1 (*Continued*)

| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| *Ruta graveolens* L.[1,3], aerial parts | *Apiganos ΠΗΓΑΝΟΝ* (=*PIGANON*) | GR (W) | If | M: 1 (obesity), NP: 1 (anti-convulsive, calmative), UG: 3 (abortive, prostate), O: 1 (anti-poisoning) | TR (2), MOD (1) |
| Salicaceae | | | | | |
| *Salix* spp.[1], leaves, bark | *Itia ITEA* (=*ITEA*) | GR (W) | D, If | GI: 3 (constipation, intestinal parasites), S: 2 (rheumatisms), O: 4 (antipyretic) | TR (2), MOD (2) |
| Santalaceae | | | | | |
| *Santalum album* L., bark | *Sandaloksylo* | IM | D, EA (Ws) | NP: 1 (brain stimulant, depression), SK: 1 (antiseptic), O: 1 (stimulant) | MOD (1) |
| Sapindaceae | | | | | |
| *Paullinia cupana* Kunth, seeds | Gouarana | IM | IF, Ig (pulverized) | O: 2 (aphrodisiac, stimulant) | MOD (2) |
| Scrophulariaceae | | | | | |
| *Euphrasia salisburgensis* Funck[1], aerial parts | Ephrazia | GR (W) | EA (Cm, Ws) | ENT: 1 (igmoritis), OP: 1 (eye irritation, eyelid inflammation, conjunctivitis, photophobia), R: 1 (common cold) | TR (1), MOD (1) |
| *Verbascum* spp.[1,3], aerial parts | Verbasko, *flomos*, *flomohorto ΦΛΟΜΜΟΣ* (=*FLOMMOS*) | GR (W) | If, EA (Cm) | CV: 1 (haemorrhoids), GI: 1 (constipation), R: 3 (asthma, common cold), S: 1 (arthritis, rheumatisms), SK: 1 (sanative), UG: 1 (diuretic) | TR (1), MOD (2), OM (1) |
| Simarubaceae | | | | | |
| *Quassia amara* L., wood | *Pikroxylo* | IM | If, D | E: 9 (diabetes), M: 1 (cholesterol) | TR (6), MOD (3) |
| Solanaceae | | | | | |
| *Capsicum anuum* L., pulverized fruits | *Piperi kokkino* | GR (C), IM | S | CV: 2 (blood circulation stimulant, heart stimulant), M: 1 (cholesterol) | TR (1), MOD (1) |
| *Datura stramonium* L., aerial parts | Datoura | GR (W) | D | NP: 1 (nymphomania) | TR (1) |
| Theaceae | | | | | |
| *Camellia sinensis* (L.) Kuntze, leaves | *Tsai prasino*[p], *tsai mavro*[q] | IM | If | CV[p]: 1 (arteriosclerosis), E[p]: 2 (diabetes, thyroid), GI: 9 (diarrhoea[q], liver disorders[p]), M: 3 (cholesterol[p], obesity[q], triglycerides[p]), N[p]: 4 (anti-cancer capacities), NP[p]: 2 (brain stimulant, calmative), OP[q]: 1 (eye inflammation), O: 2 (anti-poisoning[q], blood purification[p], stimulant[p,q]) | TR (5), MOD (3), OM (3) |
| Tiliaceae | | | | | |
| *Tilia* spp.[3], bracts, leaves, inflorescences | *Tilio*, *flamouri* | GR (W, C) | If, D, EA (Ws) | CV: 3 (arteriosclerosis, hypertension, phlebitis), E: 2 (diabetes), GI: 3 (spasmolytic), IN: 1 (influenza), M: 3 (uric acid), NP: 12 (anti-convulsive, calmative, headache, migraine), R: 9 (common cold), S: 1 (arthritis), SK: 3 (hair tonic, sanative), UG: 6 (kidney stones, renal disorders), O: 5 (antipyretic) | TR (6), MOD (5), OM (5) |
| Ulmaceae | | | | | |
| *Ulmus minor* Miller, leaves, bark | Ptelea, *ftelia*, *karagatsi ΠΤΕΛΕΑ* (=*PTELEA*) | IM | If | E: 1 (diabetes), GI: 1 (constipation), UG: 2 (diuretic, prostate), O: 1 (antipyretic, stimulant) | MOD (2) |
| Umbelliferae | | | | | |
| *Anethum graveolens* L., leaves | *Anithos ΑΝΗΘΟΝ* (=*ANITHON*) | GR (C) | If, D, S | GI: 3 (dyspepsia, spasmolytic, stomach ulcer, intestinal parasites), M: 1 (obesity), NP: 2 (calmative), UG: 1 (diuretic, dysmenorrhoea) | TR (1), MOD (2) |

| | | | | | |
|---|---|---|---|---|---|
| *Angelica sylvestris* L., aerial parts | Angeliki, arhangeliki | GR (W) | D | CV: 1 (heart stimulant), GI: 2 (bloating, dyspepsia, enteritis, liver disorders), H: 2 (anaemia), M: 1 (obesity), NP: 1 (depression), S: 2 (arthritis, rheumatisms), R: 1 (common cold), O: 3 (stimulant) | TR (3), MOD (1) |
| *Apium graveolens* L., root, leaves, seeds | *Selino ΕΛΕΙΟCΕΛΙΝΟΝ* (=*ELIOSELINON*) | GR (C) | Ig (root), If (leaves), EA (M) | E: 2 (diabetes), GI: 1 (liver disorders), M: 2 (uric acid), NP: 2 (anti-convulsive), S: 2 (rheumatisms), SK: 2 (frost bites), UG: 2 (diuretic), O: 2 (aphrodisiac, stimulant) | TR (1), MOD (1) |
| *Carum carvi* L., fruits | Karo | IM | S | GI: 1 (stomach disorders) | MOD (1) |
| *Cuminum cyminum* L.[1], fruits | *Kymino ΚΥΜΙΝΟΝ ΗΜΕΡΟΝ* (=*KYMINON IMERON*) | GR (C) | S | E: 1 (diabetes), ENT: 1 (deafness), GI: 2 (dyspepsia, spasmolytic), UG: 2 (diuretic, orchitis) | TR (2), MOD (1) |
| *Centella asiatica* (L.) Urban (Syn.: *Hydrocotyle asiatica* L.), leaves | Sentella | IM | If | CV: 1 (blood circulation stimulant, hypertension, phlebitis), M 1 (uric acid), SK: 1 (cellulites), UG: 1 (menstruation disorders) | MOD (1) |
| *Coriandrum sativum* L., whole or pulverized fruits | *Koliandros ΚΟΡΙΟΝ* (=*KORION*) | GR (C), IM | D, If, S | GI: 4 (bloating, dyspepsia, spasmolytic, stomach disorders), R: 1 (common cold), S: 1 (rheumatisms), O: 2 (appetizer, aphrodisiac, stimulant) | TR (2), MOD (1), OM (1) |
| *Foeniculum vulgare* Miller[1,3], aerial parts, seeds | *Marathos ΜΑΡΑΘΟΝ* (=*MARATHON*) | GR (C) | D | GI: 4 (bloating, dyspepsia, gall disorders, spasmolytic), M: 4 (obesity), R: 1 (common cold), UG: 3 (kidney stones) | TR (2), MOD (2), OM (1) |
| *Petroselinum crispum* (Miller) A. W. Hill (Syn.: *Petroselinum sativum* Hoffm.)[1,2], leaves | *Maidanos ΠΕΤΡΟCΕΛΙΝΟΝ* (=*PETROSELINON*) | GR (C) | If | CV: 1 (hypertension), UG: 4 (cystitis, kidney stones, prostate), O: 4 (antipyretic, aphrodisiac, appetizer, blood purification, stimulant) | TR (3), MOD (2), OM (1) |
| *Pimpinella anisum* L.[1], fruits | *Glykanisos ΑΝΙCΟΝ* (=*ANISON*) | GR (C), IM | If, D, EA (Ws) | GI: 14 (bloating, constipation, dyspepsia, intestinal disorders, spasmolytic), NP: 5 (calmative), R: 3 (asthma, bronchitis, common cold), SK: 1 (antiseptic), UG: 1 (diuretic) | TR (6), MOD (5), OM (4) |
| Urticaceae | | | | | |
| *Parietaria officinalis* L.[2,3], aerial parts | Parietaria, *perdikaki ΠΕΡΔΙΚΙΟΝ* (=*PERDIKION*) | GR (W) | If | UG: 2 (diuretic, renal disorders, prostate) | TR (1), MOD (1) |
| *Urtica* spp.[r,1,2,3], aerial parts, root | *Tsouknida ΚΝΗΦΗ, ΚΝΗΔΗ* (=*KNIPHI, KNIDI*) | GR (W) | If, D, EA (Cm, M, P, Ws) | CV: 2 (blood circulation stimulant, haemorrhoids, hypertension), E: 2 (diabetes), GI: 6 (constipation, gallstones, liver disorders), H: 8 (anaemia, haemostatic), M: 6 (cholesterol, uric acid), NP: 1 (headache), S: 5 (arthritis, rheumatisms), SK: 9 (dandruffs, eczema, hair loss), UG: 11 (dysmenorrhoea, kidney stones), O: 5 (aphrodisiac, blood purification stimulant) | TR (6), MOD (6), OM (4) |
| Valerianaceae | | | | | |
| *Valeriana officinalis* L. subsp. *officinalis*[3], aerial parts, root | Valeriana *ΟΡΕΙΝΗ ΝΑΡΔΟC* ? (=*ORINI NARDOS*) | GR (W), IM | If, D, EA (P, Cm) | CV: 5 (hypertension), M: 1 (obesity), NP: 12 (anti-convulsive, calmative, headache, neuralgia), R: 1 (common cold), SK: 1 (sanative), UG: 2 (diuretic) | TR (6), MOD (4), OM (2) |
| Verbenaceae | | | | | |
| *Aloysia triphylla* (L' Her.) Britton [Syn.: *Aloysia citriodora* Ortega ex Pers., *Lippia citriodora* (Lam.) Kunth], leaves | *Louiza* | GR (C), IM | If, D | E: 2 (diabetes), GI: 8 (constipation, diarrhoea, dyspepsia, gall disorders, intestinal disorders, spasmolytic), M: 17 (cholesterol, obesity, triglycerides, uric acid), NP: 6 (anti-convulsive, calmative, depression), R: 1 (common cold), UG: 10 (kidney stones, menstruation disorders), O: 3 (antipyretic, stimulant) | TR (6), MOD (5), OM (5) |

Table 1 (*Continued*)

| Taxon and part used | Commercial name and name in Dioscurides | Source | Preparation | Medicinal popular use—number of mentions | Market spots |
|---|---|---|---|---|---|
| Valeriana officinalis, leaves | Vervena *ΠΕΡΙΣΤΕΡΕΩΝ ΥΠΤΙΟΣ* (=*PERISTEREON YPTIOS*) | GR (W), IM | If | IN: 1 (influenza), NP: 2 (calmative, depression, epilepsy, headache), R: 2 (asthma, common cold, pneumonia), S: 1 (rheumatisms), UG: 1 (dysmenorrhoea), O: 3 (antipyretic) | TR (3), MOD (1) |
| Vitaceae | | | | | |
| *Vitis vinifera* L. subsp. *Vinifera*, leaves | *Ampeli ΑΜΠΕΛΟΣ* (=*AMPELOS*) | GR (C) | D | M: 1 (cholesterol), UG: 1 (renal disorders) | TR (1) |
| Zingiberaceae | | | | | |
| *Curcuma longa* L. (Syn.: *Curcuma domestica* Val.), pulverized root | *Kourkoumas*, *kitrinoriza* | IM | If, S | GI: 2 (dyspepsia, gall disorders, liver disorders), M: 1 (cholesterol), N: 1 (anti-cancer capacities), S: 1 (arthritis, rheumatisms), UG: 1 (urinary infection), O: 1 (antibacterial and antifungal activities, stimulant) | TR (1), MOD (1) |
| *Elettaria cardamomum* (L.) Maton, fruits | *Kardamo*, *kakoule* | IM | D, S, EA (Cm, Ws) | CV: 1 (heart stimulant), R: 1 (common cold), SK: 2 (freckles, hair tonic), O: 4 (aphrodisiac, stimulant) | TR (4), MOD (1) |
| *Zingiber officinale* Roscoe, chopped or pulverized root | Ginger, *piperoriza* | IM | D, If, S, EA (Ws) | GI: 1 (bloating, dyspepsia, gall disorders, spasmolytic), IN: 1 (influenza), NP: 1 (neuralgia), R: 3 (common cold), S: 1 (arthritis, rheumatisms), SK: 1 (antiseptic), O: 5 (antibacterial activities, aphrodisiac, appetizer, stimulant) | TR (4), MOD (4) |

Plants cited in [1] Fragaki (1969), [2] Tamarro and Xepapadakis (1986) and [3] Vokou et al. (1993); commercial name in lower case italics is also a local name; question mark (?) next to Dioscurides name shows that the species identity is uncertain. GR, Greek origin; IM, imported; W, collected from the wild; C, cultivated; Ch, chewing; D, decoction; EA, external application (Cm, compress; Fb, footbath; M, massage; P, poultice; Ws, washings); F, food; If, infusion; Ih, inhalation; Ig, ingestion; S, seasoning; TR, traditional shops; MOD, modern shops; OM, open-air market stalls. For the abbreviations of the disease names, see Section 2.

[a] Refers to the fruits.
[b] Refers to the root.
[c] Refers to the fruits.
[d] *Mentha longifolia* L. or *Mentha spicata* L. or *Mentha villoso-nervata* Opiz (*Mentha longifolia* x *Mentha spicata*).
[e] *S. euboea* Heldr. or *Sideritis perfoliata* L. subsp. *perfoliata* or *Sideritis raeseri* Boiss. & Heldr. subsp. *raeseri* or *Sideritis scardica* Griseb. or *Sideritis syriaca* L. subsp. *syriaca*.
[f] *Thymus capitatus* (L.) Hoffmanns. & Link or *Thymus sibthorpii* Bentham.
[g] Refers to *Thymus capitatus*.
[h] Refers to *Thymus sibthorpii*.
[i] Refers to the leaves.
[j] Refers to the fruits.
[k] All uses refer to the leaves except SK which refers to both.
[l] Refers to the fruits.
[m] Refers to the leaves.
[n] Refers to the fruits.
[o] Refers to the petals.
[p] Refers to the green tea.
[q] Refers to the black tea.
[r] *Urtica dioica* L. or *Urtica piulifera* L. or *Urtica urens* L.

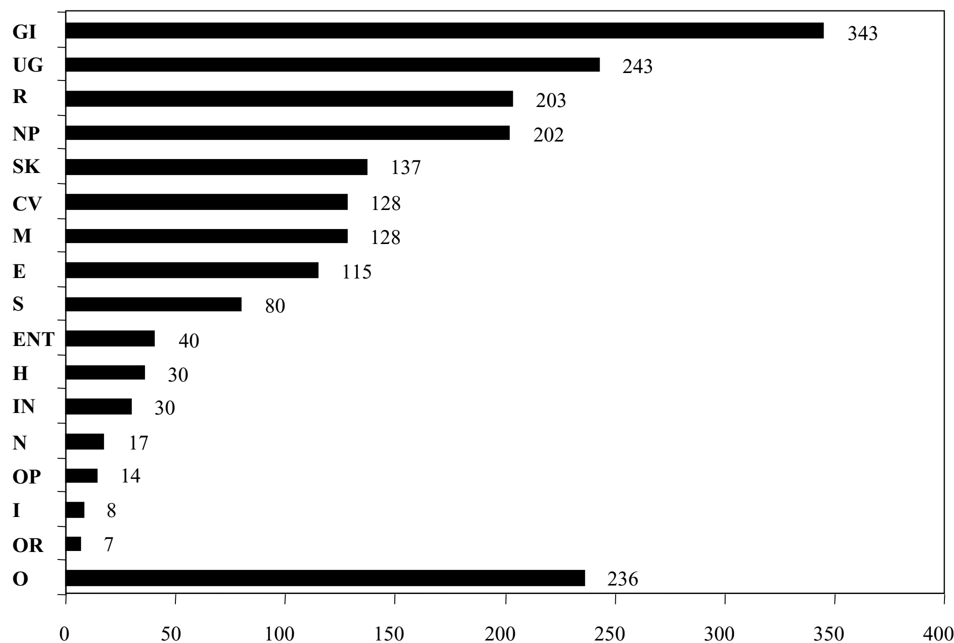


Fig. 1. Total number of mentions of the therapeutic uses in each group of diseases (for explanation of the abbreviations see Section 2).

with therapeutic properties. External application of the decoction or the infusion for washings, massage, footbaths or compresses is recommended for 61 herbs, while application of poultices is recommended for 17 herbs.

The more frequently reported medicinal uses are related to diseases of the gastrointestinal tract, the urogenital and respiratory systems and to neuropsychiatric diseases (in descending order), which represent more than 50% of the total uses (Fig. 1). Most of the herbs are recommended by the traders to cure a wide array of ailments (Table 1). Identical uses are reported in almost all market spots for many of them (e.g. *Aloysia triphylla* recommended against metabolic diseases in 16 spots, *Linum usitatissimum* recommended for respiratory ailments in 14 spots). Only a few herbs have exclusively one use, constantly mentioned in all market spots examined (e.g. *Senna alexandrina*, recommended against constipation in all 15 market spots traded). In some cases the therapeutic uses reported are in contradiction, as different traders recommend the same herb for conflicting ailments (e.g. *Lavandula angustifolia* against both hypertension and hypotension, *Zea mays* as diuretic and against incontinence).

The most frequently traded herbs, occurring in at least the 2/3 of the market spots examined, are listed in Table 2. They are all of Greek origin (with the exception of *Senna alexandrina*), mainly found in the traditional shops and most of them are gathered from the wild. As can be seen these popular herbs are recommended for a variety of ailments (except *Senna alexandrina*), ranging from 8 (in *Sideritis* spp.) up to 22 (in *Chamomilla recutita* and *Taraxacum* spp.). Among them there are many aromatic taxa used to prepare teas with therapeutic properties, e.g. *Salvia fruticosa*, *Melissa officinalis*, *Origanum majorana*, *Tilia* spp., *Aloysia triphylla*,

Table 2
The most frequently traded herbs in the market of Thessaloniki, found in at least the 2/3 of the market spots examined and number of ailments for which they are recommended

| Taxon | Number of ailments |
|---|---|
| *Chamomilla recutita*[1,2,4] | 22 (54) |
| *Taraxacum* spp.[2] | 22 (42) |
| *Acinos suaveolens* | 21 (43) |
| *Urtica* spp.[1,2,3,4] | 21 (55) |
| *Achillea millefolium*[1,4] | 20 (34) |
| *Aloysia triphylla* | 19 (47) |
| *Rosa* spp.[1,2,3,4] | 19 (43) |
| *Salvia fruticosa*[1,2] | 19 (51) |
| *Rosmarinus officinalis*[1,2] | 18 (37) |
| *Teucrium polium*[1,2,3,4] | 18 (33) |
| *Thymus* spp.[1,2,3] | 18 (32) |
| *Tilia* spp.[4] | 18 (48) |
| *Hypericum perforatum*[1,2,3,4] | 17 (36) |
| *Melissa officinalis* subsp. *Officinalis*[1,2,4] | 16 (35) |
| *Origanum vulgare* subsp. *Hirtum*[1,2,3] | 16 (19) |
| *Malva sylvestris*[1,2,4] | 15 (27) |
| *Lavandula angustifolia* subsp. *Angustifolia* | 14 (24) |
| *Origanum dictamnus*[1,2] | 14 (35) |
| *Origanum majorana*[1,2] | 14 (32) |
| *Sambucus nigra*[1,4] | 14 (31) |
| *Mentha* spp.[1,2,3] | 13 (33) |
| *Ocimum basilicum*[1,2] | 13 (21) |
| *Linum usitatissimum*[1,2] | 12 (24) |
| *Micromeria juliana*[1,2] | 12 (20) |
| *Pimpinella anisum*[1,2] | 11 (24) |
| *Elymus repens*[4] | 9 (21) |
| *Eucalyptus globulus*[2] | 9 (30) |
| *Valeriana officinalis* subsp. *Officinalis*[1,4] | 9 (22) |
| *Sideritis* spp.[2,4] | 8 (24) |
| *Senna alexandrina* | 1 (15) |

Cited in [1] Dioscurides, [2] Fragaki (1969), [3] Tamarro and Xepapadakis (1986), [4] Vokou et al. (1993).
Numbers in brackets denote total number of mentions.

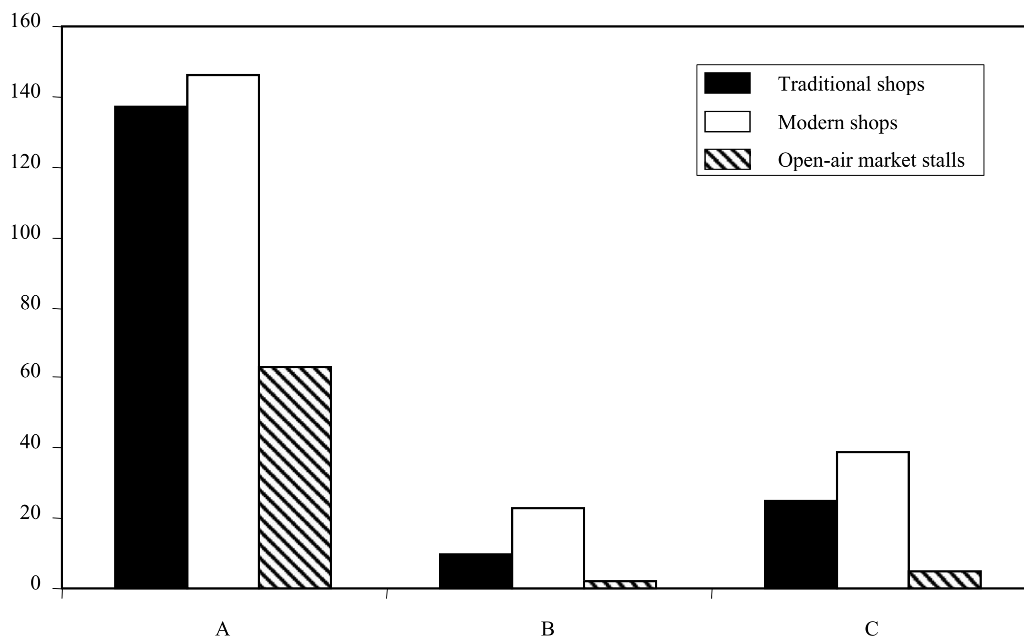


Fig. 2. Differences of the market spots examined. (A) Total number of herbs traded in each group of market spots. (B) Number of herbs confined in one group of market spots. (C) Number of exclusively imported herbs traded in each group of market spots.

*Origanum dictamnus* (a Greek endemic species), *Acinos suaveolens* (a Balkan mint odoured Labiatae), etc.

The differences of the market spots examined are summarized in Fig. 2. As can be seen the three groups differ in:

(i) The total number of herbs traded. The higher number (146 herbs) was found in the modern shops, while 137 and 63 herbs were found in the traditional shops and in the open-air market stalls, respectively.
(ii) The number of herbs confined in one group of market spots. Again the higher number was found in the modern shops, where 23 herbs are exclusively traded. These are mostly imported (e.g. *Centella asiatica*, *Ginkgo biloba*, *Hydrastis canadensis*, *Paullinia cupana*) or Greek taxa, rather rarely used in the country (e.g. *Medicago sativa*, *Primula veris*, *Trifolium pratense*). Ten herbs were confined in the traditional shops (e.g. *Ceratonia siliqua*, *Datura stramonium*, *Ficus carica* (latex)), while only two (*Origanum vulgare* subsp. *vulgare* and *Syringa vulgaris*) were found exclusively in open-air market stalls.
(iii) The number of the exclusively imported herbs. They were mainly found in the modern and the traditional shops (39 and 25, respectively), while only six were found in the open-air market stalls.

Medicinal plants used traditionally in Greece are, as a rule, recognizable from the following features: (i) they are native or if imported, they have been consolidated in Greek tradition long time ago; (ii) they are found in the market with a Greek local name (sometimes, as a modern influence, a commercial name deriving from the scientific name is also used); (iii) they are mostly sold in the traditional shops and in the open-air market stalls. The use of most of these plants for therapeutic purposes dates from antiquity. A remarkable number of medicinal plants (93 taxa) found in the market of Thessaloniki is cited by Dioscurides, while 81 taxa are reported in recent ethnobotanical studies from different parts of Greece with rich ethnobotanical tradition (Table 1) (Fragaki, 1969; Tamarro and Xepapadakis, 1986; Vokou et al., 1993). Furthermore the majority of the most frequently traded herbs are included in the ethnobotanical works mentioned above (Table 2). It is notable that the recent publications share 66 taxa with the ancient treatise, showing that the utilization of many of Dioscurides plants is uninterrupted until today (e.g. *Artemisia absinthium*, *Asplenium ceterach*, *Centaurium erythraea*, *Foeniculum vulgare*, *Hedera helix*, *Hypericum perforatum*, *Juniperus oxycedrus*, *Laurus nobilis*, *Ruta graveolens*, *Teucrium polium*, etc.). According to information provided by Theophrastus (4th century B.C.) and Dioscurides, the herbs imported from areas of West Asia and other parts of the Mediterranean area since ancient times are *Acorus calamus*, *Aloe vera*, *Crocus sativus*, *Cuminum cyminum*, *Elettaria cardamomum*, *Lawsonia inermis*, *Ocimum basilicum* and *Piper nigrum*. Finally, some taxa originating from East Asia, C and S Africa and the New World have been used in traditional medicine at least since 18th century (e.g. *Cinchona pubescens*, *Cinnamomum verum*, *Eugenia caryophyllata*, *Rheum palmatum*) (cf. Svoronos, 1956; Vakalopoulos, 1983).

Medicinal herbs recently introduced in the Greek market have the following features: (i) they are imported or if native, their use has started the last few years, since they are not reported in the ancient Greek treatises or in the more

recent ethnobotanical publications mentioned; (ii) they lack a Greek local name and their commercial name derives, as a rule, from their scientific name; (iii) they are sold in a few, mostly modern shops. Examples of plants recently introduced to Greece, under the influence of worldwide trends, are the imported *Astragalus membranaceus*, *Centella asiatica*, *Eleutherococcus senticosus*, *Ginkgo biloba*, *Hydrastis canadensis*, *Paullinia cupana*, *Peumus boldus*, *Spiraea japonica*, *Yucca filamentosa* (Table 1).

Finally, it is interesting to note that many of the herbs sold in Thessaloniki are also found in the markets of other East Mediterranean countries like Turkey (67 herbs in common) (Bingöl, 1995), Israel (59 herbs) (Lev and Amar, 2000; Said et al., 2002) and Jordan (46 herbs) (Lev and Amar, 2002). These affinities are attributed to the similar environmental conditions of this area, resulting to the highly allied native floras of these countries (the main source of the medicinal plants), as well as to the broad commercial and cultural exchanges among the Mediterranean people, existing since ancient times.

In conclusion, our results show that Thessaloniki has a well-developed herb market, which is flourishing to some extent under the influence of the worldwide trend on increasing use of natural products for health care. However, it appears that the herb trade is largely based on the traditional knowledge, which has survived since ancient times.

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# Analgesic and antiinflammatory activities of *Erigeron floribundus*

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**Abstract**

This study was intended to evaluate the analgesic and antiinflammatory activities of an aqueous extract of *Erigeron floribundus* (H.B. & K) or (syn": *Conyza sumatrensis* (Retz) E.K. Walker) (Asteraceae). Phytochemical analysis was carried out using standard methodologies. The analgesic investigations were carried out against two types of noxious stimuli, chemical (formalin-induced pain and acetic acid-induced writhing) and thermal (hotplate and tail immersion tests). The effects following aspirin and naloxone pretreatments were also studied. For the antiinflammatory activities, the carrageenan-induced oedema of the hindpaw of rats was used and the paw volume measured plethysmometrically from 0 to 24 h after injection. This was compared to a standard drug indomethacin (10 mg/kg). The results were subjected to statistical analysis. The plant had saponins, flavonoids, glycosides, alkaloids, oils, phenols and tannins and significantly increased the reaction time of hotplate and immersion tests. It decreased the writhings of acetic acid-induced abdominal contractions and lickings of formalin-induced pain. Aspirin had no effect on hotplate and tail immersion tests but showed an effect on writhing test. These results showed that the plant had both central and peripheral acting effects and this was confirmed by its effect on both phases of formalin-induced pain. The extract also significantly decreased the rat paw oedema volume at 50 mg/kg and above. In conclusion, *Erigeron floribundus* has central and peripheral analgesic properties as well as antiinflammatory activities.
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*Keywords:* *Erigeron floribundus*; *Conyza sumatrensis*; Analgesic; Antiinflammatory; Antinociceptive

## 1. Introduction

*Erigeron floribundus* (H.B. & K) or (syn": *Conyza sumatrensis* (Retz) E.K. Walker) is a terrestrial herbaceous plant of the family Asteraceae. The plant has multiple traditional uses including rheumatism, gout, cystitis, nephritis, dysmenorrhoea, dental pain, headache (Valnet, 1983; Burkill, 1985). For dental pain, fresh leaves are ground to paste and applied whenever necessary to the painful tooth. For dysmenorrhoea, 200 g of dried leaves are boil in 1 l of water for 2 h, cooled, filtered and 100 ml taken thrice a day.

Work has been done on other species and but little on *Erigeron Floribundus*. This plant has been shown to have no in vitro effect on poliovirus replication (Abad et al., 1999) but found to have antiinflammatory activity on mice (De Las Heras et al., 1998).

Since the plant is used traditionally in the treatment of painful illnesses like dysmenorrhoea and antiinflammatory diseases like gout, cystitis, nephritis and rheumatic arthritis, it became worthwhile to evaluate its antinociceptive and antiinflammatory activities in rats and mice.

## 2. Methodology

### 2.1. Plant collection and identification

Fresh plants of *Erigeron floribundus* (H.B. & K) or (syn": *Conyza sumatrensis* (Retz) E.K. Walker) (Asteraceae) were collected in Nsimeyong area of Yaounde, Cameroon in March 2002 and identified in the National Herbarium, Yaounde. The voucher number was HNC 201.

### 2.2. Preparation of extract

Eight hundred grams of oven-dried (40 °C) pulverized leaves of the plant were boiled in a litre of distilled water for 2 h and kept to cool for 24 h. The extract was later filtered, volume of extract measured and concentrated in a

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rotor evaporator and later in an oven (50 °C). The yield obtained was 18.0%.

### 2.3. Chemicals

Acetic acid (0.6%), carrageenan (Sigma, St. Louis, MO, USA), indomethacin (Indocid$^R$, Merck Sharp-Dolme Chibret, Fr.), aspirin (Laboratoires 3M, Fr.), morphine sulphate, naloxone (Narcan$^R$ Du Pont de Nemours SA, Fr.).

### 2.4. Animals

Female Wistar rats (180–200 g) and albino mice (18–25 g) were obtained from the Animal House of Faculty of Medicine and Biomedical Sciences of the University of Yaounde 1. Prior to and after treatment, the animals were fasted for 12 and 10 h, respectively. However, water was made available ad libitum.

### 2.5. Phytochemical analysis

#### 2.5.1. Preliminary chemical tests

Phytochemical properties of the extract were tested using the following chemicals and reagents (Trease and Evans, 1983). Alkaloids with Mayer and Dragendoff's reagents, saponins (frothing test), tannins ($FeCl_3$), glycosides (NaCl and Fehling's solution A and B), cardiac glycosides (Salkowski test), flavonoids (NaCl and HCl), anthraquinones (Borntrager's reaction) phenols ($FeCl_3$ and $K_3Fe(CN)_6$), and lipids (filter paper).

### 2.6. Pharmacological activities

#### 2.6.1. Nociceptive activity

*2.6.1.1. Acetic acid writhing reflex.* This was performed according to Gaertner et al. (1999). Mice (six per group) were injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg. The extract (50, 100, 200, 400 800 mg/kg, p.o.), morphine (5 mg/kg, s.c.), naloxone + extract (1 mg/kg, i.p. + 800 mg/kg, p.o.) and distilled water (p.o.) were administered 30 min prior to treatment with acetic acid. The writhings induced by the acid, consisting of abdominal constrictions and hind limbs stretchings, were counted for 30 min after a latency period of 5 min. The percentage analgesic activity was calculated as follows:

$$\text{Percentage analgesic activity} = \frac{N - N^{\mathrm{I}} \times 100}{N}$$

where $N$ is the average number of stretchings of control per group. $N^{\mathrm{I}}$ is the average number of stretchings of test per group.

*2.6.1.2. Formalin-induced pain.* The procedure described by Santos et al. (1994) was used but with slight modifications. Pain was induced by injecting 0.05 ml of 2.5% formalin (40% formaldehyde) in distilled water in the subplantar of the right hindpaw. Rats (six per group) were given extract (50, 100, 200, 400, 800 mg/kg, p.o.), aspirin (100 mg/kg), morphine (5 mg/kg, s.c.), and distilled water (p.o.) 30 min prior to injecting formalin. These rats were individually placed in a transparent Plexiglass cage (25 cm × 15 cm × 15 cm) observation chamber. The amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0 to 5 min (first phase) and 15–30 min (second phase) were counted after injection of formalin. These phases represented neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987).

*2.6.1.3. Hotplate.* Briefly the device consisted of a water bath in which was placed, a metallic cylinder (diameter 20 cm and 10 cm high). The temperature of the cylinder was set at 55 ± 0.5 °C (Lanhers et al., 1991). Each mouse (6 per group) acted as its own control. Prior to treatment, the reaction time of each mouse (licking of the forepaws or jumping response) was done at 0- and 10-min interval. The average of the two readings was obtained as the initial reaction time ($T_b$). The reaction time ($T_a$) following the administration of the extract (50, 100, 200, 400, 800 mg/kg, p.o.), aspirin (100 mg/kg), morphine (5 mg/kg, s.c.), naloxone + extract (1 mg/kg, i.p. + 800 mg/kg) and distilled water (p.o.), was measured at 0.5, 1, 2, 4, and 6 h after a latency period of 30 mins. The following calculation was:

$$\text{Percentage analgesic activity} = \frac{T_a - T_b}{T_b} \times 100\%$$

*2.6.1.4. Tail immersion.* Tail immersion was conducted as described by Aydin et al. (1999). Rats (six per group) were used. This involved immersing extreme 3 cm of the rat's tail in a water bath containing water at a temperature of 55 ± 0.5 °C. Within a few minutes, the rat reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0- and 10-min interval. The average of the two values was the initial reaction time ($T_b$). The test groups were given extract (50, 100, 200, 400, 800 mg/kg, p.o.), aspirin (100 mg/kg), morphine (5 mg/kg, s.c.), naloxone + extract (1 mg/kg, i.p. + 800 mg/kg) and distilled water (p.o.). The reaction time ($T_a$) for the test groups was taken at intervals 0.5, 1, 2, 4 and 6 h after a latency period of 30 min following the administration of the extract and drugs (Vogel and Vogel, 1997). The cut-off time, i.e. time of no response was put at 120 s. The reaction time was measured and calculated as in Section 2.6.1.3 and expressed as mean ± S.E.M.

#### 2.6.2. Antiinflammatory activity

*2.6.2.1. Carrageenan-induced oedema test.* Seven groups of six animals per group were used. The plant extract was administered orally at doses 50, 100, 200, 400, 800 mg/kg

as well as 10 mg/kg of indomethacin. Controls received distilled water. The administration of extract and drugs was 30 min prior to injection of 0.05 ml, 1% carrageenan (Lanhers et al., 1991) in the right hindpaw subplantar of each rat. The paw volume was measured plethysmometrically (model 7150, Ugo Basil, Italy). Prior to injection of carrageenan, the average volume ($V_0$) of the right hindpaw of each rat was calculated from 3 readings which did not deviate more than 4%. At 0.5, 1, 2, 4, 6 and 24 h after injection of the phlogistic agent, only one reading was obtained for each rat ($V_t$).

The percentage inhibition for each rat and each group was obtained as follows:

$$\text{Percentage inhibition} = (V_t - V_0)\text{control} - \frac{(V_t - V_0)\text{treated}}{(V_t - V_0)\text{control}} \times 100$$

*2.6.3. Statistical test*

Data were presented as mean ± S.E.M. Statistical differences between control and treated groups were tested by one way ANOVA followed by Student's $t$-test. The differences were considered significant at $P < 0.05$.

## 3. Results

### 3.1. Phytochemistry

Alkaloids, saponins, tannins, glycosides, phenols flavonoids and oils were identified whereas cardiac glycosides, and anthraquinones were absent.

### 3.2. Analgesic activities

*3.2.1. Acetic acid writhing reflex*

*Erigeron floribundus* significantly reduced writhings and stretchings induced by 0.6% acetic acid at a dose of 10 ml/kg (see Table 1). The significant protective effect was dose dependent with 15.31% ($P < 0.05$) reduction observed for 100 mg/kg and 63.56% ($P < 0.001$) seen for 800 mg/kg dose. The $ED_{50}$ of the extract was found to be 518.99 mg/kg with a confidence limit of 496.27–541.71 mg/kg. Aspirin (100 mg/kg) had only 45.50% ($P < 0.001$) inhibition and morphine (a centrally acting analgesic) had 58.75% ($P < 0.001$) inhibition. Pretreatment with naloxone blocked the protective effect of the extract.

Table 1
Effect of *Erigeron floribundus* leaves aqueous extract, aspirin and morphine on writhing induced by acetic acid

| Treatment | Dose (mg/kg) | N | Number of writhings within 30 min | Percentage inhibition |
|---|---|---|---|---|
| Control (acetic acid) | 0 | 6 | 74.20 ± 8.25 | 0 |
| Extract | 50 | 6 | 71.03 ± 6.86 | 4.27 |
| Extract | 100 | 6 | 62.84 ± 12.94 | 15.31* |
| Extract | 200 | 6 | 52.38 ± 4.65 | 29.41* |
| Extract | 400 | 6 | 41.02 ± 2.04 | 44.72§ |
| Extract | 800 | 6 | 27.04 ± 1.02 | 63.56§ |
| Aspirin | 100 | 6 | 40.44 ± 5.63 | 45.50§ |
| Morphine | 5 | 6 | 30.61 ± 0.53 | 58.75§ |
| Naloxone + extract | 1 + 800 | 6 | 55.66 ± 0.73 | 25.01** |

Values expressed as mean ± S.E.M.
* $P < 0.05$.
** $P < 0.01$.
§ $P < 0.001$.

Table 2
Effect of *Erigeron floribundus* leaves aqueous extract on formalin-induced pain

| Treatment | Dose (mg/kg) | Number of licks (s) | | | |
|---|---|---|---|---|---|
| | | 0–5 min | | 15–30 min | |
| | | Score of pain | Percentage inhibition | Score of pain | Percentage inhibition |
| Control | 0 | 64.67 ± 6.84 | 0 | 134.52 ± 6.04 | 0 |
| Extract | 50 | 61.49 ± 5.88 | 4.92 | 133.23 ± 5.15 | 0.94 |
| Extract | 100 | 61.80 ± 5.54 | 4.48 | 132.91 ± 6.22 | 1.20 |
| Extract | 200 | 58.06 ± 4.63 | 8.67 | 119.84 ± 5.56 | 10.91* |
| Extract | 400 | 61.71 ± 5.67 | 4.57 | 101.37 ± 4.66 | 24.64** |
| Extract | 800 | 38.70 ± 3.80 | 40.16§ | 95.36 ± 3.93 | 29.11** |
| Indomethacin | 10 | 57.44 ± 7.86 | 11.18 | 89.71 ± 4.21 | 33.31** |

Values expressed as mean ± S.E.M.
* $P < 0.05$.
** $P < 0.01$.
§ $P < 0.001$.

Table 3
Effect of *Erigeron floribundus* leaves aqueous extract, aspirin and morphine on pain induced by hotplate

| Treatment | Dose (mg/kg) | Latency period (h) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.5 | 1 | 2 | 3 | 4 | 6 |
| Control | 0 | 4.78 ± 0.29 | 4.38 ± 0.11 (−8.37) | 3.61 ± 0.12 (−24.48) | 3.88 ± 0.11 (−18.83) | 3.84 ± 0.14 (−19.67) | 3.92 ± 0.17 (−17.99) | 3.71 ± 0.13 (−22.38) |
| Extract | 50 | 4.57 ± 0.57 | 4.63 ± 0.43 (1.31) | 4.82 ± 0.52 (5.47) | 4.98 ± 0.61 (9.19) | 4.59 ± 0.27 (0.48) | 4.55 ± 0.74 (−0.44) | 4.52 ± 0.44 (−1.09) |
| Extract | 100 | 4.20 ± 0.43 | 4.70 ± 0.71 (11.90) | 4.44 ± 0.51 (5.71) | 5.56 ± 0.71 (32.38)** | 4.22 ± 0.46 (0.48) | 4.08 ± 0.39 (−26.67) | 3.64 ± 0.34 (−13.33) |
| Extract | 200 | 4.06 ± 0.40 | 5.13 ± 0.51 (26.35)* | 5.50 ± 0.42 (35.47)** | 7.07 ± 0.86 (74.14)§ | 5.38 ± 0.94 (32.5)** | 3.98 ± 0.25 (1.97) | 3.79 ± 0.62 (5.17) |
| Extract | 400 | 3.79 ± 0.20 | 5.20 ± 0.86 (37.20)** | 5.80 ± 0.68 (53.03)§ | 6.42 ± 0.58 (69.39)§ | 5.14 ± 0.24 (35.62)** | 3.74 ± 0.32 (1.32) | 3.59 ± 0.47 (5.28) |
| Extract | 800 | 3.70 ± 0.15 | 4.93 ± 0.48 (33.20)** | 6.77 ± 0.66 (82.97)§ | 8.47 ± 0.31 (128.92)§ | 5.87 ± 0.11 (36.97)** | 3.95 ± 0.41 (6.76) | 3.89 ± 0.39 (5.14) |
| Aspirin | 100 | 4.84 ± 0.05 | 4.92 ± 0.16 (1.79) | 4.81 ± 0.14 (−0.62) | 4.89 ± 0.11 (−0.61) | 4.83 ± 0.13 (−0.21) | 4.80 ± 0.44 (−0.83) | 4.08 ± 0.41 (−15.70) |
| Morphine | 5 | 4.36 ± 0.05 | 6.18 ± 0.67 (41.75)§ | 7.35 ± 0.69 (68.58)§ | 8.29 ± 0.66 (90.14)§ | 6.64 ± 0.90 (52.22)§ | 5.18 ± 0.36 (18.80)* | 4.10 ± 0.52 (0.06) |
| Naloxone + extract | 1 + 800 | 4.82 ± 0.21 | 4.92 ± 0.20 (2.90) | 4.41 ± 0.22 (−8.51) | 4.81 ± 0.23 (−0.21) | 5.05 ± 0.21 (4.77) | 4.91 ± 0.35 (1.87) | 4.43 ± 0.62 (8.09) |

Values expressed as mean ± S.E.M. and units are in seconds. Percentages of protection against thermally induced pain by hotplate are in parentheses.

* $P < 0.05$.

** $P < 0.01$.

§ $P < 0.001$.

Table 4
Effect of *Erigeron floribundus* leaves aqueous extract, aspirin and morphine on pain using tail immersion test

| Treatment | Dose (mg/kg) | Latency period (h) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.5 | 1 | 2 | 3 | 4 | 6 |
| Control | 0 | 2.49 ± 0.12 | 2.56 ± 0.09 (2.81) | 2.67 ± 0.20 (7.23) | 2.41 ± 0.08 (−3.21) | 2.52 ± 0.08 (1.20) | 2.63 ± 0.11 (6.02) | 2.39 ± 0.11 (−4.02) |
| Extract | 50 | 3.11 ± 0.31 | 3.01 ± 0.21 (−3.22) | 2.95 ± 0.20 (−5.14) | 3.10 ± 0.16 (−0.32) | 2.98 ± 0.22 (−4.18) | 2.99 ± 0.19 (−3.86) | 3.13 ± 0.15 (0.64) |
| Extract | 100 | 3.00 ± 0.20 | 2.72 ± 0.30 (−9.33) | 2.97 ± 0.05 (1.00) | 2.31 ± 0.25 (−23.00) | 2.02 ± 0.02 (−32.67) | 2.02 ± 0.02 (−32.67) | 2.21 ± 0.02 (−26.33) |
| Extract | 200 | 2.31 ± 0.08 | 2.70 ± 0.07 (16.69)* | 2.68 ± 0.04 (16.45)* | 2.94 ± 0.12 (27.27)** | 2.34 ± 0.13 (1.29) | 2.31 ± 0.06 (0.0) | 2.33 ± 0.04 (0.87) |
| Extract | 400 | 2.26 ± 0.04 | 2.42 ± 0.04 (15.93)* | 2.89 ± 0.18 (27.88)** | 3.19 ± 0.04 (41.15)§ | 2.27 ± 0.10 (0.44) | 2.14 ± 0.02 (−5.31) | 2.21 ± 0.02 (−2.20) |
| Extract | 800 | 2.20 ± 0.00 | 2.59 ± 0.06 (17.73)* | 3.00 ± 0.13 (40.00)§ | 3.38 ± 0.11 (58.18)§ | 3.04 ± 0.11 (38.18)** | 2.32 ± 0.14 (5.45) | 2.19 ± 0.03 (−0.45) |
| Aspirin | 100 | 3.13 ± 0.23 | 3.04 ± 0.09 (−2.88) | 2.91 ± 0.33 (−7.03) | 3.03 ± 0.10 (−3.19) | 3.33 ± 0.24 (6.39) | 3.25 ± 0.76 (3.83) | 3.17 ± 0.28 (1.60) |
| Morphine | 5 | 4.10 ± 0.54 | 9.45 ± 1.71 (130.49)§ | 9.74 ± 0.81 (141.00)§ | 8.21 ± 1.05 (100.24)§ | 6.83 ± 0.71 (66.59)§ | 5.92 ± 1.34 (44.39)§ | 4.62 ± 0.15 (12.90)* |
| Naloxone + extract | 1 + 800 | 2.48 ± 0.32 | 2.41 ± 0.14 (−2.82) | 2.47 ± 0.13 (−0.35) | 2.51 ± 0.14 (1.21) | 2.54 ± 0.56 (2.42) | 2.51 ± 0.37 (1.21) | 2.53 ± 0.15 (2.02) |

Values expressed as mean ± S.E.M. and are in seconds. Percentages of protection against thermally induced pain by warm water are in parentheses. Morphine showed very significant analgesic effects beginning from 0.5 to 4 h post-treatment. The extract showed its maximal analgesic effects 2 h post-treatment with percentage inhibition of 58.18% (800 mg/kg). Negative values indicate that the tails were withdrawn faster than the controls.

* $P < 0.05$.

** $P < 0.01$.

§ $P < 0.001$.

Table 5
Effect of *Erigeron floribundus* leaves aqueous extract and indomethacin on carrageenan-induced oedema

| Treatment | Dose (mg/kg) | Right hindpaw volume (mean ± S.D.) (ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 h | 0.5 h) | 1 h | 2 h | 4 h | 6 h | 24 h |
| Control | 0 | 0.74 ± 0.1 | 0.88 ± 0.01 (18.92) | 0.90 ± 0.02 (21.62) | 0.98 ± 0.02 (32.43) | 1.07 ± 0.03 (44.59) | 0.96 ± 0.01 (29.73) | 0.91 ± 0.02 (22.97) |
| Extract | 50 | 1.08 ± 0.02 | 1.24 ± 0.02 (14.81) | 1.26 ± 0.01 (16.67) | 1.29 ± 0.01 (19.44)* | 1.35 ± 0.02 (25.00)* | 1.26 ± 0.01 (16.67)* | 1.19 ± 0.03 (10.91)* |
| Extract | 100 | 1.09 ± 0.01 | 1.19 ± 0.01 (9.17)** | 1.22 ± 0.01 (11.9)** | 1.26 ± 0.02 (15.60)** | 1.30 ± 0.02 (19.27)** | 1.22 ± 0.01 (11.90)§ | 1.18 ± 0.04 (8.26)§ |
| Extract | 200 | 0.89 ± 0.01 | 0.95 ± 0.03 (6.71)§ | 0.97 ± 0.01 (9.00)§ | 0.99 ± 0.01 (11.24)§ | 1.04 ± 0.00 (16.85)§ | 0.98 ± 0.01 (10.11)§ | 0.96 ± 0.02 (7.87)§ |
| Extract | 400 | 0.95 ± 0.01 | 0.99 ± 0.02 (4.21)§ | 1.03 ± 0.02 (8.42)§ | 1.06 ± 0.00 (11.58)§ | 1.07 ± 0.00 (12.63)§ | 1.04 ± 0.01 (9.47)§ | 0.98 ± 0.03 (3.16)§ |
| Extract | 800 | 0.72 ± 0.01 | 0.74 ± 0.03 (2.78)§ | 0.76 ± 0.01 (5.56)§ | 0.78 ± 0.01 (8.33)§ | 0.80 ± 0.01 (11.11)§ | 0.79 ± 0.01 (9.72)§ | 0.75 ± 0.04 (4.17)§ |
| Indomethacin | 10 | 0.84 ± 0.02 | 0.85 ± 0.04 (1.19)§ | 0.87 ± 0.03 (3.57)§ | 0.89 ± 0.02 (5.95)§ | 0.89 ± 0.02 (5.95)§ | 0.90 ± 0.01 (7.14)§ | 0.86 ± 0.03 (2.38)§ |

Control values at time zero was an average of three values. Percentages of oedema are in parentheses.
* $P < 0.05$.
** $P < 0.01$.
§ $P < 0.001$.

*3.2.2. Formalin-induced pain*

The extract had analgesic effects on both first (0–5 min) and second phases (15–30 min) of formalin test as shown on Table 2. These phases corresponded to neurogenic and inflammatory pains, respectively. Its neurogenic-induced pain blockade, occurred only at 800 mg/kg (40.16%, $P < 0.001$) whereas beginning from 200 mg/kg, the extract significantly blocked pain emanating from inflammation. The extract was found to inhibit the neurogenic-induced pain (40.16%, $P < 0.001$) better than the pain resulting from inflammation (29.11%, $P < 0.01$). Indomethacine was significantly active (33.33%, $P < 0.01$) only on the second phase.

*3.2.3. Hotplate*

The results of the hotplate test (Table 3) revealed that the reaction time for the mice was significantly increased from the dose of 100 mg/kg (32.38%, $P < 0.01$) and above. This was somewhat dose dependent. This augmentation in reaction time reached 128.92% ($P < 0.001$) with 800 mg/kg. Pretreatment with naloxone (1 mg/kg) drastically reduced the analgesic potentials of the extract (−8.51 to −8.09%). The $ED_{50}$ of the extract after 2 h was estimated to be 223.87 mg/kg with confidence limits of 189.68–258.06. In the presence of naloxone, the effect of the extract was inhibited. Acetylsalicylic acid or aspirin at 100 mg/kg (−15.70 to 1.79%), did not offer any protection against the heat-induced pain. Morphine sulphate at 5 mg/kg significantly showed its maximum protective effect of 90.14% ($P < 0.001$) after 2 h compared to 128.92% ($P < 0.001$) for 800 mg/kg of the extract.

*3.2.4. Tail immersion*

After a latency period of 0.5 h following oral administration of the extract at a dose of 200 mg/kg (16.69%, $P < 0.05$), there was a significant reduction of painful sensation due to tail immersion in warm water and it was dose dependent, see Table 4. The inhibitory effects of the extract became pronounced between 1 and 3 h post-dosing and reached a maximum of 58.18% ($P < 0.001$) with the dose of 800 mg/kg. The antinociceptive properties of the extract at 800 mg/kg (38.18–58.18%) was not as effective as that of morphine (44.39–141.00%). Aspirin had no effect on this test. The analgesic activity of the extract was blocked by naloxone.

*3.3. Antiinflammatory activities*

*3.3.1. Carrageenan-induced oedema*

The average right backpaws volumes and percentages of oedema are presented in Table 5. The percentages of inhibition are reported in Table 6. For the control group, the injection of the phlogistic agent caused localised oedema, 30 min after. The swelling increased progressively after 4 h to a maximum volume of 1.07 ± 0.03 ml (44.59%) and remained obvious 24 h after injection. Rats pretreated with *Erigeron floribundus* significantly decreased the carrageenan-induced oedema 30 min post-dosing beginning with 100 mg/kg and

Table 6
Percentage of inhibition of *Erigeron floribundus* leaves aqueous extract and indomethacin on carrageenan-induced oedema

| Treatment | Dose (mg/kg) | Interval (h) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 2 | 4 | 6 | 24 |
| Extract | 50 | −14.29 | −12.50 | 12.50** | 18.18* | 18.18* | 35.29** |
| Extract | 100 | 28.57** | 28.75** | 29.17** | 36.37** | 40.91§ | 47.06§ |
| Extract | 200 | 57.14§ | 50.00§ | 58.33§ | 54.54§ | 59.09§ | 58.82§ |
| Extract | 400 | 71.43§ | 50.00§ | 54.17§ | 63.63§ | 59.09§ | 82.35§ |
| Extract | 800 | 85.71§ | 75.00§ | 75.00§ | 75.76§ | 68.18§ | 82.35§ |
| Indomethacin | 10 | 92.86§ | 81.25§ | 79.17§ | 84.85§ | 72.73§ | 88.24§ |

Values expressed as mean ± S.E.M. The extract at doses greater than 50 mg/kg, showed significant inhibition of oedema due to carrageenan. The negative values indicate that the absolute oedema volume change for the control was less than that of the treated group.
* $P < 0.05$.
** $P < 0.01$.
§ $P < 0.001$.

in a dose related manner. At 50 mg/kg, the extract showed significant inhibition of oedema formation after 2 h (12.50%, $P < 0.05$) and continued for 24 h (35.29%, $P < 0.01$). At 200 and 400 mg/kg, the extract achieved its maximal inhibitory effects of 59.06% ($P < 0.001$) and 82.35% ($P < 0.001$), respectively, after 6 and 24 h. At 800 mg/kg, the extract (85.71%, $P < 0.001$) and indomethacine (92.86%, $P < 0.001$) attained their maximal effects 30 min post-dosing. Although both the extract and indomethacine reduced the swellings, they still remain significantly visible 24 h after.

## 4. Discussion

The study indicated that *Erigeron floribundus* aqueous extract has both peripheral and central analgesic properties. Its peripheral analgesic activity was deduced from its inhibitory effects on chemical (acetic acid and formalin, inflammatory phase) induced nociceptive stimuli. At 400 mg/kg (44.72%), the peripheral analgesic action of the extract on acetic acid induced pain was found to be comparable to 100 mg/kg (45.50%) of aspirin but less so with inflammatory phase of formalin test (29.11%). The centrally acting protective effects of the extract were corroborated by the first phase of formalin-induced pain, hotplate and immersion tests results. In these experiments, aspirin or indomethacin were inactive. The tail immersion test indicated that the pharmacological actions were mediated by mu (μ) opioid receptors rather than kappa (κ) and delta (δ) receptors (Schmauss and Yaksh, 1984; Aydin et al., 1999).

A comparison done on acetic acid-induced pain showed that morphine at 5 mg/kg had 58.75% ($P < 0.001$) protection similar to 800 mg/kg of the extract (63.56%, $P < 0.001$). Similar comparison between effects of morphine sulphate and *Erigeron floribundus* on tail immersion test showed that at 5 mg/kg, morphine sulphate had very potent analgesic effect with significant percentages of inhibition ranging from 44.39 to 141.00% compared to the extract (38.18–58.18%). This showed that the extract was a weaker opioid receptor agonist. The fact that the neurogenic (0–5 min) algesia was significantly blocked by the extract meant that it also acted through opioid receptors which were more centrally located than peripheral. Due to their central location, a higher therapeutic concentration (800 mg/kg) of the extract was therefore required for the analgesia as revealed by the first phase of formalin-induced pain test.

The antiinflammatory effects of the extract on acute inflammatory process such as carrageenan-induced oedema in rat paw was dose dependent. At 200 mg/kg, the extract showed at least 50% inhibitory activity throughout the measurement intervals and the efficacy of indomethacin (10 mg/kg) was comparable to 800 mg/kg of the extract. De Las Heras et al. (1998) had also demonstrated the antiinflammatory activity of this plant in mice. Similar results of dual analgesic actions have been reported for *Vitex negundo* (Telang et al., 1999). At present, no literature has been found describing the side effects such as gastric ulcer, of this plant.

These data validated the traditional uses of this plant to assuage pain resulting from headache, dysmenorrhoea, and toothache as well as inflammatory diseases like gout, rheumatism, cystitis and nephritis. Although these inflammatory diseases are chronic in nature, this study has focused mainly on the acute inflammatory properties of the plant.

Based on 800 mg/kg dose used in this experiment, calculations showed that a mouse (25 g) received a total of about 20 mg of extract a day which corresponded to 0.11 g of dried leaves. A 200 g rat received about 160 mg per day corresponding to 0.88 g of dried leaves. Now following the tradi-practitioner's posology for treating dysmenorrhoea in a 70 kg woman, 100 ml of decoction contained about 2.05 ± 0.45 g of extract. Thus, for the thrice daily doses, a total of 6.15 ± 1.28 g extract was needed to be taken per day, corresponding to 37.42 ± 4.13 g of dried leaves.

## 5. Conclusion

It can be concluded that *Erigeron floribundus* is endowed with peripheral and centrally acting analgesic properties as

well as antiinflammatory activity on acute inflammatory processes.

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# Study of the hypoglycaemic activity of *Fraxinus excelsior* and *Silybum marianum* in an animal model of type 1 diabetes mellitus

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**Abstract**

The hypoglycaemic effect of the aqueous extracts of *Fraxinus excelsior* (FE) seed and *Silybum marianum* (SM) aerial part was investigated in normal and streptozotocin (STZ) diabetic rats. After a single dose or 15 daily doses, oral administration of the aqueous extracts (20 mg/kg) produced a significant decrease of blood glucose levels in both normal and STZ diabetic rats ($P < 0.001$). From the first week, the body weight was increased in normal rats ($P < 0.05$) and decreased in STZ rats ($P < 0.01$) after FE administration. In addition, no changes were observed in basal plasma insulin concentrations after both FE and SM treatments in either normal and STZ diabetic rats indicating that these plants exert their pharmacological activity without affecting insulin secretion.

We conclude that the aqueous extracts of FE and SM exhibit potent hypoglycaemic and anti-hyperglycaemic activities in normal and STZ rats, respectively, without affecting basal plasma insulin concentrations.

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*Keywords: Fraxinus excelsior*; *Silybum marianum*; Diabetes mellitus; Streptozotocin; Aqueous extract; Oral administration; Blood glucose

## 1. Introduction

From a few years, we have undertaken a study focused on the repertory of the main medicinal plants used in the treatment of diabetes mellitus around Morocco (Jouad et al., 2001a; Eddouks et al., 2002, 2004). More than one hundred plants were described as anti-diabetic remedies (Eddouks et al., 2002; Jouad et al., 2001a). Until now, the pharmacological activities of some of them have been demonstrated (Jouad et al., 2000, 2002a,b, 2003b) in streptozotocin-induced diabetic rat which is considered as a precious tool for the study of both pathophysiology and pharmacology of type 1 diabetes mellitus (Burcelin et al., 1992, 1995). Such studies were and remain particularly recommended by the World Health Organization (WHO, 1978; Farnsworth, 1980) in order to enrich the therapeutic arsenal of diabetes mellitus by new potent natural hypoglycaemic agents.

*Fraxinus excelsior* (FE), locally known as “l’ssane l’ousfour”, is a native shrub widely distributed throughout the south-eastern region of Morocco (Tafilalet). This region has been shown to be an important reserve of bioactive medicinal plants in which phytotherapy knowledge has been and remains to be very developed (Eddouks et al., 2002). This plant is known in phytomedicine around the world as anti-oxidative (Schempp et al., 2000; Meyer et al., 1995), anti-inflammatory (Von Kruedener et al., 1996; El-Ghazaly et al., 1992), anti-rheumatic (Von Kruedener et al., 1995), analgesic and antipyretic (Okpanyi et al., 1989). FE has never been described or tested in the treatment of diabetes mellitus. In the south-eastern region of Morocco (Tafilalet), FE seeds are recognised as potent hypoglycaemic agents by several traditional healers (Eddouks et al., 2004). *Silybum marianum* (SM), locally known as “Chouk J’mal” or “Guandoule”, is a native shrub (less than one meter high) widely distributed throughout the south-eastern region of Morocco (Tafilalet). This plant is traditionally used in phytomedicine as anti-oxidative (Von Schonfeld et al., 1997), antihepatitis (Giese, 2001) antiulcerogenic (Khayyal et al., 2001), antifibrotic, hepatoprotective (Thyagarajan et al., 2002; Luper, 1998), anti-atherosclerosis (Bialecka, 1998; Rui, 1991), anti-inflammatory, immunomodulating

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and liver regenerative (Thyagarajan et al., 2002), hypocholesterolemic (Skottova et al., 1998) and a potent inhibitor of cyclic AMP phosphodiesterase (Koch et al., 1985). In diabetes phytotherapy, flavonoids isolated from SM have been demonstrated as preventive agents of cyclosporine A-induced toxicity in exocrine pancreas (Von Schonfeld et al., 1997), protective compounds on pancreatic damage in alloxan-induced diabetes mellitus (Soto et al., 1998) and exhibited a lowering blood glucose levels activity in type 2 diabetic patients after a long term treatment (Lirussi et al., 2002; Velussi et al., 1997). However, until now, the hypoglycaemic activity of silymarin and silibinin extracted from SM has been studied only in type 2 diabetes mellitus. The results indicated a slight hypoglycaemic effect if we take on consideration the doses used and duration of treatment in type 2 diabetic patients (Lirussi et al., 2002; Velussi et al., 1997).

The objective of this study was to evaluate the pharmacological effects of FE and SM on blood glucose levels in both normal and STZ diabetic rats. The effects of FE and SM are compared with sodium-vanadate as a reference hypoglycaemic drug. Since there is no standard therapeutic dose of any hypoglycemic drug in the streptozotocin rat model used for analysis of hypoglycaemic effect of medicinal plants, the dose of sodium-vanadate used in our study was selected to be between low doses without significant hypoglycaemic effects and very high doses which caused severe hypoglycemia in diabetic rats (Eddouks et al., 2003). The effect of FE and SM on basal plasma insulin concentrations was also analysed in order to determine a probable mechanism of action of these plants. Finally, the eventual changes of rat body weight were followed after long term FE or SM administration.

## 2. Material and methods

### 2.1. Plant material

Specimens of FE (Oleaceae) and SM (Asteraceae) were collected from the Tafilalet region (semi-arid area) of Morocco in May–June 2001, and air-dried at 40 °C. The plants were previously identified and authenticated by Pr. M. Rejdali (Agronomy and Veterinary Institute, Rabat) and a voucher specimen (ME3, MAGH3 and ME, MAGH2, respectively) were deposited at the herbarium of the Faculty of Sciences and Techniques Errachidia.

### 2.2. Preparation of the aqueous extracts

Plant material was prepared according to the traditional method used in Morocco (Eddouks et al., 2004) (decoction): 1 g of powdered FE seed and SM aerial part were mixed with 100 ml distilled water was boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extracts were filtered using a Millipore filter (Millipore, 0.2 mm, St. Quentin en Yvelines, France) to remove particulate matter. The filtrate was then freeze-dried and the desired dose (milligram of lyophilized aqueous extract of FE seed or SM aerial part per kilogram body weight) was then prepared and reconstituted in 1.5 ml of distilled water. The aqueous extracts were prepared daily, just before administration. The extracts obtained were then given orally to different groups of rats at a dose of 20 mg/kg body weight. This dose was used according to the Moroccan traditional phytotherapy. The FE extract was yellow coloured with low viscosity, the percent yield was 12%, pH 6.05 and the osmolarity was 14 mOsm/kg$H_2O$. The SM extract was green coloured with a percent yield of 16%, its average osmolarity was 50 mOsm, pH 6.4, and with a very low viscosity.

### 2.3. Experimental design

Experiments were performed in adult male Wistar rats weighing from 200 to 250 g. Animals were housed under standard environmental conditions ($23 \pm 1$ °C, $55 \pm 5$% humidity and a 12 h/12 h light/dark cycle) and maintained with free access to water and a standard laboratory diet (carbohydrates 30%; proteins 22%; lipids 12%; vitamins 3%) ad libitum.

Diabetes was induced by intravenous injection of streptozotocin (Sigma, St. Louis, MO, USA) into the tail vein at a dose of 65 mg/kg body weight (Burcelin et al., 1995). STZ was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. After 18 h, animals with fasting blood glucose levels greater than 16.5 mmol/l were considered diabetic and then included in this study.

Normal and diabetic rats were randomly assigned to four different groups ($n = 6$ in each group). The control group received distilled water; treated groups received aqueous extracts of FE or SM at a dose of 20 mg/kg or the reference drug; sodium-vanadate (Fluka, Chemica, Switzerland) at a dose of 0.8 mg/kg (Levy and Bendayan, 1991; Eddouks et al., 2003). All experiments were realized in overnight fasted rats.

The drug solutions or vehicle were administered orally by gastric intubation using a syringe once daily at 08:00 a.m. The effect of the vehicle, FE aqueous extract, SM aqueous extract or vanadate on blood glucose were determined in fasted rats, 1, 2, 4 and 6 h after a single oral administration and after 2, 4 days, 1 and 2 weeks of once daily repeated oral administration (20 mg/kg).

### 2.4. Determination of parameters

Blood samples from rats were collected from the tail vein in anaesthetised rats using ether. Blood glucose levels were determined by the glucose oxidase method using a reflective glucometer (Model GX, Ames Miles, Bayer Diagnostics, Genome Biotechnologies, Casablanca, Morocco). Basal plasma insulin concentrations were determined by radioim-

munoassay kit (Pharmacia, Uppsala, Sweden) with a Beta matic counter (Cronex, Dupont, France). The kit included human insulin as standard and $^{125}$I-labelled human insulin antibody, which cross reacts with rat insulin.

### 2.5. Statistical analysis

All the data reported are expressed as mean ± S.E.M. Statistical analysis was performed using the Student's *t*-test. The values were considered to be significantly different when the $P$ value was less than 0.05 compared to baseline values.

## 3. Results

### 3.1. Single oral administration

The effects of single oral administration of FE and SM at a dose of 20 mg/kg in normal and STZ diabetic rats are shown in Fig. 1. In normal rats, the blood glucose levels dropped significantly from the first ($P < 0.01$) to the sixth hour ($P < 0.001$) after a single oral administration of aqueous FE extract (20 mg/kg) (Fig. 1a). In addition, the blood glucose levels dropped significantly with 13.6, 21 and 25%, 2, 4 and 6 h after a single oral administration of aqueous SM

Fig. 1. Plasma glucose levels over 6 h after single oral administration of aqueous FE and SM extracts (20 mg/kg) in normal (a) and diabetic rats (b). Data are expressed as means ± S.E.M., $n = 6$ rats per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, when compared to baseline values. (□) Control; ( ) FE; ( ) SM; (■) vanadate.

extract (20 mg/kg), respectively (Fig. 1a). Frissons were noticed in some rats indicating a severe hypoglycaemic effect of these plants in normal rats. Vanadate treatment at a dose of 0.8 mg/kg did not affect blood glucose levels in normal rats (Fig. 1a).

In diabetic rats, a significant progressive decrease of blood glucose levels was noted in FE, SM, and vanadate treated groups when compared to baseline values (Fig. 1b). Pre-treatment blood glucose levels in diabetic rats dropped from the first ($P < 0.01$) to the sixth hour ($P < 0.001$) after a single oral administration of aqueous both FE and SM extracts at a dose of 20 mg/kg (Fig. 1b). Vanadate at a dose of 0.8 mg/kg caused a significant decrease in blood glucose levels after 1 h ($P < 0.05$), 2 h ($P < 0.01$), 4 h ($P < 0.001$) and 6 h ($P < 0.001$) of a single oral administration in STZ rats (Fig. 1b).

### 3.2. Repeated oral administration

The effects of once daily repeated oral administration of FE, SM (20 mg/kg) and vanadate (0.8 mg/kg) in normal and STZ diabetic rats are shown in Fig. 2. In normal rats with once daily FE oral administration repeated, the blood glucose levels showed a strong and progressive decrease from the second ($P < 0.001$) and fourth day ($P < 0.001$) to the second week ($P < 0.001$) (Fig. 2a). In addition, the blood

Fig. 2. Plasma glucose levels after once daily repeated oral administration of aqueous FE and SM extracts (20 mg/kg) for 15 days in normal (a) and diabetic rats (b). Data are expressed as means ± S.E.M., $n = 6$ rats per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, when compared to baseline values. (□) Control; ( ) FE; ( ) SM; (■) vanadate.

glucose levels showed a strong and progressive decrease from the fourth ($P < 0.001$) and seventh day ($P < 0.001$) to second week ($P < 0.001$) of once daily repeated SM oral administration (Fig. 2a). The blood glucose levels were decreased by 29, 35 and 50% at the fourth, seventh and second week of repeated oral administration, respectively (Fig. 2a). After 2 weeks of repeated oral administration of the aqueous FE and SM extracts, severe hypoglycaemic frissons were observed in normal rats. Repeated vanadate administration did not cause any change of blood glucose levels (Fig. 2a).

In STZ diabetic rats, once daily repeated oral administration of the aqueous FE extract (20 mg/kg) produced a strong reduction in blood glucose levels from the second ($P <$ 0.05), fourth day ($P < 0.01$) to the first ($P < 0.001$) and second week ($P < 0.01$). In addition, SM extract produced a strong reduction in blood glucose levels from second day to second week after repeated oral administration ($P < 0.001$). The blood glucose levels were also significantly decreased at second ($P < 0.001$), seventh ($P < 0.001$) day and second week ($P < 0.001$) after once daily oral administration of SM extract (20 mg/kg), respectively (Fig. 2b). Two weeks after repeated oral administration, FE and SM extracts (20 mg/kg) were statistically more effective than vanadate at the doses used (Fig. 2b). Moreover, some STZ rats have shown hypoglycaemic symptoms at the second week of both plants treatments. Finally, vanadate treatment caused a significant

Fig. 3. Effect of FE and SM aqueous extracts treatment (20 mg/kg) on body weight (g) in normal and diabetic rats over 2 weeks. Data are expressed as means ± S.E.M., $n = 6$ rats in each group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, when compared to baseline values. (□) Control; ( ) FE; ( ) SM; (■) vanadate.

Table 1
Basal plasma insulin concentrations (μU/ml) after repeated oral administration of aqueous FE and SM extracts at a dose of 20 mg/kg in normal and diabetic rats

| Experimental groups | Plasma insulin concentrations (μU/ml) | |
|---|---|---|
| | day 0 | day 15 |
| Normal rats | | |
| Control | 33.4 ± 3.17 | 34.45 ± 4.1 NS |
| FE | 33.16 ± 0.53 | 32.80 ± 1.20 NS |
| SM | 32.15 ± 0.43 | 32.6 ± 1.14 NS |
| Vanadate | 31.2 ± 3.1 | 31.7 ± 2.9 NS |
| Diabetic rats | | |
| Control | 6.98 ± 0.39 | 6.75 ± 0.15 NS |
| FE | 6.36 ± 0.42 | 6.55 ± 0.19 NS |
| SM | 6.22 ± 0.32 | 6.4 ± 0.13 NS |
| Vanadate | 5.5 ± 0.5 | 5.8 ± 0.52 NS |

Data are expressed as means ± S.E.M., $n = 6$ rats in each group. NS: not significant compared to baseline values (day 0 of repeated oral administration).

decrease of blood glucose levels from the second day ($P < 0.01$) to the second week ($P < 0.001$) of repeated oral administration (0.8 mg/kg) (Fig. 2b).

### 3.3. Body weight changes

The body weight changes during repeated oral administration of test samples are given in Fig. 3. In normal rats, aqueous FE and SM extracts (20 mg/kg) induced a slight increase of body weight 1 ($P < 0.05$) and 2 weeks ($P < 0.05$) after repeated oral administration (Fig. 3a). This effect was disappeared four and seven days of repeated SM administration (Fig. 3a). However, the body weight values were slightly increased after 2 weeks of repeated oral administration of aqueous SM extract ($P < 0.05$) (Fig. 3a). Vanadate treatment caused a significant decrease of body weight from the second day ($P < 0.05$) to the second week ($P < 0.001$) (Fig. 3a).

In STZ rats, aqueous FE extract treatment for 2 weeks provoked a significant decrease of the body weight values from the second ($P < 0.05$) and fourth day ($P < 0.05$) to the first ($P < 0.01$) and second week ($P < 0.01$) (Fig. 3b), while aqueous SM extract treatment for 2 weeks did not affect the body weight values. Vanadate administration caused a significant decrease of body weight values from the fourth day ($P < 0.05$) to the second week ($P < 0.001$) (Fig. 3b).

### 3.4. Basal plasma insulin concentrations

The basal plasma insulin concentrations did not differ significantly neither in FE nor SM-treated groups (20 mg/kg/d) when compared to untreated group in both normal and diabetic rats (Table 1). In addition, vanadate treatment did not affect insulin secretion in both normal and STZ rats (Table 1).

## 4. Discussion

The main pharmacological activities of FE have been shown to be anti-oxidative (Schempp et al., 2000; Meyer et al., 1995), anti-inflammatory (Von Kruedener et al., 1996; El-Ghazaly et al., 1992), anti-rheumatic (Von Kruedener et al., 1995), analgesic and antipyretic agent (Okpanyi et al., 1989). On other hand, SM has been shown to be anti-oxidative, antilipidperoxidative, antifibrotic, anti-inflammatory, immunomodulating and liver regenerative (Thyagarajan et al., 2002). The benefit role of SM in the treatment of atherosclerosis has been demonstrated in an experimental animal model (Bialecka, 1998). According to this study, bioflavonoids extracted from SM exhibited evident normalization of lipid metabolism, as estimated by total serum cholesterol, LDL-cholesterol fraction, phospholipids and triglycerides levels and inhibition of atherosclerotic changes in experimental atherosclerosis development in rabbits (Bialecka, 1998). Silymarin has been demonstrated to possess a slight hypoglycaemic effect after 12 months of treatment in type 2 diabetic patients concomitantly with a significant decrease of insulin secretion (Velussi et al., 1997) and to blunt the sustained increment in plasma glucose induced by alloxan in rat (Soto et al., 1998). Moreover, silibinin, another flavonoid extracted from SM, protected the exocrine pancreas from cyclosporine A toxicity and inhibited glucose-stimulated insulin release in vitro (Von Schonfeld et al., 1997). In addition, silybin-beta-cyclodextrin caused a minor decrease with a percentage of 14.7% on blood glucose levels in type 2 diabetic patients (Lirussi et al., 2002). However, a real anti-hyperglycaemic effect of FE and SM in either clinical or experimental type 1 diabetes mellitus has not been demonstrated previously.

Based on our previous ethnopharmacological survey in south-eastern region of Morocco (Tafilalet), FE seed and SM aerial part were currently used for the treatment of both type 1 and type 2 diabetes mellitus (Eddouks et al., 2004). Because this ethnobotanical practice has not been tested previously, the aim of the present study was to test the hypoglycaemic effect of FE and SM in normal and streptozotocin-induced diabetic rats. Vanadate was used as a reference drug because it has been reported to be a potent insulin mimetic agent (DeFronzo, 1988; Reaven, 1988). Administration of this compound to diabetic animals is known to normalize blood glucose concentration in vivo (Kashiwagi et al., 1983).

The results demonstrated that the aqueous extracts of either FE or SM exerted a significant hypoglycaemic effect in normal rats and anti-hyperglycaemic activity in STZ-diabetic rats. This strong effect is first demonstrated in this study realized in STZ rat, an experimental model of type 1 diabetes mellitus (Burcelin et al., 1995). The low dose of these extracts used (20 mg/kg) and the duration of treatment (2 weeks) were sufficient to reduce strongly the blood glucose levels in severely diabetic rats with fasting glycaemia greater than 20 mM. The glucose lowering activity of aqueous FE and SM extracts (20 mg/kg) was more

pronounced than vanadate treatment in STZ rats at the dose used 2 weeks after once daily oral treatment. The observed frissons in normal FE -treated rats indicated that the hypoglycaemic effect of both FE and SM is very potent and cumulative because, despite the counter regulatory factors physiologically involved in response to hypoglycaemia, such as glucagon, cortisol, adrenaline, the blood glucose levels have been still to be very low after both single and repeated oral administration of these plants (Gerich, 1988). In addition, the dose of extracts used in this study was very low compared to previous studies (Jouad et al., 2003).

The extract of both FE and SM had no effect on basal plasma insulin concentrations in both normal and diabetic rats. It appears that these plants exert a hypoglycaemic effect independently of insulin secretion by pancreatic β cells of Langerhans. This finding is in accordance with a previous study indicating that insulin secretion was unaffected in type 2 diabetic patients treated with silybin-beta-cyclodextrin (Lirussi et al., 2002). In the contrary, the insulin secretion has been demonstrated to be decreased after treatment with silibinin in vitro (Von Schonfeld et al., 1997). These results let suppose the intervention of other phytochemical constituents of SM in the release of insulin and/or implication of paracrine factors in insulin secretion in vivo. Recent-onset insulinopenia in STZ-diabetic rats was associated with glucose overproduction in the basal (hyperglycaemic) state (Burcelin et al., 1995). The hypoglycaemic activity of these plants may therefore be due to inhibition of hepatic glucose production (Eddouks et al., 2003) and/or stimulation of glucose utilization by peripheral tissues, especially muscle and adipose tissue. No evidence of increased insulin sensitivity was seen in the normal rats. In addition, because a state of insulin resistance was demonstrated in recent onset diabetes in STZ rats (Burcelin et al., 1995), these plants may be normalizing blood glucose levels in diabetic rats by the restoration of normal insulin sensitivity. The plants extracts could also act as inhibitors of tubular renal glucose reabsorption (Maghrani et al., 2003).

No very important effect of aqueous FE and SM extracts on body weight has been observed in this study except a slight increase after 2 weeks of once daily repeated oral administration in normal rats perhaps, due to over utilization of glucose by peripheral tissues and/or increase of food intake in response to hypoglycaemia in addition of a slight decrease after 2 weeks in STZ rats after FE treatment.

The known main isolated constituents of FE have been demonstrated to be phenols (Mammela, 2001) and fraxin (Poukens-Renwart et al., 1992). In addition phytochemical investigations of SM have demonstrated that flavonolignan represent the main compounds (Bialecka, 1998). Among these constituents, silymarin (Velussi et al., 1997), silibin (Von Schonfeld et al., 1997) and silybin-beta-cyclodextrin (Lirussi et al., 2002) have been tested in type 2 diabetic state. Their eventual hypoglycaemic effect in type 1 diabetes mellitus has not been demonstrated. However their potential effect on anti-atherosclerotic activity has been proved (Bialecka, 1998). Flavonoids are considered as active principles in many medicinal plants (Wollenweber, 1988) and natural products with positive effect for human health (Das and Ramanathan, 1966). We have previously demonstrated that flavonoids were responsible for the anti-hypertensive activity of *Spergularia purpurea* in normal and hypertensive rats (Jouad et al., 2001b). These natural compounds could act separately or synergistically to cause the hypoglycaemic effect. This could not exclude the intervention of other phytochemical constituents as bioactive hypoglycaemic agents.

In conclusion, the aqueous extracts of FE seed and SM aerial part are potent hypoglycaemic agents in normal rats and anti-hyperglycaemic solutions in STZ diabetic rats without affecting insulin secretion. This finding represents an experimental confirmation of the Moroccan traditional use of these plants. In addition, the hypoglycaemic effect of FE and SM extracts appear to be independent of insulin secretion. Finally, the precise mechanism(s) and site(s) of this activity and the active constituent(s) involved are still to be determined in addition to toxicological studies.

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# Diuretic activity of *Spilanthes acmella* flowers in rats

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**Abstract**

In the Sri Lankan traditional medicine, *Spilanthes acmella* Murr. (Family: Compositae) flowers are claimed to possess powerful diuretic activity. However, as yet, the diuretic potential of these flowers is not investigated by scientifically controlled studies. The aim of this study was to evaluate the diuretic potential of *Spilanthes acmella*flowers in rats using a cold-water extract (CWE). Different concentrations of CWE (500, 1000, 1500 mg/kg) or vehicle or furosemide (13 mg/kg) were orally administered ($N = 6$ per each treatment group) to hydrated rats and their urine output was monitored at several intervals of time (1–5 h). The highest dose of CWE significantly ($P < 0.05$) and markedly increased the urine output. The onset of this diuretic action was extremely prompt (within 1 h) and lasted throughout the studied period (up to 5 h). The peak effect was evident between 1 and 2 h. Further, the intensity of diuresis induced by the CWE in the first hour was almost similar to that of furosemide. *Spilanthes acmella* CWE also caused marked increase in urinary $Na^+$ and $K^+$ levels and a reduction in the osmolarity of urine suggesting that it is mainly acting as a loop diuretic. It may also inhibit ADH release and/or action. It is concluded that the *Spilanthes acmella* CWE has strong diuretic action as is claimed.
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*Keywords:* *Spilanthes acmella*; Diuretic; Loop diuretic; Urine output

## 1. Introduction

*Spilanthus acmella* Murr. (Family; Compositae), Acmella in Sinhala and Akkirakaran in Tamil, is an annual or short-lived perennial herb, 20–60 cm tall, with a prostrate or ascending branched cylindrical hairy stem and simple ovate opposite leaves without stipules. The flowers are yellow, non-fragrant with five petals on long glabrous peduncles. This herb is found in India, Sri Lanka and other tropical countries (Jayaweera, 1981). In Sri Lanka, it is common in moist places up to 1800 m altitude (Jayaweera, 1981).

Sri Lanka traditional physicians, especially, in the Uva province claim that the cold infusion of the flowers of *Spilanthes acmella* have potent diuretic activity and the ability to dissolve urinary calculi. The flowers are chewed or used in the form of a tincture for toothache and to stimulate flow of saliva (Jayaweera, 1981). It is also recommended for paralysis of the tongue and in stammer and sore mouth in children (Jayasinghe, 1994). Further, the flowers are used locally against itching and psoriasis (Jayaweera, 1981). Experimentally, it is shown to have larvicidal potential against *Culex quinquefasciatus*(Pitasawat et al., 1998). Phytochemically, flowers of *Spilanthes acmella* are reported to contain amino acids (Mondal et al., 1998; Peiris et al., 2001), alkaloids (Peiris et al., 2001) and N-isobutylamides (spilanthol, undeca-2E,7Z,9E-trienoic acid isobutylamide and undeca-2E-en-8,10-diynoic acid isobutylomide) (Ramsewak et al., 1999).

Since the advocated diuretic potential of *Spilanthes acmella* flowers were not tested rigorously by scientifically controlled experiments, this study was undertaken to investigate the diuretic potential of a cold water extract (CWE) of *Spilanthes acmella* flowers using rats.

## 2. Materials and methods

### 2.1. Experimental animals

Healthy adult crossbred male albino rats (weighing 200–225) from our own colony were used. They were housed in standard environmental conditions (temperature: 28–30 °C, photoperiod: approximately 12 h natural light per

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day, relative humidity: 50–55%) with free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and water.

### 2.2. Collection of flowers

Yellow coloured fresh flowers were collected from mature *Spilanthus acmella* plants at the Ayurvedic medicinal garden, Haldumulla (Uva Province), Sri Lanka, in June 2002. The identification an authentication was done by Mr. S. B. Weerakoon, Department of Ayurveda, Colombo, Sri Lanka. A voucher specimen (Am/01, 2002) is deposited at the museum of the Department of Zoology, University of Colombo, Sri Lanka.

### 2.3. Preparation of the cold water extract (CWE)

Fresh flowers (482 g) were homogenised in distilled water (400 ml) using a domestic blender (National Model MX-T, Matsushita Ltd., Tokyo, Japan) for 10 min and filtered through eight layers of muslin cloth. The resulting brown coloured filtrate was freeze-dried (17.5 g, yield: 3.6% w/w) and stored air tight at 4 °C. The freeze-dried powder was dissolved in distilled water to obtain the required dosage concentration (in terms of fresh weight) in 1 ml solution (500, 1000, or 1500 mg/kg). The highest dose tested was 7.5 times higher than that is normally recommended by the traditional practitioners of the Uva province, which is within the accepted range for the rat model (Dhawan and Srimal, 2000).

### 2.4. Evaluation of the diuretic activity

Thirty rats were deprived of water but not food for 18 h. Their urinary bladders were emptied by gentle compression of the pelvic area and by pull of their tails. Each of these rats was then orally administered with 15 ml of isotonic saline (NaCl, 0.9% w/v) to impose a uniform water load. Forty-five minutes later, these rats were randomly assigned into five groups ($N = 6$ per group) and treated orally in the following manner. Group 1: 1 ml of distilled water, group 2: 500 mg/kg of CWE, group 3: 1000 mg/kg of CWE, group 4: 1500 mg/kg of CWE and group 5: 13 mg/kg of furosemide (State Pharmaceutical Corporation, Colombo, Sri Lanka), the reference drug (Rang et al., 1995; Dharmasiri et al., 2003). Each of these rats was individually placed in metabolic cages and cumulative urine output was determined at hourly intervals for 5 h. The colour of urine was also noted.

In an attempt to ascertain the broad mechanisms of action, the urine collected from group 1 (control) and group 4 (1500 mg/kg of CWE) were subjected to the following investigations: pH (by pH meter, Toa Electronics Ltd., Tokyo, Japan), $Na^+$ and $K^+$ levels by flame photometry (compact atomic absorption spectrometer, GFS Scientific Equipment Pvt. Ltd., Sydney, Australia), osmolarity (by Osmometer, Type TW2, Advanced Instrument Inc., Massachusetts, USA), specific gravity and, glucose and proteins (using Combistrix®, Reagent strips, Bayer Diagnostics Manufacturing Ltd., Bridgend, UK). $Na^+/K^+$ ratio was then computed.

### 2.5. Evaluation of acute and sub chronic toxicity

Twelve rats were randomly assigned into two equal groups ($n = 6$). The first group was orally treated daily for 7 days with the highest dose of CWE and the other with 1 ml of distilled water. During this period, each rat was observed for overt signs of toxicity (salivation, lachrymation, ptosis, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (erection of fur and exopthalmia), behavioural abnormalities (such as impairment of spontaneous movement, climbing, cleaning of face and ataxia, and other postural changes) and aversive behaviour (biting and scratching behaviour, licking of tail, paw and penis, intense grooming behaviour and vocalization) and diarrhoea. On day 1 post treatment, these rats were anaesthetised with ether (BDH Chemical Co., Poole, UK). Blood was collected from tails using aseptic precautions, serum separated and, urea and creatinine (to examine renal toxicity), and GOT and GPT (to judge liver toxicity) levels determined using respective assay kits (Randox Laboratory Ltd., UK).

### 2.6. Statistical analysis

Data are represented as mean ± S.E.M. Statistical comparisons were made using Mann–Whitney, $U$-test. Significance was set at $P \leq 0.05$.

## 3. Results

As shown in Table 1, the highest dose of the CWE significantly ($P < 0.05$) and profoundly increased the cumulative urine out put (by 426%). Further, the increase in urine output induced by the highest dose of CWE was evident from the first hour and lasted until the termination of the experiment: 1 h (by 523%), 2 h (by 526%) 3 h (by 451%) 4 h (by 388%) and 5 h (by 348%) (see Fig. 1). The peak effect was seen at the first and the second hour. The reference drug, furosemide significantly ($P < 0.05$) increased (by 405%) the urine out

Table 1
Cumulative urine output in rats over a 5-hour period following oral administration of cold-water extract (CWE) of *Spilanthes acmella* flowers (mean ± S.E.M.)

| Treatment | Total urine output (ml) |
|---|---|
| Control (distilled water) | 10.2 ± 2.0 |
| 500 mg/kg of CWE | 8.6 ± 1.7 |
| 1000 mg/kg of CWE | 6.7 ± 0.5 |
| 1500 mg/kg of CWE | 53.4 ± 8.0* |
| 13 mg/kg of furosemide | 8.1 ± 0.8 |

* $P < 0.05$ as compared with control (Mann-Whitney, $U$-test).

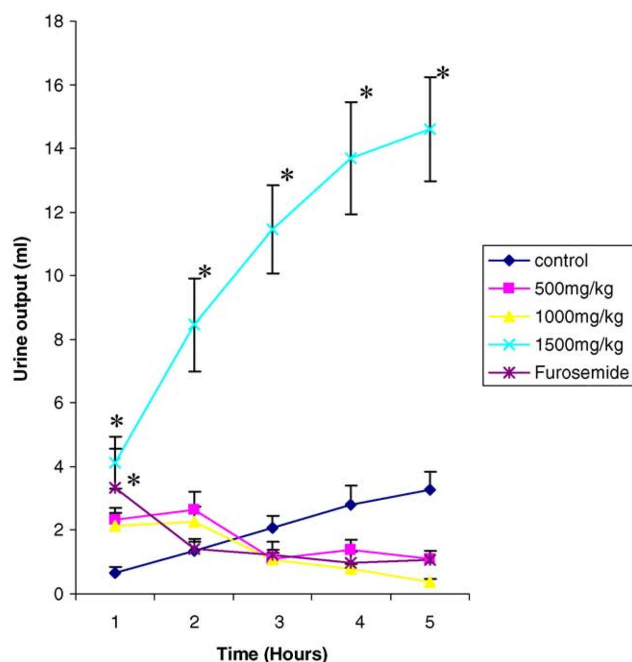


Fig. 1. Time course of diuresis in rats treated with (500, 1000 or 1500 mg/kg) different doses of cold-water extract of *Spilanthes acmella* flowers, vehicle and reference drug, furosemide. Each point represents the mean of six rats and vertical bars indicates SEM. $^*P < 0.05$.

put only at the first hour. Further, the diuresis induced by the CWE at 1 h was almost similar to that of furosemide.

As shown in Table 2, the highest dose of CWE slightly but significantly ($P < 0.05$) reduced the urinary pH. It also provoked a massive and significant ($P < 0.05$) increase in urinary excretion of $Na^+$ (by 51%) and $K^+$ (by 213%). However, the $Na^+/K^+$ ratio remained unaltered. Accompanying the increase of urine output was a significant ($P < 0.05$) reduction (by 22%) in urinary osmolarity. Additionally, the highest dose did not induce proteinurea or glucoseurea. The colour of the urine of rats in treated groups appeared almost identical to that of the control group.

In the toxicity study, the CWE did not provoke any overt signs of toxicity, stress or aversive behaviour. There was also no sign of diarrhoea and none of the treated rats died. Moreover, of the serum parameters tested only serum urea significantly ($P < 0.05$) altered: (reduced by 54%) (urea: control versus treatment: $31.8 \pm 6.6$ versus $14.6 \pm 1.6$ mg/dl, creatinine: $0.7 \pm 0.2$ versus $0.6 \pm 0.1$ mg/dl, SGOT: $22.3 \pm 5.7$ versus $22.4 \pm 4.7$ mg/dl and SGPT: $14.5 \pm 1.4$ versus $11.8 \pm 2.3$ mg/dl).

Table 2
Effect of orally administrated cold-water extract of *Spilanthes acmella*flowers (1500 mg/kg) on some urine parameters (up to 5 h) of rats (mean ± S.E.M.)

| Parameter | Control group | Treated group |
|---|---|---|
| pH | 7.1 ± 0.3 | 6.6 ± 0.25 |
| Specific gravity | 1.012 ± 0.0 | 1.023 ± 0.0 |
| $Na^+$ (ppm) | 10330 ± 1539 | 15587 ± 1149* |
| $K^+$ (ppm) | 4449 ± 845 | 13938 ± 4825* |
| $Na^+/K^+$ ratio | 2.5 ± 0.28 | 1.56 ± 0.2 |
| Glucose | Negative | Negative |
| Protein (mg/dl) | Negative | Negative |

$^*$ $P < 0.05$, compared to control (Mann-Whitney $U$-test).

## 4. Discussion

This study examined the diuretic potential of *Spilanthes acmella*flowers using a CWE. The results showed that the highest dose of CWE of flowers tested possesses strong diuretic activity when given orally in a single dose. A similar pattern of diuresis is reported with some other plant diuretics: for instance, leaves and stems of *Anisomeles indica* (Dharmasiri et al., 2003) and *Strychnos potatorum* seeds (Biswas et al., 2001). The CWE was well tolerated with an encouraging safety profile even following subchronic administration (as judged by absence of mortality, overt signs of toxicity, stress, behavioural abnormalities and increased levels of serum GOT, GPT creatinine and urea). However, hexanic extract of *Spilanthes acmella*plant in rats is reported to induce full tonic-clonic convulsions accompanied by typical electrographic seizures in the EEG (Moreica et al., 1989). It is well recognized that hexane, extracts predominately nonpolar constituents whilst CWE isolates polar constituents. This may account for the discrepancy observed between these two studies. In addition, Moreira et al. (1989) have used the whole plant while we have used fresh flowers. Interestingly, the CWE also appears to be renoprotective (in terms of serum urea levels). If the results are applicable to humans, then this is an important and clinically useful finding both locally and globally as it provides scientific evidence in favour of its claimed diuretic potential by controlled experimentation; after all, 35% of Sri Lankan (Mahindapala, 2000) and 80% of the people in developing countries of the world (Farnsworth, 1999) rely on traditional medicine, and about 85% of traditional medicine involves the use of plant extracts (Farnsworth, 1999). Undoubtedly, this would widen the options of potential diuretic therapies available to traditional practitioners.

The CWE-induced diuresis was strong and was not accompanied with a reduction in urinary $K^+$ levels. Further, there was no alkalisation of urine. Collectively, these observations suggest that the CWE is not acting as potassium-sparing diuretics (Rang et al., 1995; BNF, 2000; Kreydiyyeh and Julnar, 2002). The CWE is also unlikely to be acting as thiazide diuretics: these only increase the urinary $K^+$ level and alter the urinary $Na^+/K^+$ ratio (Rang et al., 1995; BNF, 2000). But, in this study, both urinary $Na^+$ and $K^+$ levels were increased without any alteration in the $Na^+/K^+$ ratio.

On the other hand, the diuresis induced by the CWE of *Spilanthes acmella*flowers was strong with an intensity similar to that of furosemide and accompanied by marked increases in both urinary $Na^+$ and $K^+$ levels.

Further, the urine was slightly acidified. These features strongly suggest that the CWE is acting as a loop diuretic. Loop diuretics are the most powerful of all diuretics and these inhibit the $Na^+/K^+/Cl^-$ co-transporter system in the thick ascending loop of the nephron, thereby increasing natriuresis and kaleuresis (Rang et al., 1995; BNF, 2000; Kreydiyyeh and Julnar, 2002). These diuretic also cause acidification of urine (Rang et al., 1995; Osorio and Teitelbaum, 1997; BNF, 2000). Further, the onset of the diuretic activity of the CWE was extremely rapid (within 1 h of administration) as observed with clinically used synthetic loop diuretics (Rang et al., 1995; BNF, 2000). Interestingly, in spite of the heavy loss of urinary $Na^+$ and $K^+$, there was a significant reduction in the osmolarity of urine in CWE treated rats. Thus, it is possible that the CWE, in addition, may impair the basal secretion of ADH and/or diminished the responsiveness of uriniferous tubules to the action of ADH: inhibition of ADH causes polyurea with low osmolarity (Mayne, 1994; Osorio and Teitelbaum, 1997). Phytochemically, *Spilanthes acmella* flowers are shown to contain N-isobutylamides (Ramsewak et al., 1999), alkaloids, (Peiris et al., 2001) and amino acids (Mondal et al., 1998; Peiris et al., 2001). Amino acids are resorbed in the proximal convoluted tubules of nephrons (Rang et al., 1995) and cannot function as diuretics. Thus, the diuretic activity of the CWE may be attributed to its alkaloids.

Loop diuretics are clinically used in patients with salt and water overload due to host of conditions such as pulmonary oedema, heart failure ascites, hypertension (Rang et al., 1995; BNF, 2000). Loop diuretic mode of action of the CWE of the *Spilanthes acmella* flowers indicate that *Spilanthes acmella* flowers may be useful as a non toxic natural therapeutic agent in the treatment of such conditions by traditional practitioners. The onset of the diuretic action of the CWE was extremely rapid and it also had a fairly long duration of action. This is an appealing diuretic profile as it would curtail the frequency of administration. However, there is one major limitation: an increased risk of hypokalaemia as with other therapeutically used loop diuretics.

In conclusion, this study provides first scientific evidence in favour of claimed diuretic potential of CWE of *Spilanthes acmella* flowers. It further shows that the CWE of *Spilanthes acmella* flowers mainly acts as a loop diuretic in inducing diuresis.

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# Antispasmodic and hypotensive effects of *Ferula asafoetida* gum extract

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**Abstract**

The effects of *Ferula asafoetida* gum extract on the contractile responses of the isolated guinea-pig ileum induced by acetylcholine, histamine and KCl, and on the mean arterial blood pressure of rat were investigated. In the presence of extract (3 mg/ml), the average amplitude of spontaneous contractions of the isolated guinea-pig ileum was decreased to $54 \pm 7\%$ of control. Exposure of the precontracted ileum by acetylcholine (10 μM) to *Ferula asafoetida* gum extract caused relaxation in a concentration-dependent manner. Similar relaxatory effect of the extract was observed on the precontracted ileum by histamine (10 μM) and KCl (28 mM). However, when the preparations were preincubated with indomethacin (100 nM) and different antagonists, such as propranolol (1 μM), atropine (100 nM), chlorpheniramine (25 nM) then were contracted with KCl, exposure to the extract (3 mg/ml) did not cause any relaxation. Furthermore, *Ferula asafoetida* gum extract (0.3–2.2 mg/100 g body weight) significantly reduced the mean arterial blood pressure in anaesthetised rats. It might be concluded that the relaxant compounds in *Ferula asafoetida* gum extract interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilisation of calcium ions required for smooth muscle contraction non-specificly.
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*Abbreviations: Ferula asafoetida*; Gum extract; Ileum; Blood pressure; Relaxation; Hypotension

## 1. Introduction

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs. The Ferula genus from the family of Umbelliferae has been found to be a rich source of gum-resin (Fernch, 1971). *Ferula asafoetida* (*Ferula assa-foetida* L.) is a herbaceous wild plant native to Iran. In Iranian traditional medicine *Ferula asafoetida* gum extract has been used as a remedy for abdominal pain, constipation, diarrhea and as an antihelminthic. Several fractions such as gum fraction (25%, inculding glucose, galactose, L-arabinose, rhamnose and glucuronic acid), resin (40–64%, which contains ferulic acid esters (60%), free ferulic acid (1.3%), coumarin derivatives (e.g. umbelliferone), volatile oils (3–17%) including sulphur-containing compounds, and various monoterpenes have been isolated from this plant (Kajimoto, 1989).

Although there is some evidence for anticoagulant action of *Ferula asafoetida* gum extract (Leung, 1980) its pharmacological effects on intestinal smooth muscles and blood pressure have not been established yet. The present study was performed as a starting point for examining the folkloric claims regarding the beneficial effects of gum obtained from *Ferula asafoetida* in gastrointestinal and haemodynamic disorders. Here, we report our observations on the effects of *Ferula asafoetida* gum extract on the contractile responses of guinea-pig ileum induced by various stimuli (in vitro), and on blood pressure recorded from the anaesthetised rats (in vivo).

## 2. Material and methods

### 2.1. Plant gum extract

*Ferula asafoetida* gum was collected from Gonabad region (South of Khorasan province) during the summer. The plant was identified at the Botany Department, Faculty of Science, Mashhad University, Mashhad, Iran. A voucher specimen number was kept in record (293-0606-2) at the Department of Pharmacognosy, Faculty of Pharmacy, Mashhad University of Medical Sciences. The powdered dried gum

*Abbreviations:* Ach, acetylcholine; At, atropine; Ch, chlorpheniramine; Fa, *Ferula asafoetida* gum extract; g BW, gram body weight; His, histamine; Ind, indomethacin; MABP, mean arterial blood pressure; Pr, propranolol

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(10 g) was soaked overnight in distilled water (100 ml) at room temperature and filtered for daily use. From 10 g powdered gum, 4.7 g was insoluble in water. Concentrations and doses of the aqueous extract are expressed as total amount of the dried gum used in preparing the extract.

### 2.2. Drugs

Acetylcholine chloride, histamine, propranolol, chlorpheniramine, indomethacin and atropine sulphate were purchased from Sigma. Thiopental was obtained from Biochemie GmbH (Austria). All drugs except indomethacin were dissolved in distilled water and added in a volume less than 1 ml to the organ bath. Indomethacin was dissolved in ethanol. At the final bath concentration (0.01%, v/v), ethanol had no effect on the ileum responses.

### 2.3. Experimental protocol

To perform in vitro studies, guinea-pigs were killed by a blow to the head and exsanguination. The ileum was exteriorised, the ileo-caecal junction located and approximately 30 cm removed. A 8–10 cm segment of the terminal portion was discarded before the contents in the remaining ileum were flushed out with modified Krebs–Henseleit solution (mM): NaCl 118.4, KCl 4.7, $MgSO_4{\cdot}7H_2O$ 1.4, $KH_2PO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11.1. Segments of ileum (2 cm) were set up under 1 g of tension in 10 ml tissue baths, ready for isometric recording using an isometric transducer connected to an Oscillograph 400 MD/2. Preparations were incubated in Krebs–Henseleit solution gassed (95% $O_2$/5% $CO_2$) at 37 °C, and allowed for about 1 h to equilibrate prior to testing with a priming concentration of the gum extract or any other drugs.

For in vivo studies, experimental protocol was according to the 'Guiding Principles in the Use of Animals', adopted by the WHO. Sprauge–Dawley rats (250–300 g) and guinea-pigs (400–500 g) of either sex were anaesthetised with thiopental (15 mg/kg, i.p.). The trachea was cannulated and animals were artificially respired with room air using a Harvard small animal ventilation pump (at a rate of 54 strokes per minute, volume of 1 ml/100 g body weight). Body temperature was maintained at $37 \pm 0.5$ °C by using an incandescent lamp placed over the abdomen and coupled to a rectal thermistor probe. The right common carotid artery and right jugular vein were canulated using a polythene cannula for the measurement of arterial blood pressure and administration of the extract, respectively. Blood pressure was recorded continuously using an elcomatic EM751 pressure transducer on a Physiograph (Harvard Universal Oscillograph).

### 2.4. Analysis of data

Values in the text refer to mean ± S.E.M. Changes in MABP were compared using Student's *t*-test. Differences between groups have been compared using one-way ANOVA followed by a Tukey–Kramer multiple comparison test. A *P*-value of less than 0.05 was considered as significant.

## 3. Results

### 3.1. Effect of the extract on spontaneous contractions of the isolated guinea-pig ileum

The isolated guinea-pig ileum preparations had spontaneous contractions when they were mounted in the tissue bath under 1 g tension. Exposure of the preparations to 1, 2, 3, 5 and 7 mg/ml of the extract reduced the average amplitude of the spontaneous contractions to $83 \pm 6\%$, $68 \pm 5\%$, $54 \pm 7\%$, $21 \pm 9\%$ and $9 \pm 3\%$ of control, respectively ($n = 11$).

### 3.2. Effect of the extract on evoked contractions of the isolated guinea-pig ileum

The contractions were elicited by acetylcholine (Ach), histamine and KCl. Addition of these agents produced rapidly developing increase in tension with minimal increase in rhythmic activity. Returning to resting tension was rapid on washout of the organ bath.

In tissues precontracted with different agents, *Ferula asafoetida* gum extract induced concentration-dependent relaxations (Fig. 1A). The relaxatory effect of the extract in the preparations precontracted with histamine (25 μM) was more pronounced than those precontracted with Ach (25 μM), and or KCl (28 mM).

### 3.3. No relaxatory effect in the presence of indometacin and different antagonists

When preparations were preincubated with indomethacin (100 nM), propranolol (1 μM), atropine (100 nM) and chlorpheniramine (25 nM), then contracted by KCl (28 mM), the extract had no relaxatory effect (Fig. 1B). In the presence of indomethacin, propranolol, atropine and chlorpheniramine, addition of the extract increased tension of the preparations contracted by KCl. As shown in Fig. 1B, contractile responses of the preparations preincubated with indomethacin, propranolol, atropine and chlorpheniramine to the KCl (28 mM) and the extract were $156 \pm 17\%$, $157 \pm 2\%$, $126 \pm 9\%$, $178 \pm 28\%$ of response to 1 μM acetylcholine, respectively. When the preparations were incubated with indomethacin and the antagonists simultaneously and then exposed to the extract a contraction ($167 \pm 5\%$ of response to acetylcholine) was observed (Fig. 1B).

### 3.4. Effects of the extract on rat blood pressure

Basal blood pressure ($109 \pm 7$ mmHg) and heart rate ($464 \pm 17$) of five anaesthetised rats used in this study remained unchanged over the experimental period. Sev-

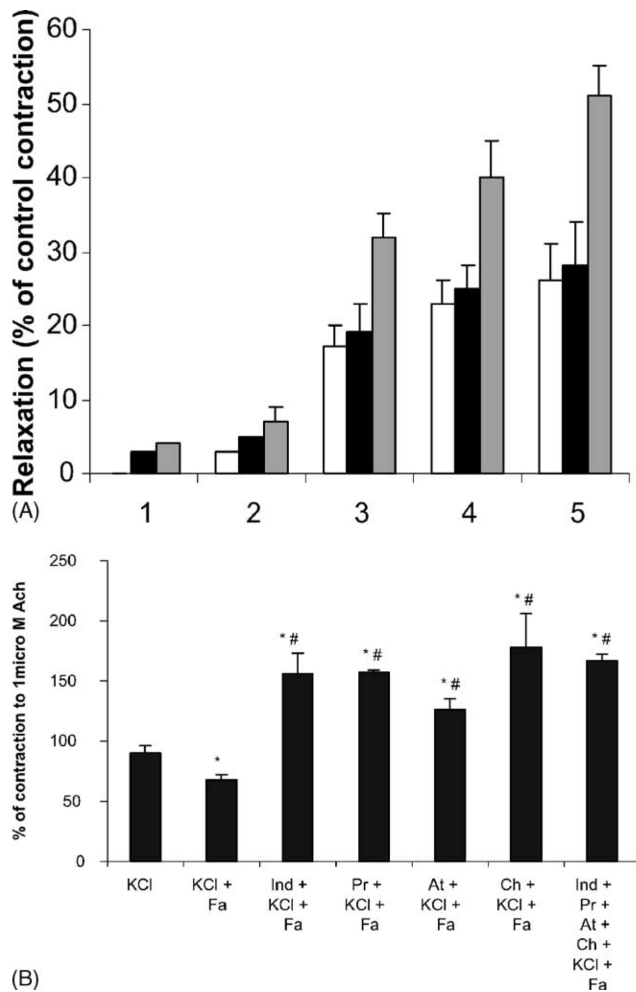


Fig. 1. (A) The relaxatory effect of *Ferula asafoetida* gum extract (1–7 mg/ml) on guinea-pig isolated ileum precontracted with KCl (28 mM, $n = 6$, white bars), acetylcholine (20 μM, $n = 6$, black bars), and histamine (20 μM, $n = 5$, gray bars). The category axis (the bar clusters) of 1–5 represent concentrations of 1, 2, 3, 5 and 7 mg/ml of the extract, respectively. Relaxations induced by the extract (3–7 mg/ml) were significant compared to equivalent volume of normal saline ($P < 0.05$). (B) Effects of different antagonists and a cyclooxygenase inhibitor on responses to *Ferula asafoetida* gum extract (3 mg/ml) responses in guinea-pig isolated ileum precontracted with KCl (28 mM). Prior to addition of *Ferula asafoetida* gum extract (Fa), the preparations were incubated with indomethacin (Ind, 100 nM; $n = 4$), propranolol (Pr, 1 μM; $n = 4$), atropine (At, 100 nM; $n = 4$) and chlorpheniramine (Ch, 25 nM; $n = 4$), then preparations were contracted with KCl (28 mM). This procedure completely inhibited the relaxatory effect of Fa and a contractile response was observed. $^{*}P < 0.05$ vs. KCl, $^{\#}P < 0.01$ vs. KCl + Fa.

eral doses of the extract (0.3–2.2 mg/100 g body weight in 0.4 ml) decreased rat blood pressure in a dose-dependent manner (Fig. 2).

## 4. Discussion

In the present study, *Ferula asafoetida* gum extract was found to reduce the spontaneous contraction of the isolated

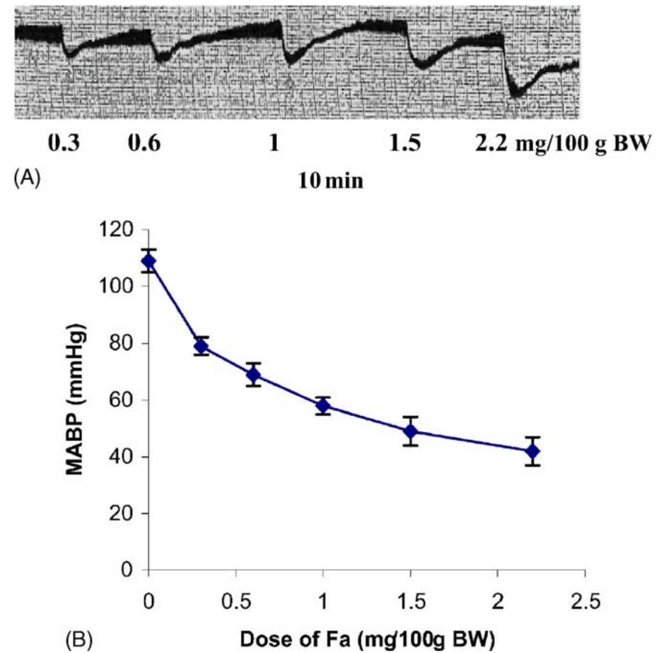


Fig. 2. (A) Representative tracing of the effect of increasing doses of *Ferula asafoetida* gum extract (0.3–2.2 mg/100 g body weight) on the arterial blood pressure of an anaesthetised rat. (B) Effects of different doses of *Ferula asafoetida* gum extract on the mean arterial blood pressure of anaesthetised rats.

guinea-pig ileum. It has been established that the spontaneous contractions of the intestinal smooth muscle are regulated by cycles of depolarisation and repolarisation. Action potentials are generated at the peak of depolarisation and constitute a fast influx of calcium ions through the voltage-activated calcium channels (Walsh and Singer, 1980; Brading, 1981). Therefore, it is possible that the extract contains some compounds which, interfere with the calcium channels activity. The extract also decreases contractions induced by acetylcholine, histamine and KCl in the isolated guinea-pig ileum. This effect was concentration-dependent and reversible after washing. Similar inhibitory effects of *Ferula sinaica* (another species from *Ferula* genus) root extract on rat and guinea-pig uterine smooth muscle contractions was reported by Aqel et al. (1991). However, the exact mechanism of action of *Ferula sinaica* extract remained unknown. Acetylcholine and histamine cause depolarisation and tonic contractions of intestinal smooth muscles. It is generally accepted that an increase in concentration of cytoplasmic-free calcium ions is indispensable for smooth muscle contraction. The activation of muscarinic receptors of longitudinal smooth muscle of guinea-pig small intestine produces an increased frequency of action potential discharge and depolarisation which results in a contraction (Reddy et al., 1995). The acetylcholine-evoked contraction is generally regarded as mediated via $M_3$ subtype of muscarinic receptor although the muscle has a preponderance of $M_2$ subtype muscarinic binding sites. Whereas, histamine-induced contraction happens via $H_1$ receptor ac-

tivation (Zavecz and Yellin, 1982), and contraction induced by KCl is due to an increase in $K^+$ and depolarisation of smooth muscle fibers, leading to increased influx of calcium through L-type voltage-operated channels (Gilani et al., 1994). In short, calcium ions gain access to the cytoplasm through voltage-activated or receptor-operated calcium channels (Triggle, 1985). According to our observations, when the isolated guinea-pig ileum preparations were precontracted with histamine, the relaxation induced by the extract was very much higher than that in the presence of Ach. However, the spasmolytic activity of the extract could not be attributed solely to any pure antagonistic effect, since the tissue contracted by KCl was also relaxed after exposure to the extract. Considering lack of the relaxatory effect of the extract in the presence of atropin, chlorpheniramine and propranolol, one might suggest that these antagonists competed with the relaxant compounds of the extract for binding to their acceptors. Therefore, there is no good reason to exclude any interaction between some compounds of the extract and cholinergic, histaminergic and adrenergic receptors. On the other hand, smooth muscle contractile tone can be relaxed by increased levels of adenosine 3′,5′-cyclic monophosphate (cAMP) (Berridge, 1975). Therefore, the extract may have its relaxatory effect through an increase in cAMP independent of any specific receptor activity, then a reduction in $Ca^{2+}$ levels.

Furthermore, we observed that indomethacin (a cyclooxygenase inhibitor) significantly inhibited the relaxatory effect of *Ferula asafoetida* gum extract in isolated guinea-pig ileum, suggesting that cyclooxygenase metabolites (e.g. prostaglandins such as PGE2, PGD2 and PGI2) may be involved in this relaxation.

The present study also demonstrates that *Ferula asafoetida* gum extract is effective in reducing blood pressure in anaesthetised normotensive rats. This effect is shown to be dose-related and rapid in onset. At higher doses, the duration of the depressor response to *Ferula asafoetida* gum extract was long-lasting. Taken together, the relaxatory effects of the *Ferula asafoetida* gum extract on vascular smooth muscle as well as on ileum smooth muscle may suggest that, this natural product reduce the cytosolic $Ca^{2+}$ in a non-specific manner.

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# Evaluation of acute and chronic treatments with *Harpagophytum procumbens* on Freund's adjuvant-induced arthritis in rats

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**Abstract**

The extract of *Harpagophytum procumbens*, widely utilized in Europe and, more recently, in other countries, is traditionally indicated to treat inflammatory processes. *Harpagophytum procumbens* acts by way of interleukins and leukocyte migration to the painful and inflamed joint area. Chemically, its secondary tuberous roots contains iridoid glycosides, harpagogide, procumbide, and harpagoside, as the active principle. The purpose of the present study was evaluate the therapeutic potential as anti-inflammatory and analgesic agent in rat model of Freund's adjuvant-induced arthritis both in the acute and chronic phases. The animals were injected with Freund's adjuvant in sub-plantar tissue of the right posterior paw and randomly assigned in acute (25, 50, or 100 mg/kg) or chronic (100 mg/kg) treatments with *Harpagophytum procumbens* solution test or vehicle. Then, submitted to behavioral test and assessment of body weight and right paw's measurements. The results show that *Harpagophytum procumbens* extract increased the animals 'latency of paws' withdrawal, indicating a protective effect against the pain induced by the thermal stimulus, both in acute and chronic treatments. In addition to reduction in the right paw edema in the experimental groups when compared to control group. Thus, the data showed anti-inflammatory and peripheral analgesic properties of *Harpagophytum procumbens* extract with all doses tested, thus confirming its indication for inflammatory processes.
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*Keywords:* *Harpagophytum procumbens*; Freund's adjuvant-induced arthritis; Anti-inflammatory properties; Antinociceptive effects; Rats

## 1. Introduction

Popularly known as "devil's claws," *Harpagophytum procumbens* D.C. (de Candolle; Volk, 1953) is a plant, originated from Southern Africa, specifically, from the Kalahari Desert and Namibia steppes. The plant counting to family of Pedaliaceae has been widely utilized for anti-rheumatic treatment in European countries and has recently been introduced in Brazil. *Harpagophytum procumbens* acts by way of interleukins and leukocyte migration to the painful and inflamed joint area (Copelman, 1996). It does not interfere with the prostaglandins' cascade, favoring the indication of this plant in ostheoarthritis, arthroses and arthritis processes, as well as in arthralgias of inflammatory etiology and myalgias (Copelman, 1996). Chemically, its secondary tuberous roots contains iridoid glycosides, mainly harpagoside, harpagogide and procumbide (Van Haelen, 1986).

Several clinical and animal studies have demonstrated the efficacy of *Harpagophytum procumbens* in rheumatic diseases, in addition to its analgesic properties (Lanhers et al., 1991). In regard to its anti-inflammatory effects, Van Haelen (1986) reported that *Harpagophytum procumbens* presents efficient results in sub-acute processes. Other studies, however, have not confirmed these findings (McLeod et al., 1979; Grahame and Robinson, 1981; Whitehouse et al., 1983).

Freund's adjuvant-induced arthritis have been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance for the study of pathophysiological and pharmacological control of inflammatory processes, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs (Butler et al., 1992; Besson and Guilbaud, 1988). One of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agents in this model and in rheumatoid arthritis in humans. The arthritis is induced by a sub-cutaneous injection of Freund's adjuvant. The denatured *Mycobacterium butyricum* suspended in mineral oil can be injected sub-cutaneously at the base of the rat's tail or in the paw's plantar surface, or by intra-joint via.

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The adjuvant elicits arthritis predominantly in the joints of hind limbs, promoting significant reduction of motor activity and increased itching and scratching behaviors (Calvino et al., 1987). In addition, increased sensitivity of the affected paw to pressure or flexion and extension of the inflamed joints and weight loss are observed (Besson and Guilbaud, 1988). The arthritis observed in rats is associated with a hyperalgesia phenomenon (Calvino et al., 1987) and spontaneous behaviors, such as protection of the affected paw, evidenced by curving and/or elevation of the paw, as well as avoidance of supporting the body on the paw (Clatworthy et al., 1995). The hyperalgesia is more evident during the acute inflammatory phase, when spontaneous behaviors, indicative of painful response are more pronounced (Calvino et al., 1987). Increased paws' diameter (posterior and anterior), due to inflammation and edema is also observed (Cain et al., 1997). The initial inflammatory response is developed within hours, but more critical clinical signals emerge from the 10th post-inoculation day and thereafter, and the alterations remain detectable for several weeks (Colpaert et al., 1982). According to Abbadie and Besson (1994), maximum arthritic response is obtained in 3 weeks.

Considering the anti-inflammatory effects of *Harpagophytum procumbens* have only been described on sub-acute and on acute processes, and the controversial data related to these anti-inflammatory and analgesic effects, the purposes of the present study were:

(a) to examine the anti-inflammatory effects of *Harpagophytum procumbens* in rats submitted to the model of Freund's adjuvant-induced arthritis;
(b) to evaluate its possible analgesic effects;
(c) to differentiate between acute and chronic treatments.

## 2. Methodology

### 2.1. Animals

Ninety-day-old Wistar male rats, bred, and raised in the Animal Facility of the Department of Psychobiology of Universidade Federal de São Paulo were used in this study. They were maintained under constant automatically control 12 h/12 h light/dark cycle (lights on from 07.00 a.m. to 07.00 p.m.) and environmental temperature ($23 \pm 1\,^{\circ}C$). Rat chow and tap water were provided ad libitum in standard propylene cages. Cage cleaning consisted of daily change of sawdust bedding. At the end of the study, animals were sacrificed with an overdose of chloral hydrate.

### 2.2. Number of animals

Due to the painful condition imposed on the animals, the number of subjects used was restricted to the minimum that allowed reliable statistical analysis of the results. All procedures were submitted to and approved by the Ethics Committee of Universidade Federal de São Paulo (process #063/99) and followed the recommendations of the Research and Ethics Committee of IASP (1983). Each group was composed of 10 animals.

Fig. 1. Chemical structure of harpagoside, the main component of *Harpagophytum procumbens*.

### 2.3. Plant material

*Harpagophytum procumbens* D.C. roots were obtained from Börner GmbH (Berlin, Germany) as a dry extract and under the care of Zenimport Ltd. (Rio de Janeiro, Brazil). A voucher specimen (*Harpagophytum procumbens* ASC 2102) is kept in the National Herbarium of Namibia, National Botanical Research Institute, Namibia, and Africa. The active principle as harpagoside as shown in Fig. 1.

### 2.4. Extraction procedure

Plant materials were extracted with 60% ethanol and stirred mechanically. The ethanol was evaporated and the volume brought to the desired concentration before use. The native extract (harpagoside) proportion is 80–90% and the weight ratio "extract/drug" of *Harpagophytum procumbens* and rad. sicc. is 4:1/60%. The yield was 10% in average, based on the presence of solid residues. The analytical method used was the High Performance Liquid Chromatography (HPLC) with Diode Array detector. The quality of the plant extract is guaranteed in the framework of the assay in the final product by the HPLC method by comparing the retention times and the content of harpagoside in the HPLC-chromatogram with the standard substance and the content in the controlled and released material (extract). The evidence of harpagoside in the extract is done according to the European Pharmacopoeia (1997), monograph for Harpagophyti radix. The purity tests showed content of harpagoside 1.5%. (Note: According to the monograph published in European Pharmacopoeia (1997), the requirement is that "it contains not less than 1.2% of harpagoside, calculated with reference to the dried drug.")

### 2.5. Pharmacological experiments

All experiments were conducted in a quiet room between 09.00 and 11.00 a.m. at constant temperature of $23 \pm 1\,^{\circ}C$. All solutions were daily prepared in distilled water and stirred until the residues were completely dissolved. The

volume injected was 1 ml/kg of body weight. Control animals received the same volume of distilled water.

### 2.6. Induction of arthritis

Following anesthesia (140 mg/kg of ketamina chloride i.p.), 0.1 ml of Freund's adjuvant (complete fraction of *Mycobacterium butyricum* suspended in mineral oil; Sigma Chemical Co., USA) was injected in the sub-plantar tissue of the right posterior paw. One hundred percent of the animals developed arthritis. Everyday animals were carefully and thoroughly inspected, by examining the affected paw and the animal's general status. Evaluation of the anti-inflammatory effects of *Harpagophytum procumbens* was performed by monitoring the edema in the right paw. In control animals, sub-plantar injection of Freund's adjuvant produced a pronounced local edema after a few hours with a progressive increase reaching its maximum in the eighth day after inoculation (Experiment 1) or reaching an average of 12 mm in the 40th day after induction of the arthritis (Experiment 2).

### 2.7. Acute treatment with Harpagophytum procumbens

On Day 0, all animals were subjected to behavioral tests, assessment of body weight and measurements of the right paw (Test 1). Subsequently, Freund's adjuvant was injected in the right paw. Five days after administration of Freund's adjuvant, animals were again subjected to the tests (Test 2) and were randomly assigned to one of four groups: control (CTRL), which received distilled water (1 ml/kg); experimental Group 1, 25 mg/kg (H-25); Group 2, 50 mg/kg (H-50); and Group 3, 100 mg/kg (H-100) of *Harpagophytum procumbens* preparation, followed by the tests. On Days 6, 7, and 8 administration of the test solution were repeated (Tests 3, 4, and 5, respectively).

### 2.8. Chronic treatment with Harpagophytum procumbens

On Day 0, all animals were subjected to behavioral test and assessment of body weight and right paw's measurements, followed by the injection of Freund's adjuvant in the right paw (Test 1). On the 10th day after adjuvant administration, all tests were repeated (Test 2). On the 20th day, animals were randomly distributed into two groups: control, treated with distilled water (1 ml/kg of body weight), and experimental (100 mg/kg of *Harpagophytum procumbens*) and again subjected to the tests the next day (Test 3). The test solution was administered daily and testing application was done on Days 24, 29, 34, and 40 after injection of Freund's adjuvant (Tests 4, 5, 6 and 7, respectively).

### 2.9. Anti-nociceptive activity—Hot-plate test

The apparatus consisted of a hot plate on which the rat was placed for testing (Ugo Basile Biological Research Apparatus Company, Comerio, Italy). The apparatus consists of a 20-cm diameter metal hot-plate surface set at 50 °C, a Plexiglas cage that fits the hot metal surface, and a timer operated by a foot-switch. Pain threshold was determined by the latency for nociceptive response (licking of any paw) with a maximum cut-off time of 35 s.

### 2.10. Measurement of the right paw

The width and height of the paw and width of the joints were measured with a caliper ruler before and on subsequent testing days, after induction of arthritis.

### 2.11. Statistical analysis

Non-parametric tests (Kruskal–Wallis) followed by the Mann–Whitney test were used in Experiment 1 or a two-way ANOVA, followed by the Tukey's multiple range test in Experiment 2. The significance level was set at 0.05.

## 3. Results

### 3.1. Antinociceptive effects

#### 3.1.1. Acute treatment

The results illustrated in Fig. 2A and B show that *Harpagophytum procumbens* extract increased the animals 'latency of paws' withdrawal, indicating a protective effect against the pain induced by the thermal stimulus, both in acute (Fig. 2A) and chronic treatments (Fig. 2B). For Fig. 2A, statistical analysis of the data showed that the groups did not differ among each other on Tests 1 (basal) and 2 (5th day after induction of arthritis). In Test 3, all groups treated with H (*Harpagophytum procumbens*; doses of H-25, H-50, and H-100 mg/kg, respectively) presented augmented paw(s) withdrawal latency compared to control group ($P < 0.001$). When the hot-plate test was again carried out (Test 4), the results showed a significant difference between H-25 and H-50 treated groups and control group ($P < 0.01$), and no further differences with a dose of H-100. On the 8th day after induction of arthritis (Test 5), administration of all doses of *Harpagophytum procumbens* produced significant differences of the pain threshold to a hot stimulus when compared to the control group ($P < 0.001$).

#### 3.1.2. Chronic treatment

ANOVA detected a main effect of group (control and experimental; $F_{(1,18)} = 30.26$; $P < 0.001$) and of time-points (behavioral tests; $F_{(6,108)} = 6.53$; $P < 0.001$), in addition to an interaction between these factors ($F_{(6,108)} = 17.14$; $P < 0.001$). Follow-up analysis revealed that groups did not differ from each other on Tests 1 and 2; however from Test 5 on (29 days after injection of Freund's adjuvant), there was a difference among the groups. Establishing Test 2 as the baseline measure for comparison resulted in no difference throughout the testing, whereas group H-100 presented an

Fig. 2. Influence of different doses of *Harpagophytum procumbens* on the anti-nociceptive response. (A) *Acute*: control group ($n = 9$) and experimental groups H-25 ($n = 10$), H-50 ($n = 9$), and H-100 ($n = 10$). Test 1: basal measurement; Test 2: 5 days after administration of Freund's adjuvant; Test 3: 1 day after onset of treatment and 6th day of arthritis; Test 4: 7th day of arthritis; Test 5: 8th day of arthritis. (B) *Chronic*: control ($n = 10$) and H-100 ($n = 10$). Test 1: basal measurement; Test 2: 10th day after administration of Freund's adjuvant; Test 3: 1 day after onset of treatment and 21st day of arthritis; Test 4: 24th day after induction of arthritis; Test 5: 29th day of arthritis; Test 6: 34th day of arthritis; Test 7: 40th day of arthritis. Data are expressed as mean ± S.E.M. $^*P < 0.05$ compared with control group. An arrow (↑) indicates beginning of the effects of Freund's adjuvant administration.

increase in the threshold of withdrawal of the paw(s) from Test 4 on, indicating the analgesic effect of *Harpagophytum procumbens* test solution.

### 3.2. Anti-inflammatory effect

#### 3.2.1. Acute treatment

Treatment with *Harpagophytum procumbens* significantly reduced the right paw edema in all animals of the experimental group in a dose-dependent manner, indicating its anti-inflammatory effect. The results showed that all doses of *Harpagophytum procumbens* significantly reduced the edema from Test 3 on, compared with saline-treated control group (Fig. 3A–C). Specifically, paw width (Measurement A) did not differ among the doses. However, on Tests 4 and 5, the dose H-100 was significantly different from the other doses. The paw height (Measurement B) showed a decrease with treatment. Doses H-50 and H-100 were more effective than dose H-25 on Test 3 ($P < 0.001$ in both comparisons). On Test 4, only dose H-100 was significantly different from H-25 ($P < 0.006$) and on Test 5, group H-100 was different from both H-25 ($P < 0.001$) and H-50 ($P < 0.01$). Regarding the measurement of the joint (Measurement C), all doses of *Harpagophytum procumbens* decreased the joint size compared to control group ($P < 0.001$). Comparison among the doses with the Mann–Whitney *U* Test revealed that doses H-50 and H-100 did not differ from each other, and dose H-100 differed statistically from dose H-25 on Tests 3, 4, and 5 ($P < 0.001$, 0.001, and 0.04, respectively).

Fig. 3. Influence of acute treatment with *Harpagophytum procumbens* on Freund's adjuvant-induced edema in rats. Panels A–C represent the different measurements of, respectively, paw width, paw height, and joint width (in mm) of control, H-25, H-50, and H-100 experimental groups throughout a 5-test period. Values are expressed as mean ± S.E.M. (∗) Different from control group; (#) different from H-25 group; (§) different from H-50 group. An arrow (↑) indicates beginning of induced arthritis.

*3.2.2. Chronic treatment*

ANOVA showed a main effect of group (control and experimental; $F_{(1,18)} = 198.2$; $P < 0.001$) and of time-points (behavioral tests; $F_{(6,108)} = 109.2$; $P < 0.001$) and an interaction between these factors (group × tests; $F_{(6,108)} = 42.4$; $P < 0.001$) on paw width (Measurement A). Follow-up analysis of this interaction showed that starting from Test 4 on, control and H-100 groups differed from each other. Compared to Test 2, control group presented an increase in paw measurement starting with Test 3 to the end of the study. Test 3 shows that, although administration of *Harpagophytum procumbens* had begun 24 h before, there was still an increase in Measurement A, suggesting that on Day 21, Freund's adjuvant still produced edema. Analysis of right paw's height (Measurement B) revealed a difference between the three groups starting from Test 3 on. Compared to the 10th day of inoculation of Freund's adjuvant, control group showed an augmented edema on Tests 5, 6, and 7. On the other hand, group H-100 presented a decrease in Measurement B starting from Test 3 on. In the same manner, ANOVA revealed that, as regard Measurement C, control and H-100 groups showed a difference from each other starting from Test 3 on. Group H-100 presented a reduction of the paw's edema on Tests 4–7 (Fig. 4C).

Fig. 4. Influence of chronic treatment with *Harpagophytum procumbens* on Freund's adjuvant-induced edema in rats. Panels A–C represent the different measurements of, respectively, paw width, paw height, and joint width (in mm) of control group, and H-25, H-50, and H-100 experimental groups throughout a 5-test period. Values are expressed as mean ± S.E.M. (∗) Different from control group. An arrow (↑) indicates onset of induced arthritis.

## 4. Discussion

The results of the present study indicate that the dry extract of *Harpagophytum procumbens* exhibits anti-inflammatory and antinociceptive effects in rats with Freund's adjuvant-induced arthritis, either on its acute as well as its chronic phase.

The model of adjuvant-induced arthritis in rats has been extensively used in the study of inflammatory processes (Jones and Ward, 1966) and validated as a model of chronic pain (Colpaert et al., 1982). This fact is corroborated by evidence of spontaneous pain behaviors in arthritic rats, such as reduced locomotor activity and increased itching and scratching behaviors in the affected paw (Calvino et al., 1987) and an attempt at protection of the affected paw, as evidenced by curving and/or elevation, as well as avoidance to support its own weight (Clatworthy et al., 1995). In addition, altered sleep pattern has been observed in these animals (Landis et al., 1989; Andersen and Tufik, 2000). Fig. 2 illustrates the significant difference for acute and chronic treatments, demonstrating that the method used for induction of arthritis by administering Freund's adjuvant was effective, reducing the pain threshold in the injected animals, thus, revealing the applicability of induced arthritis as an experimental model of chronic pain.

Several studies using arthritic rats as a model of chronic pain evaluates hyperalgesia in different ways. In 1988, Hargreaves et al. stated that the method of nociceptive thermal stimulus, such as the hot plate used in the present study, provides a quantitative measurement of hyperalgesia-related behaviors. Previous studies show that reduction of the latency for the animal's reaction corresponds to augmented sensitivity to pain (Fig. 2).

In the medical literature, several reports are founded on substances which have already been tested in the induced arthritis in rats, either for its anti-inflammatory or for its analgesic effects. In the present study, we evaluated *Harpagophytum procumbens* extract, despite the numerous reports on its anti-inflammatory and/or analgesic properties, because these reports are controversial. Therefore, this study was performed in order to provide further evidence on the effectiveness of *Harpagophytum procumbens* or "devil's claw,"

as well as to provide further demonstration on its acute and chronic effects.

The acute treatment showed that all three doses of *Harpagophytum procumbens* (25, 50, and 100 mg/kg) increased the paw withdrawal latency, indicating a protective effect against the pain induced by a thermal stimulus on Tests 3 and 5, i.e., 1 and 3 days after the onset of treatment, respectively. Although the dose of 100 mg/kg of *Harpagophytum procumbens* did not differ from the control group on Test 4, two animals showed the maximum latency (35 s) on Tests 4 and 5, indicating that this dose induced a complete analgesia in the animals. We believe that daily evaluation of pain threshold may not be the most adequate procedure to use, because such method could mask the values observed through the repetition of the hot-plate test, due probably possibly to tissue lesion. Therefore, the present result suggests that the experimental design, including independent groups, or dependent groups with longer inter-test intervals, would be more adequate.

Regarding the chronic administration of *Harpagophytum procumbens*, it proved that this plant is efficient in producing an analgesic effect. It should be emphasized that day, following Freund's adjuvant administration.

The anti-inflammatory effect was obtained with all doses of *Harpagophytum procumbens*. From the beginning of the treatment, the doses used were capable to abolish the progressive increase of the paw's dimensions, observed in the control group, either during the acute (6 days after induction of arthritis) or the chronic study (21 days after adjuvant administration). Previous studies demonstrate that *Harpagophytum procumbens* appears to be efficient in sub-acute inflammatory processes, not presenting any or only minimum effects in acute processes (McLeod et al., 1979; Whitehouse et al., 1983).

## 5. Conclusions

The results presently discussed demonstrate the peripheral anti-inflammatory and analgesic effects of *Harpagophytum procumbens* extract in the Freund's adjuvant-induced arthritis in rats, both in its acute, as well as in its chronic phases.

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# Ethnopharmacognostic survey on the natural ingredients used in folk cosmetics, cosmeceuticals and remedies for healing skin diseases in the inland Marches, Central-Eastern Italy☆

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**Abstract**

An ethnopharmaceutical study focused on domestic cosmetics, cosmeceuticals, and remedies to heal skin diseases traditionally used in the inland part of the Marches region (Central-Eastern Italy) has been conducted. At present, traditional knowledge concerning home-made phytocosmetics is represented by both the remnants of an orally transmitted folk heritage and also by new forms of knowledge, sometimes coming from popular phytotherapeutical books and the mass media (out of the scope of this survey), but also as a result of recent migration trends from Eastern Europe.

We recorded approximately 135 cosmetic or cosmeceutical preparations prepared from more than 70 botanical species and a very few animal or mineral ingredients. Among the recorded preparations, developing a clear distinction amongst cosmetics, cosmeceuticals and pharmaceuticals for skin diseases is very problematic, confirming that in folk knowledge systems medicinal products for healing skin diseases and cosmetics have often been perceived as two poles of a continuum.

Many of the quoted species represented well-known medicinal plants of the European phytotherapy, although we also recorded a few unusual plant taxa, which are briefly discussed under the perspective of their eventual phytochemical and/or phytopharmacological potentialities. Exotic drugs or precious essences, even native of the Mediterranean, were not quoted as ingredients for preparing perfumes and fragrances by the interviewees of the present study, thus indicating that popular cosmetic practices in rural Central Italy have taken a much separated path away from the cosmetic "know-how" of the aristocracy and high bourgeois classes of the last centuries.
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*Keywords:* Ethnobotany; Cosmetics; Cosmeceuticals; Ethnopharmacology; Folklore; Migrations

## 1. Introduction

### 1.1. Cosmetics

"Cosmetic products" have been defined by the European Directive 93/35/EEC (European Commission, 1993) as "any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good conditions". Over time, people have developed and used cosmetic products as fragrances and perfumes (Brunello, 1989; Manniche and Forman, 1999; Morris, 1999; Aftel, 2002). Differently from the widely spread idea that diffusion of cosmetics is a by-product of acculturated rich Western so-

☆ This article is dedicated to the memory of Jo Castle, pharmacy historian and passionate researcher of the history of cosmetics, unforgettable colleague and friend, who died January 9th 2004.

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cieties, or have been only used by aristocratic and upper middle classes in the recent centuries in Europe, they also belong to the heritage of indigenous cultures (Vigueras and Portillo, 2001) and popular classes as well, as, for example, in southern Europe (Tammaro and Xepapadakis, 1986; Guarrera, 1994), although field investigations on these products have been much neglected in recent ethnobiological and ethnopharmaceutical studies.

### 1.2. Cosmeceuticals

If "drugs" have been defined as compounds used in the treatment and prevention of disease, or are intended to affect a physiologic function or structure of the body, and "cosmetics" have been labelled as substances that cleanse, or enhance the appearance of the skin without therapeutic benefits, there is also a "grey" area bordering these two fields, for which the term *cosmeceuticals* has been defined. Cosmeceuticals represent hybrids between drugs and cosmetic products and are intended to enhance both the health and beauty of the skin by external application (Elsner and Maibach, 2000; Millikan, 2001). As in the case of *food-medicines*, where food plants are consumed because they are thought to have more or less specific beneficial effects on health (Etkin, 1996; Pieroni, 2000, 2001; Pieroni et al., 2002), *cosmeceuticals* cover a border field between pharmaceuticals for skin diseases and cosmetics (Fig. 1).

### 1.3. Traditional knowledge (TK) on cosmetics and cosmeceuticals

The traditional use of plants against skin diseases and especially for "cosmeceutical" purposes is a common practice in the domestic medicine of many cultures. However, medical anthropological aspects of the aetiology of some skin affections are very complex and are not always completely understood (Grabner, 1963; Bartoli et al., 1997; Quave and Pieroni, 2001). In contrast to *food-medicines*, *cosmeceuticals* are much more difficult to be defined due to the fact that the concept of "improving the aesthetic value of the body" can change considerably by subjects even inside the same cultural framework. On the other hand, cosmetics as perfumes and deodorants have always been used by women in the rural peasant societies in southern Europe. Specific interdisciplinary studies on traditional knowledge (TK) related with cosmetic products have been never carried out so far.

### 1.4. The Marches

In this field study, we recorded the traditional use of home-made cosmetic and dermatological products in the inland territory of the Marches, in Central Italy. The Marches is the region of Central Italy located between the Adriatic side and the Umbria-Marches Apennins, it is mainly mountainous (highest mountain is the Mount Vettore, 2476 m, part of the Sibillini Mountains on the Umbrian border), but from the ridge of the Apennines it slopes gradually towards the Adriatic coast, which for long stretches is flat and straight, a narrow ribbon of sand lying against the fringes of the hills beyond. The natural vegetation has been greatly modified by man, originally to obtain arable land, later for tourist resorts. The woods that once spread over most of the area now cover only 16% of the region.

A demographic analysis of the regional population does not indicate a great degree of urbanisation. Ancona, the administrative centre, is the only town with over 100,000 inhabitants; and even the populations in the four provinces are fairly balanced. Intra-regional migration has thus been towards the many craft businesses on the hills and the factories and tourist attractions along the coast. Apart from those in the provinces, the main urban settlements are Fano, Iesi, Fermo, Civitanova Marche, San Benedetto del Tronto and Senigallia, all with over 30,000 inhabitants.

The Marches dialects can be divided into four main groups: the Emilia-Romagna dialect prevails to the north (province of Pesaro and Urbino, and part of the province of Ancona); in the centre (Ancona, Loreto, Iesi, Fabriano) the dialect is a mixture of northern and Umbrian–Tuscan fea-

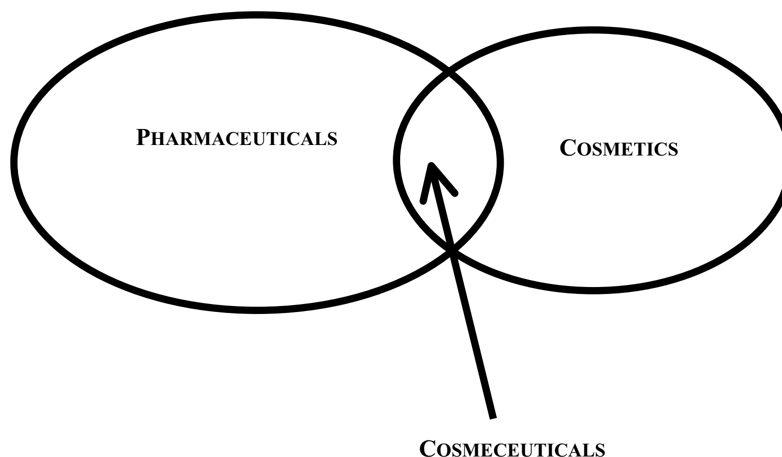


Fig. 1. Cosmeceuticals.

tures; a little farther south (province of Macerata, Fermo) the Umbrian–Latian type prevails; in the far south (province of Ascoli) the inflection acquires southern characteristics, especially those of Abruzzo.

Environmental conditions appear to be fairly satisfactory, given the absence of large industrial and urban concentrations, generally the source of high levels of pollution. Though farming methods are not particularly efficient, agriculture still employs one-sixth of the working population. There are two main reasons for this: farmers are strongly attached to their land and small craft businesses (nearly 50,000) which often provide part-time jobs or work at home, so that farming is a spare time activity. Livestock resources are nowadays rather limited.

Industrial development has spread mainly along the coast and mussels are traditionally harvested through a semi-craft system, although in the last few decades, large concerns have evolved regarding development, particularly near ports. Industry plays an increasing role in the local economy—and especially in the inland areas of the region—by middle and small industries, producing footwear, textiles, furniture, and paper (Fabriano). The development of these new sources of profit has affected the traditional socio-economic structure and a vast portion of traditional agro-pastoral activity has disappeared, sometimes substituted by newly conceived organic farming and integrated agro-tourist systems.

### *1.5. Studies on traditional ethnobotanical knowledge in the Marches*

Very few ethnobotanical field studies have been carried out in the Marches in the last 50 years (Guarrera, 1981; Bellomaria, 1982; Bellomaria and Della Mora, 1982; Bellomaria and Lattanzi, 1982; Guarrera, 1982, 1990; Leporatti et al., 1985) and they have mainly investigated medicinal plants. The present work addresses anthropological issues regarding the remembrances of remedies and means of healing skin disease in the last 20th Century, whose practices today for the most part have been abandoned. In addition, it offers a look at the new dynamics and changes of TK in rural societies: in the inland Marches, as in many other rural areas in Western Europe, migrations and cultural *metissage* phenomena are greatly changing and transforming domestic *know-hows* and supposed "traditions". In this case, recent migration flows from Albania, Kossova, Macedonia, Romania, Poland and Ukraine (Caritas, 2002) have become particularly relevant.

## 2. Methods

Field work was conducted during the period, March–October 2002 and March–June 2003 in a few municipalities, mainly located in the inland part of the Marches (Central Italy, Fig. 2): Pergola, San Lorenzo in Campo, Serra Sant'Abbondio (Pesaro and Urbino Province), Arcevia, Cerreto d'Esi, Genga, Fabriano, Montecarotto, Sassoferrato, Senigallia (Ancona Province), Bolognola, Camerino, Corridonia, Civitanova Marche, San Severino Marche, Tolentino (Macerata Province), Montegiorgio, S. Elpidio a Mare (Ascoli Piceno Province) as well in the bordering Osimo (Ancona Province) and Gualdo Tadino (Perugia Province, Umbria region) territories.

Information concerning cosmeceuticals was collected using semi-structured interviews with 97 consenting participants (61 women, 36 men) whose age ranged from 28 to 94 years, and who still retain TK. A few nursing homes for the elderly were visited as well.

Interviewees were asked to quote all the home-made preparations which are or have been used for cosmetic purposes (for the hygiene of the face, the hair, the skin, the oral tract, including gargles and other antiseptic means for treating inflammations of the mouth), for the maintenance

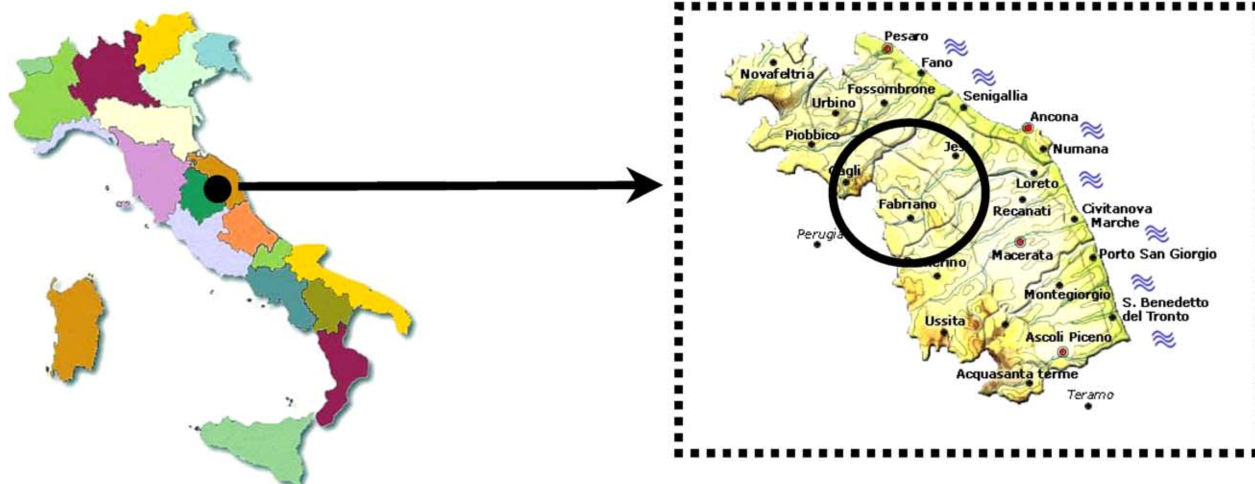


Fig. 2. The studied area.

of skin health or to increase its beauty (proper cosmetic field) or for healing skin diseases.

Species and uses that were cited with very precise quantitative details, and for which the knowledge probably came from popular media are not reported here. This kind of problem, due to a "contamination" of a supposed original knowledge with modern phytotherapeutical news, spread by the media (television and newspapers), is very well-known among ethnobiologists in the methodological approaches in rural Southern Europe (Cappelletti et al., 1983).

Only uses described by at least two diverse informants were considered. Moreover, data regarding the personal history of the informants, as well as migrant status, was recorded. Each non-cultivated botanical species recognised by the interviewees to be used for cosmetic or related aims was collected and identification was carried out by the first author; nomenclature follows the standard botanical work for the Italian flora (Pignatti, 2002). Voucher specimens were gathered and are deposited at the first author's address.

## 3. Results

Natural cosmeceuticals in the rural Marches are nowadays more frequently bought from herbal shops ("*erboristerie*") or from pharmacies. Nevertheless, in a few cases they are still prepared at home, especially for minor illnesses (gargles against sore throats, topical preparations for burns or skin inflammations), while the remembrance of many domestic practices is still popular amongst the elderly population.

The knowledge of domestic phytocosmetics is represented nowadays by both remnants of a folk heritage orally transmitted and also by new forms of knowledge, often originating in modern phytotherapeutical popular books and/or mass media (which we did not included in this study), and also by recent migration phenomena from Eastern Europe.

In Table 1, the phytocosmetics, phytocosmeceuticals and plant remedies traditionally used for healing skin diseases in the studied area are reported. Plant species representing the major ingredients of these compositions (and often the unique "active principle") are organized in the table in alphabetic order, while excipients (such as olive oil, bees wax or pig fat) are discussed only in the description of the preparations, with the exception of the cases in which they also represent the possible "active ingredients" of the formulation.

Plant-derived home-made cosmetics, cosmeceuticals and remedies for skin diseases include approximately 135 preparations coming from roughly 70 botanical species. A few animal or mineral derived remedies are listed in Table 2.

Distribution of TK about cosmetics, cosmeceuticals and remedies to treat skin diseases is widely spread among the studied population, although the variability of this knowledge is very high, suggesting that many remedies represent a sort of "familiar" tradition, where the domestic *know-how* of the women of the household was not regularly exchanged with those of other households inside the same community. Women played a primary role in the preparation and administration of many remedies. Their home-made remedies were prepared for the whole family in the form of simple poultices or compresses, often using bees wax, pig lard, butter or olive oil as excipients. Women were also the only producers and "consumers" of home-made cosmetics, including simple fragrances and perfumes.

Among the plant-derived preparations, it is very difficult to clearly distinguish between plants used as cosmetics, cosmeceuticals and pharmaceuticals for skin diseases, confirming that amongst popular cultures, these categories are indeed quite artificial. In other words, in traditional folk medical systems, medicinal products for healing skin diseases and cosmetics seem to have been often perceived as two poles of a continuum.

### *3.1. Folk cosmetics and aesthetic values among rural classes*

Cosmetic preparations used for toning or colouring the skin, lightening or colouring the hair, inhibiting hair loss, or to perfume the skin have been recorded in our field study. They have been mainly used in the past and primarily by women. Most of the plant ingredients (oat, walnuts, camomile, pot marigold, carrot, almonds, cucumber, lavender, bay leaves, mint, rose and sweet violet petals) are used also today in the modern phytocosmetics (Roth and Kormann, 1996; D'Amelio, 1999), while a few ingredients (mainly represented by aromatic species) are less known nowadays for cosmetic purposes (basil, yarrow, corn flowers, lemon verbena, marshmallow, silver birch, hazel, apple fruits, corn poppy petals, oregano, thyme). A similar picture in the tradition of preparing popular "perfumed waters" has been recorded in Latium (Central-Western Italy, Guarrera, 1994 and in Central Spain, Verde López, 2002).

Under an anthropological point of view, it is interesting to note how until the recent past, women of rural classes tended to find diverse ingredients for the whitening of their skin, because a dark colour of the face and hand skin was considered synonymous with poverty and self-stigmatised: so the use of boiled chestnuts and potatoes, elderberry flowers and rice infusions indicate changes in the cultural concepts of aesthetics, which has taken place in the last decades. Nowadays, in fact, to have darkened skin, tanned by sunshine or cosmetic products, is normally considered a sign of wellness in Western societies.

On the other hand, art and aesthetics are definable only inside given cultural categories, and they are greatly affected by political, economic, and socio-cultural dimensions. The same definitions of art, representation, expression, beauty, quality, style, signs and meaning (semiotics) are historically and culturally dependent: for example, the fresh sensation of perfumes prepared with a prevalence of flower essences (as in *Eau de Cologne*, *Eau de Cananga*, *Eau de Floride*, *Eau de Lubin*) was very much appreciated by the bourgeois

Table 1
Plant-derived folk cosmetics, cosmeceuticals and remedies to heal skin diseases in the inland Marches

| Botanical taxon (voucher specimens code) | Botanical family | Vernacular name in the Marches | English name | Quotation frequency (referred to the species) | Status | Part(s) used | Preparation | Popular use |
|---|---|---|---|---|---|---|---|---|
| *Achillea millefolium* L. (FABACHI) | Asteraceae | *Erba del soldato; erba pennina* | Yarrow | ♣♣ | W | Flowering tops | Macerate in white wine (ca. 10 days), in external application | To heal Chapped skin; cicatrising |
| | | | | | | | Decoction, in external washes | Haemostatic; cicatrising |
| | | | | | | Flowers | Infusion, in external application | To “clean” the skin |
| *Aesculus hippocastanum* L. (FABAES) | Hippocastanaceae | *Castagna bastarda* | Horse chestnut | ♣♣ | W | Bark and leaves | Ointment made by mixing the decoction with olive oil or pig fat | Anti-haemorrhoids |
| *Allium cepa* L. | Liliaceae | *Cipolla* | Onion | ♣♣♣ | C | Bulb | Boiled and eaten as a soup | To heal throat and vocal chord inflammations |
| | | | | | | | Slices are rubbed on the skin | To heal insect bites; anti-burns**; against black-heads |
| | | | | | | | Compress made by grinding the bulb with salt | To heal finger bruises |
| | | | | | | | Compress made by adding ground onions to roasted soap and cheese | To heal furuncles |
| *Allium sativum* L. | Liliaceae | *Aglio* | Garlic | ♣♣ | C | Bulb | Cold macerate prepared from the crushed bulb, sometimes adding also bread and olive oil | Antiseptic; to heal dry and flaky skin of feet |
| | | | | | | | Nails scrape into the bulb | To strengthen the nails |
| | | | | | | | Cut, and externally applied | To heal rheumatisms and corns |
| | | | | | | | Rubbed on the skin | To relieve insect bites |
| | | | | | | | Macerate in olive oil | Anti-rheumatic; anti-otitis |
| | | | | | | Bulb membrane | Applied in the mouth | To heal mouth ulcers |
| *Althaea officinalis* L. (FABALT) | Malvaceae | *Altea* | Marsh mallow | ♣ | W | Root | Decoction, in external washes | To prevent hair loss |
| *Avena sativa* L. | Graminae | *Biada* | Oats | ♣♣♣ | C | Seeds (oat meal) | Compress made by mixing one spoon of oat meal with one egg yolk and a spoon of honey | To treat “tired” skin |
| *Balsamita major* (L.) Desf. (FABBAL) (syn.: *Tanacetum balsamita* L.) | Asteraceae | *Erba della Madonna “lilla”* | Alecost; costmary | ♣♣♣ | C | Leaves | Dried, added in the bath (sometimes also adding yeast); as ingredient of the “St. John’s water” (cold macerate in water of a few species, prepared at St. John’s night, on 24th June) | To strengthen the skin of babies; skin toner and perfuming (ritual) |
| *Beta vulgaris* Alef | Chenopodiaceae | *Bieta* | Beet | ♣ | C | Leaves | Compress | Anti-haemorrhoids |
| *Betula pendula* Roth (FABBET) | Betulaceae | *Bidollo betulla* | Silver birch | ♣ | W | Bark | Decoction, in external washes | To prevent hair loss |
| | | | | | | Buds and leaves | Decoction, in external application | cicatrising |
| *Borago officinalis* L. (FABBOR) | Boraginaceae | *Borragine* | Borage | ♣ | W | Leaves | Compress of raw or boiled leaves | To heal eczema and acne; anti-burns |
| *Brassica oleracea* L. | Brassicaceae | *Cavolo* | Cabbage | ♣♣ | C | Leaves | Topical applications | Cicatrising; to heal mastitis; anti-rheumatism |
| *Bryonia dioica* Jacq. (FABBRY) | Cucurbitaceae | *Zucca selvatica* | Bryony | ♣ | W | Fruits | Compress of crushed fruits | To heal bone pains; against toothache |
| *Calendula officinalis* L. (FABCAL) | Asteraceae | *Fiorarancio* | Pot marigold | ♣♣♣ | C | Flowers | Infusion, in external washes | Skin toner; to heal skin tears; anti-burns |
| | | | | | | | Ointment made with olive oil by infusion in the sunshine (ca. 5 days long) | To treat reddened skin |
| | | | | | | Flower juice | Topical application | To enhance hair growth* |
| *Capsicum annum* L. | Solanaceae | *Peperoncino* | Chilli | ♣ | C | Fruits | Macerate in olive oil | Anti-rheumatic; anti-otitis |
| *Carica papaya* L. | Caricaceae | *Papaya* | Papaya | ♣ | C | Fruits flesh | Topical application | To lighten the skin; emollient*** |
| *Castanea sativa* L. | Fagaceae | *Castagna* | Chestnut | ♣♣♣ | SC | Fruits | Decoction, in external washes | To enhance the colour of light hair and give a brown gloss |
| | | | | | | | Compress made from the boiled fruit pulp | Emollient; to whiten facial skin |
| | | | | | | Bark | Decoction, in external washes | To treat reddened and inflamed skin |
| *Centaurea cyanus* L. (FABCEN) | Asteraceae | *Fiordaliso* | Cornflower | ♣♣♣ | W | Flowers | Infusion, in external application | To treat reddened eyes |
| | | | | | | | Infusion, in local application | To heal eye inflammations; to give a special gloss and blue nuance to grey and white hair (avoiding the yellowing of hair) |
| *Ceratonia siliqua* L. | Fabaceae | *Teghe marine*[pl] | Carob | ♣ | C | Seeds | Decoction, also made with almond epicarp and corn poppy stems and fruits | Against sore throat |
| *Chelidonium major* L. | Papaveraceae | *Celidonia* | Greater celandine | ♣♣ | W | Sap | Topical application | Against warts and calluses |
| *Cirsium arvense* (L.) Scop. (FABCIR) | Asteraceae | *Strummelli*[pl] *stoppoloni*[pl] | Creeping thistle | ♣♣ | W | Leaves | Compress of leaves juice or topic applications of chewed leaves | Antiseptic; cicatrising |
| *Citrus aurantium* L. | Rutaceae | *Arancio amaro* | Bitter orange | ♣ | C | Epicarp | Decoction | To heal cold# |

Table 1 (*Continued*)

| Botanical taxon (voucher specimens code) | Botanical family | Vernacular name in the Marches | English name | Quotation frequency (referred to the species) | Status | Part(s) used | Preparation | Popular use |
|---|---|---|---|---|---|---|---|---|
| *Citrus limon* (L.) Burm. f. | Rutaceae | *Limone* | Lemon | ♣♣ | C | Fruit juice | Poultice made with a boiled potato | To whiten the skin of the hands |
| | | | | | | | Compress made by mixing the juice with scrambled egg albumen | To soften facial skin |
| | | | | | | | Mixed with oil, in external application | To give a special gloss to the hair |
| | | | | | | | Gargles | To heal sore throat |
| | | | | | | | Instilled in the eye | To given a special effect to the glance |
| | | | | | | Leaves | Compresses | To heal furuncles |
| *Citrus sinensis* (L.) Osneck | Rutaceae | *Arancio* | Orange | ♣ | C | Leaves | Compresses | to heal furuncles |
| *Clematis vitalba* L. (FABCLE) | Ranuncolaceae | *Vitalba* | Traveller's joy | ♣ | W | Stems | Decoction of the stems, after having eliminated the bark, in external washes | Anti-warts |
| *Cocos nucifera* L. | Palmae | *Cocco* | Coconut | ♣ | C | Flesh | Ground, applied externally on the hair | To give a special gloss and maintain soft hair*** |
| *Corylus avellana* L. (FABCOR) | Betulaceae | *Ciaccarelle*[pl] | Hazel | ♣ | W | Leaves | Decoction, in external washes | To make the skin "younger" and to give it colour |
| *Crataegus monogyna* Jacq. (FABCRA) | Rosaceae | *Biancospino; perelle rosse*[pl] | Hawthorn | ♣♣ | W | Fruits | Decoction, drunk | Against sore throat |
| | | | | | | Flowers | Compress of boiled crushed flowers | Skin emollient |
| *Cucumis sativus* L. | Cucurbitaceae | *Milangula* | Cucumber | ♣♣ | C | Seeds | Compress of crushed seeds | against wrinkles |
| *Cydonia oblonga* Mill. | Rosaceae | *Mela cotogna* | Quince | ♣ | C | Fruits | Decoction | Emollient for the skin |
| *Daucus carota* L. | Apiaceae | *Carota* | Carrot | ♣♣♣ | C | Root | Compress prepared with crushed boiled carrots, a spoonful of honey and the water remaining after boiling rice | Skin toner; against Burns |
| | | | | | | | Ground, in external application | Against burns |
| | | | | | | | Decoction, topical application | Against burns; cicatrising* |
| *Dianthus caryophyllus* L. | Caryophyllaceae | *Garofano* | Carnation | ♣ | C | Petals | As an ingredient of the "St. John's water" (cold macerate in water of a few species, prepared at St. John's night, on 24th June) | Skin toner and perfuming (ritual) |
| *Equisetum arvense* L. (FABEQUI) | Equisetaceae | *Coda di cavallo* | Field horsetail | ♣♣ | W | Aerial parts | Decoction, instilled in nose | Haemostatic |
| | | | | | | | Compresses | To strengthen the hair |
| | | | | | | | Compress of crushed aerial parts and honey | To reinforce finger and toe nails |
| *Eupatorium cannabinum* L. (FABEUP) | Asteraceae | *Erba rozza* | Hemp agrimony | ♣ | W | Flowering tops | Ointment prepared by grinding flowering tops with pig fat in a mortar | Cicatrising |
| *Euphorbia* sp. | Euphorbiaceae | | | ♣ | W | Latex | Topical application | Anti-warts |
| *Ficus carica* L. (FABFIC) | Moraceae | *Fico* | Fig | ♣♣ | W | Latex | Applied externally | Cicatrising; anti-warts (also#); to heal calluses/corns on the skin; to whiten dark skin spots |
| | | | | | | Leaves | As an ingredient of the "St. John's water" (cold macerate in water of a few species, prepared at St. John's night, on 24th June) | Skin toner and perfuming (ritual) |
| *Fragaria vesca* L. (FABFRA) | Rosaceae | *Fragola* | Strawberry | ♣ | C & W | Leaves | Decoction, gargled | Against sore throat* |
| *Hedera helix* L. (FABHED) | Araliaceae | *Edera; ellera abbracciabosco* | Ivy | ♣♣♣ | W | Leaves | Infusion, in external washes | To give a gloss or colour to white hair |
| | | | | | | | Infusion, in external washes | To treat swollen feet and legs |
| | | | | | | | Decoction, in external washes | to Strengthen the hair |
| | | | | | | | Macerate in olive oil | To heal haematoma |
| *Hypericum perforatum* L. (FABHYP) | Guttiferae | *Scacciadiavoli; erba di San Giovanni; erbe del perico* | St. John's wort | ♣♣♣ | W | Flowers | Macerate in olive oil with German camomile flowers, in external application | To heal eye inflammations; to heal shingles |
| | | | | | | Flowering tops (sometimes also adding in a second phase the fruits) | Macerate in olive oil | To heal burns; to treat skin tears; vulnerary |
| | | | | | | Leaves | As an ingredient of the "St. John's water" (cold macerate in water of a few species, prepared at St. John's night, on 24th June) | Skin toner and perfuming (ritual) |
| | | | | | | Leaves | Compress | To strengthen legs of babies |

| *Juglans regia* L. | Juglandaceae | *Noce* | Walnut | ♣♣♣ | SC | Leaves | Decoction, in external compress | Cicatrising; antiseptic of genital skin parts |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Leaves | As an ingredient of the “St. John’s water” (cold macerate in water of a few species, prepared at St. John’s night, on 24th June) | Skin toner and perfuming (ritual) |
| | | | | | | Unripe fruits | Compress | To colour the hair (brown) |
| *Laurus nobilis* L. | Lauraceae | *Lauro* | Bay tree | ♣♣ | SC | Leaves | Decoction, in external washes | To prevent hair loss |
| | | | | | | | Compress made by grinding the leaves with wheat flour | To treat various skin inflammations |
| | | | | | | Leaves | As an ingredient of the “St. John’s water” (cold macerate in water of a few species, prepared at St. John’s night, on 24th June) | Skin toner and perfuming (ritual) |
| *Lavandula angustifolia* Mill. | Lamiaceae | *Lavanda* | Lavender | ♣♣♣ | C | Flowering tops | Macerate in cold water together with rosemary and thyme flowering tops, then mixed with alcohol | Perfume |
| | | | | | | | Beaten with a stone and applied externally | Against viper bite |
| | | | | | | | Macerate in cold water (sometimes also adding rose petals) | To perfume and tonify the skin |
| *Lavandula latifolia* Medicus | Lamiaceae | *Spigolo* | Spike lavender | ♣ | C | Aerial parts | As an ingredient of the “St. John’s water” (cold macerate in water of a few species, prepared at St. John’s night, on 24th June) | Skin toner and perfuming (ritual) |
| *Lawsonia inermis* L. | Lythraceae | *Hennè* | Henna | ♣ | C | Leaves | Ground and suspended in water, compresses | To dye hair+ |
| *Linaria vulgaris* Mill. (FABLIN) | Schrophulariaceae | *Linaiola* | Yellow toadflax | ♣ | W | Aerial parts | Compress made from a macerate of fresh aerial parts | Anti-haemorrhoids |
| *Linum bienne* Miller (FABLINU) | Linaceae | *Linu sarvaggiu* | Wild flax | ♣ | W | Seeds | Compress made by boiling the seeds in water | Anti-acne; against ear pains |
| *Linum usatatissimum* L. | Linaceae | *Linu* | Flax | ♣ | C | Seeds | Compress made by boiling the seeds in water | To relieve shoulder pains; anti-otitis |
| Lippia triphylla (L’Hér.) O. Kuntze | Verbenaceae | *Limoncella* | Lemon verbena | ♣♣ | C | Leaves and flowers | Rubbed on the skin and the clothes | Perfume |
| | | | | | | Leaves | Poultice prepared with olive oil and petrol for application to the hair | Anti-lice; to give a special brightness to the hair |
| *Lupinus albus* L. | Fabaceae | *Lupino* | White lupin | ♣ | C | Seeds | Compress made by macerating the dried seeds in cold water for one day | Anti-lice |
| *Lycopodium clavatum* L. (FABLYC) | Lycopodiaceae | *Erba strega* | Common club moos | ♣ | | Spores | Ointment made by mixing and heating olive oil with the spores | Against dermatitis |
| | | | | | | | Ground, external topical application | To treat reddened skin in babies |
| *Malus domestica* borkh. | Rosaceae | *Mela* | Apple | ♣ | C | Fruit | Very thin slices applied externally | Facial skin toner |
| *Malva sylvestris* L. (FABMAL) | Malvaceae | *Marva* | Mallow | ♣♣♣ | W | Leaves and flowers | Chewed | Against toothache |
| | | | | | | | Decoction, in gargles or washes | To treat gingival inflammations*; against sore throat and mouth inflammations; to treat diverse skin inflammations |
| | | | | | | | Decoction, in external washes or compresses | To treat diverse skin inflammations (especially on the face skin) |
| | | | | | | | Compress made crushing the leaves with sweet violet flowers and wax in a mortar | To perfume facial skin |
| | | | | | | Aerial parts | Compress obtained by boiling the plant (sometimes also adding bread) | To heal furuncles and abscesses; cicatrising; to heal nail infections against gingival inflammations and toothache |
| *Matricaria recutita* L. (FABMAT) | Asteraceae | *Camomilla* | German camomile | ♣♣♣ | W | Flowering tops | Infusion or decoction, in washes or gargles | To make the hair blond; to heal skin, eyes and mouth inflammations; |
| | | | | | | | Infusion | To heal sore throat and eye inflammations |
| | | | | | | | Macerate in olive oil with St. John’s wort flowers, in external application | To heal eye inflammations; to heal shingles |
| | | | | | | Root | Decoction, in gargles | Against throat inflammations |
| *Melissa officinalis* L. (FABMEL) | Lamiaceae | *Melissa* | Lemon balm | ♣ | W | Leaves | Compress of crushed fresh leaves | To heal insect bite |
| *Mentha spicata* L. | Lamiaceae | *Menta* | Spearmint | ♣♣ | C | Leaves | Infusion, in washes or gargles | Mouth antiseptic; perfume for skin and mouth |
| *Mentha suaveolens* Ehrh. (FABMEN) | Lamiaceae | *Mentone* | Round-leaved mint | ♣ | W | Leaves | As an ingredient of the “St. John’s water” (cold macerate in water of a few species, prepared at St. John’s night, on 24th June) | Skin toner and perfuming (ritual) |
| *Mespilus germanica* L. | Rosaceae | *Nespolo* | Medlar | ♣ | W | Leaves | Decoction, in gargles | Against sore throat and mouth inflammations |
| *Morus alba* L. | Moraceae | *Gelso* | Mulberry | ♣ | SC | Leaves and fruits | Compresses | To heal toothache |
| *Ocimum basilicum* L. | Lamiaceae | *Basilico* | Basil | ♣♣♣ | C | Leaves | Inserted behind the ear | Perfuming |

Table 1 (*Continued*)

| Botanical taxon (voucher specimens code) | Botanical family | Vernacular name in the Marches | English name | Quotation frequency (referred to the species) | Status | Part(s) used | Preparation | Popular use |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Infusion, in gargles | Against sore throat |
| | | | | | | | Compress made from crushed leaves | To heal skin inflammations |
| | | | | | | | Infusion, in baths | Skin toner and perfume |
| | | | | | | Leaves | As an ingredient of the "St. John's water" (cold macerate in water of a few species, prepared at St. John's night, on 24th June) | Skin toner and perfuming (ritual) |
| | | | | | | | Crushed | To relieve the pain caused by insect bites |
| | | | | | | | Decoction, in external application | suppurative |
| *Olea europaea* L. | Oleaceae | *Olivo* | Olive tree | ♣♣ | C | Fruits → oil | Topical application | Anti-lice (children); emollient for the skin; to heal labial herpes |
| | | | | | | | Topical application | Skin toner in case of inflammations caused by averse atmospheric events or in babies |
| | | | | | | | Mixed with water, in external application | Anti-burns; to relieve anal inflammations in babies |
| *Origanum vulgare* L. | Lamiaceae | *Origano* | Oregano | ♣ | C | Aerial parts | Added in baths | Body perfume |
| *Oryza sativa* L. | Graminae | *Riso* | Rice | ♣♣ | C | Seeds | Decoction, in external washes | To "whiten" facial skin |
| | | | | | | | Boiled and mixed with egg yolk, compresses | To relieve bruises |
| *Papaver rhoeas* L. (FABPAP) | Papaveraceae | *Papavero* | Corn poppy | ♣♣♣ | W | Stems and fruits | Decoction, also made with walnut epicarp and carob seeds | Against sore throat |
| | | | | | | Petals | Decoction, in external washes | Against sore throat |
| | | | | | | | Rubbed on the skin | To colour the cheeks |
| *Parietaria officinalis* L. (FABPAR) | Urticaecae | *Erba muraria; erba muraiola; vetriola* | Pellitory of the wall | ♣♣ | W | Leaves | Compress of crushed fresh leaves | To heal arthritis and rheumatic pains; anti-haematomas; against furuncles |
| *Petroselinum crispus* (Mill.) Nyman ex AW Hill. | Apiaceae | *Prezzemolo* | Parsley | ♣ | C | Leaves | Decoction | To give the hair a special gloss |
| *Plantago laceolata* L. (FABPLA) | Plantaginaceae | *Orecchie di lepre rapocciò* | Ribwort plantain | ♣♣ | W | Leaves | Applied externally | Cicatrising; against furuncles; against snake and insect bites |
| *Prunus cerasus* L. | Rosaceae | *Cerase*[pl] | Sour cherry | ♣♣ | SC | Fruits | Poultice made from fruit flesh and lemon juice | To refresh and lighten the colour of the skin |
| *Prunus dulcis* (Miller) D.A. Webb | Rosaceae | *Mandulini*[pl] | Almond | ♣♣♣ | C | Epicarp | Decoction, also made with carob seeds and corn poppy stems and fruits | Against sore throat |
| | | | | | | Endocarp | Compress made by mixing crushed almonds with an egg yolk | To treat "tired skin" |
| | | | | | | | Compress made by mixing crushed almonds with honey | To "clean" the skin |
| *Punica granatum* L. | Punicaceae | *Melagranata* | Pomegranate | ♣ | C | Fruit juice | Applied externally | To "clean" the face; to eliminate black heads |
| *Quercus* sp. | Fagaceae | *Cerqua* | Oak | ♣ | W | Bark (of young branches) | Decoction, in external washes | To treat oily hair |
| *Ranunculus bulbosum* L. (FABRAN1) | Ranuncolaceae | *Ranuncolo* | Bulbous buttercup | ♣ | W | Bulb | Slices rubbed carefully on the skin (not too much time: this could generate burns) | Anti-warts |
| *Ranunculus sceleratus* L. (FABRAN2) | Ranuncolaceae | *Ranuncolo* | Celery-leaved buttercup | ♣ | W | Aerial parts | Compress | Anti-sciatica |
| *Raphanus sativus* L. (FABRAP) | Brassicaceae | *Ravanello* | Radish | ♣ | C | Root juice | Juice, drunk | Against sore throat |
| *Rapistrum rugosum* (L.) All. | Brassicaceae | *Rapetta* | Wild radish | ♣ | W | Leaves | Externally applied | To heal legs furuncles |
| *Rosa canina* L. (FABROS) and *R. damascena* L. | Rosaceae | *Rosa* | Rose | ♣♣♣ | W C | Petals | Macerate in cold water (sometimes also adding lavender flowers or a few drops of vinegar) | Skin toner and perfume |
| | | | | | | | As an ingredient of the "St. John's water" (cold macerate in water of a few species, prepared at St. John's night, on 24th June) | Skin toner and perfuming (ritual) |
| *Rosmarinus officinalis* L. | Lamiaceae | *Rosmarino; rosmarinu* | Rosemary | ♣♣♣ | C | Leaves | Infuse with sage leaves and nettle roots, then make into an ointment with castor oil; macerate in olive oil | To treat oily hair; to strengthen the hair |
| | | | | | | | Decoctions, in bath | Tonic for the skin |
| | | | | | | Flowering tops | Macerate in cold water together with thyme and lavender flowering tops, then mix with alcohol | Perfume |
| | | | | | | | Infusion | To "clean" and "smoothen" facial skin |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Rubus fruticosus* L. (FABRUB) | Rosaceae | *Rovo* | Blackberry | ♣♣♣ | W | Leaves | Compress of crushed fresh leaves | Against furuncles; suppurative; cicatrising; anti-bruises; anti-haemorrhoids |
| | | | | | | | Macerate in olive oil; compress with pig fat (sometimes after letting the fat go bad or rot) | Suppurative |
| | | | | | | | Decoction | Anti-sore thoat |
| *Rumex crispus* L. (FABRUM) | Polygonaceae | *Romice* | Curled dock | ♣ | W | Leaves | Compress obtained by quickly boiling the leaves | Against furuncles; to heal bruises and haematomas |
| *Ruta graveolens* L. | Rutaceae | *Ruta* | Rue | ♣ | C | Leaves | Poultice made by crushing the leaves in a mortar | Anti-acne |
| | | | | | | | Macerate in alcohol, in external applications | To heal muscular pains, and as anti-diaphoretic[##] |
| *Salvia glutinosa* L. (FABSAL) | Lamiaceae | *Erba delle emmorroidi* | Jupiter's distaff | ♣ | W | Roots | Macerate in olive oil | Anti-haemorrhoids |
| *Salvia officinalis* L. | Lamiaceae | *Salvia sarvia* | Sage | ♣♣♣ | C | Leaves | Infusion (sometimes also with rosemary leaves and nettle roots, and then in an ointment with castor oil) | To heal gingival and mouth inflammations; antiseptic |
| | | | | | | | External rubbing on the teeth | To whiten the teeth |
| | | | | | | | Decoction, externally applied | Antiseptic on wounds |
| *Sambucus nigra* L. (FABSAM) | Caprifoliaceae | *Sammucu* | Elderberry | ♣♣♣ | W | Flowers | Decoction | Skin toner and whitener |
| | | | | | | | Compress of boiled crushed flowers | Skin emollient |
| | | | | | | Flowers and leaves | Poultice made by mixing and boiling the plant parts with milk | Anti-haemorrhoids |
| | | | | | | Leaves | Dried, then grounded and inserted in the nose | Haemostatic |
| | | | | | | | Boiled, in compresses | Anti-burns |
| | | | | | | Bark of young branches | Cut in small slices and externally applied | To heal swollen feet and hands |
| | | | | | | | Poultice | Anti-burns |
| *Satureja montana* L. (FABSAT) | Lamiaceae | *Persichina rosa, santoreggia* | Wild savory | ♣♣ | W | Aerial parts (including flowering tops) | As an ingredient of the "St. John's water" (cold macerate in water of a few species, prepared at st. John's night, on 24th june) | Skin toner and perfuming (ritual) |
| *Solanum tuberosum* L. | Solanaceae | *Patata; patielli*[pl] | Potato | ♣♣♣ | C | Tubers | Tuber slices applied externally | To heal eye inflammations, eye sockets and conjunctivitis; anti-burns; cicatrising; anti-bruises |
| | | | | | | | Poultice made from a boiled potato and lemon juice | To whiten the skin of the hands |
| *Stachys* sp. (FABSTA) | Scrophulariaceae | *Erba della Madonna* | | ♣ | W | Aerial parts with flowers | Compresses | Anti-headache |
| *Spartium junceum* L. (FABSPA) | Fabaceae | *Ginestra* | Spanish broom | ♣♣♣ | W | Flowers | Crushed, in external application | Anti-lice |
| | | | | | | Leaves | As an ingredient of the "St. John's water" (cold macerate in water of a few species, prepared at St. John's night, on 24th June) | Skin toner and perfuming (ritual) |
| *Symphytum officinale* L. (FABSYM) | Boraginaceae | *Erba di San Lorenzo* | Comfrey | ♣ | W | Leaves | Crushed, in topical applications | Cicatrising; anti-burns |
| *Syzygium aromaticum* (L.) MERR. ET PERRY | Myrtaceae | *Garofano* | Clove | ♣ | C | Flower bud | Inserted around the teeth | Against toothache |
| *Taraxacum officinale* WEB. (FABTAR) | Asteraceae | *Piscialletto; soffione* | Dandelion | ♣ | W | Whole plant | Decoction, in washes | Anti-haemorrhoids; to heal varicose veins; to treat diverse skin inflammations |
| *Tilia cordata* MILL. | Tiliaceae | *Tiglio* | Lime | ♣♣ | SC | Flowers | Infusion, in gargles or washes | To heal sore throat and skin inflammations |
| *Tricticum aestivum* L. | Poaceae | *Grano* | Wheat | ♣ | C | Seeds | Heated, in compresses | Anti-arthrosis |
| *Thymus vulgaris* L. | Lamiaceae | *Timo* | Thyme | ♣ | C | Flowering tops | Cold water infusion together with rosemary and lavender flowering tops, then mixed with alcohol | Perfume |
| *Ulmus minor* MILL. (FABULM) | Ulmaceae | *Olmo* | Elm | ♣ | W | Bark | Decoction | Cicatrizing |
| | | | | | | Bark (especially that extracted from young stem) | Topical application | Anti-wounds |
| | | | | | | Galls | The internal content externally applied | Anti-wounds |
| *Urtica dioica* L. (FABURT) | Urticaceae | *Ortica* | Nettle | ♣♣♣ | W | Leaves | Decoction, drunk or more often externally applied in washes | Anti-dandruff; to strengthen hair and prevent hair loss; anti-haemorrhoids |

Table 1 (*Continued*)

| Botanical taxon (voucher specimens code) | Botanical family | Vernacular name in the Marches | English name | Quotation frequency (referred to the species) | Status | Part(s) used | Preparation | Popular use |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Decoction, externally applied to the skin | Emollient |
| | | | | | | Aerial parts (with fruits) | Rubbed on the skin | To heal swollen feet an legs; against rheumatism; anti-sciatica |
| | | | | | | Roots | Infusion with rosemary and sage leaves, then made into an ointment with castor oil | To treat oily hair |
| *Vicia faba* L. | Fabaceae | *Fava* | Broad bean | ♣ | C | Fruit | Compress made with the dried and ground legume and egg albumen | Anti-bruises |
| | | | | | | Seed | Macerate in water, than applied on the eye | To heal haemorrhages in the eye |
| *Viola odorata* L. (FABVIO) | Violaceae | *Violetta* | Sweet violet | ♣♣♣ | W | Flowers | Macerate in cold water; compress made crushing the leaves with mallow leaves and wax in a mortar | Facial skin toner and perfume |
| *Vitis vinifera* L. | Vitaceae | *Vite* | Grape | ♣♣♣ | C | Sap (from young shoots) | Topical application | To treat eye inflammations; to prevent split ends in hair |
| | | | | | | Wine | Drunk by the mother, retained in the mouth, then expelled, applied in external washes, adding corn meal | To strengthen the legs of babies |
| | | | | | | | Washes | To strengthen the legs |
| | | | | | | Wine that is going to become vinegar | Externally applied | Antiseptic for wounds |
| | | | | | | Vinegar | Applied externally (sometimes with petrol) | Anti-lice; prevent hair loss |
| | | | | | | | Gargles with salt | To heal sore throat |
| | | | | | | | Applied externally for one week | To treat hard, flaky skin of the feet |
| *Zea mais* L. | Graminae | *Granturco* | Corn | ♣♣ | C | Corn meal and semolina | Added in the bath | Skin toner, especially used for babies |
| diverse tree species | | | | ♣♣ | | Coal; ashes | Put in water, and then filtered, in washes | To clean the hair; against scabies |

pl: plural; C: cultivated; SC: semi-cultivated (including plants “managed in the wild”); W: wild; *quotation frequency*: ♣: quoted by less than 10% of the informants; ♣♣: quoted by more than 10% and less than 40% of the informants; ♣♣♣: quoted by more than 40% of the informants. (∗) usage quoted by German migrants; (∗∗) usage quoted by Ukrainian migrants; (∗∗∗) usage quoted by Philipino migrants; (#) usage quoted by Paraguayan migrants; (##) usage quoted by Spanish migrants; (+) usage quoted by Moroccan migrants.

Table 2
Animal or mineral derived folk cosmeceuticals and popular remedies to heal skin diseases in the inland Marches

| Ingredient | Preparation | Folk use |
|---|---|---|
| Antinomy and lead sulphides | Externally applied | To decorate eyes$^{+}$ relieve burning eyes$^{+}$ |
| Ashes | Externally applied | To heal sore throat |
| | With water applied on the hair | To give hair a special gloss and softness |
| Bees wax | Externally applied | To heal the hard, flaky skin of feet |
| Brick | Heated and externally applied on the breast | Anti-tussive |
| Calcium bicarbonate | Mixed with honey | Facial skin emollient |
| Charcoal | Pulverized, and applied on the teeth | To whiten the teeth |
| Clay | Suspended in water, topically applied | To give a special softness to skin and hair |
| Cobweb | Topical application | Cicatrising |
| Cow faeces | Topical application | Anti-burnes |
| Egg yolk | Applied externally | To strengthen the hair |
| Egg shell | Crushed and mixed with olive oil, in external application | Anti-otitis |
| Honey | Externally applied | To avoid swollen skin after a syringe injection |
| | Mixed with flour and egg yolk, externally applied | Suppurative for furuncles |
| | Mixed with calcium bicarbonate | Facial skin emollient |
| Human milk | A few drops instilled in the ear of babies | Anti-otitis |
| Petrol | Topical application | Anti-lice |
| Pig fat | Let rot and use as an excipient | Suppurative |
| Rabbit internal skin | Dried and used as a plaster, adhesive agent | Cicatrising |
| Salted water | Topical application | Cicatrising |
| Sand | Heated and applied on the lumbar region | To heal respiratory infections |
| Sea stone | Heated and applied on the breast | Against cough |
| Shell | Topically applied | To relieve sore throat (ritual use) |
| Snail | Topical application | Anti-warts$^{\#}$ |
| Snake skin | Compresses on the head | To heal headache |
| Sulphur | Mixed with pig fat, externally applied | To heal scabies |
| Urine | Externally applied | Cicatrising |
| | Washes | Against wrinkles and chilblains |
| Water (naturally) containing colloidal sulphur | Compresses | Anti-wounds and anti-acne |

(#) Usage quoted by Spanish migrants; (+) usage quoted by Moroccan migrants.

during the 19th Century (Cristiano, 2001) but it is not appreciated by the youngest generation nowadays. A broad spectrum of field studies would be necessary for analysing aesthetic systems under a cross-cultural perspective in order to better understand the socio-cultural significance of, and dynamic historical changes in, the use of traditional appearance-changing products.

### 3.2. Ethnocosmeceuticals

Many recorded formulations belong to the middle field of cosmeceuticals: they have been or still are used in fact to both enhance the appearance of the skin and produce not well definable benefits (as emollients, skin toners and strengtheners). Little is known about the phytopharmacology of the ingredients used in these preparations, they often had an emollient action, and were thought to restore and optimise the functions of the skin and skin annexes, which were highly affected by a lifestyle characterised by hard daily agro-pastoral activities.

This group of remedies includes onions, alecost, lime, hawthorn, nettle, ivy, flax, cherry fruits, radish, rosemary, pomegranate, rue, potatoes and even wine. A few of these species are in fact medicinal plants *tout-court*, very well-known in the European modern evidence-based phytotherapy (Schilcher and Kammerer, 2000; Barnes et al., 2002; Fintelmann and Weiss, 2002; Wichtl, 2002; Jäniche et al., 2003), and also in the most important southern European herbal treatise of the past five centuries (Mattioli, 1578).

### 3.3. Skin phytotherapeuticals

This third group of plants includes species used to heal well-defined afflictions of the skin apparatus. Some of these well-known medicinal plants are, in fact, widely used in modern phytotheraphy to heal skin diseases, and include horse chestnut, St. John's wort, mallow, and plantain.

An external or topical application of some of these plants has been never recorded before in modern ethnobotanical studies in Italy or the Marches: this is the case for such plants as borage, bulbous buttercup, briony, cabbage, common club moose, cornflower, Jupiter's distaff, and Spanish broom.

### 3.4. Phytochemical and phytopharmacological considerations

In the following paragraphs the most uncommon species recorded, and whose phytochemistry and phytopharmacology should be maybe better investigated in future surveys, are discussed.

#### 3.4.1. Balsamita major

*Balsamita major* (alecost or costmary, also known as *Tanacetum balsamita* L.) represents a species that has been very popular among rural classes: it was and is mainly cultivated in the home-garden. It has been used in Italy not only for cosmetic and cosmeceutical purpose (as in the Marches), but also as sedative, anti-tussive, carminative, diuretic (Gastaldo, 1987) and even for aromatizing food in home-made omelettes (Sella, 1992). Nevertheless, its phytopharmacology is poorly known: while sesquiterpene lactones (Todorova and Ognyanov, 1989) and many volatile compounds (among them, carvone and α-thujone, as major constituents; Bylaite et al., 2000; Monfared et al., 2002) have recently been identified in the leaves and in the essential oil of this species, so far, biological assays have only shown a certain anti-microbial (Kubo and Kubo, 1995), antioxidant activity in rapeseed oil (Bandoniene et al., 2000), and insect anti-feedant (Kubo et al., 1996) activities.

#### 3.4.2. Centaurea cyanus

The cornflower also represents a species that, despite its widespread use in the Italian medical phytotherapy for minor ocular inflammations (Campanini, 1998) (in the Marches we recorded both this use and a hair dyeing usage, which gives a special gloss blue nuance to the white hair of elderly women), is not still very well-known phytochemically.

Recently, polysaccharides found to be mainly composed of galacturonic acid, arabinose, glucose, rhamnose and galactose, and extracted from its flower-head, have shown anti-inflammatory and immunological effects (Garbaci et al., 1999).

#### 3.4.3. Lycopodium clavatum

Common club moss (*Lycopodium clavatum*) has been subject to thorough toxicological screening because of its poisoning alkaloids (Roth and Kormann, 1996), and also for having been often confused in Central Europe with the very toxic fir club moss (*Lycopodium selago*), which contains a potent inhibitor of the acetylcholinesterase, huperzine A (Felgenhauer et al., 2000).

Nevertheless, recently methanolic extracts from the species have shown propyl endopeptidase inhibitory activity (Tezuka et al., 1999). This enzyme plays a role in metabolism of proline-containing neuropeptides (PEP), such as vasopressin, substance P and thyrotropin-releasing hormone (TRH), which are suggested to be involved with learning and memory processes and the specific inhibitors of PEP are expected to have antiamnesic effects.

Moreover, two new serraten triterpenes isolated from an ethanolic extract of *Lycopodium cernuum* have shown propyl endopeptidase inhibitory effects against *Candida albicans* secreted aspartic proteases (Zhang et al., 2002). Interestingly, in the studied area we recorded a very rare use of the spores of *Lycopodium clavatum* for preparing ointment to heal dermatitis. Further pharmacological studies taking account of this traditional use could be worthwhile.

### 3.5. Ingredients of animal and mineral origin

Bee products (wax and honey), pig fat, eggs, and even urine and cobwebs represent the most commonly reported ingredients of animal origin in the cosmetic apparatus of rural women (Table 2). A few of these ingredients were used as excipients and active ingredients at the same time, mainly as emollients.

### 3.6. Historical considerations

From the gathered data, it is possible to point out that traditional knowledge in producing domestic home-made cosmetics, cosmeceuticals and remedies for healing skin diseases as well, never includes exotic drugs (as Oriental spices or other Southern American or Asian essences such as vetiver, patchouli, Perù and Tolu balsams, benzoin, etc.), nor expensive ingredients from the Mediterranean (such us saffron, laudanum, iris, bergamot) or exclusive animal essences (as like civet, ambra, castoreum). The only exception to this is represented by the use of violets and roses.

In this sense, the folk cosmetic practices have taken a much separated path away from the historical "schools" of cosmetics. While this is also generally true in ethnopharmaceutical studies in the Mediterranean (Pieroni, 2000), the differences in the field of cosmetics are even more dramatic. This could be explained by the fact that while in the phytotherapy a certain osmosis between practices of the poor classes and those of the aristocrats and upper bourgeois existed during the Middle ages, especially due to the gardens of the monasteries, which passed through many forms of "high knowledge" to rural people, a similar process did not happen at all in the field of cosmetics. Moreover, many of the plant species, from which the most precious essences were and are extracted, and widely used in the last four centuries in the cosmetics, have never been widely cultivated in the South-European areas. The difficulty in acquisition of such exotic plants could also explain why they have been considered rare and have represented objects of prestige and symbols of status. Moreover, folk cosmetics in the Marches do not have also much in common with the natural ingredients that the industry nowadays generally uses for cosmetic preparations (Aburjai and Natseh, 2003).

### 3.7. Folklore versus multicultural societies: anthropological considerations

Today, the traditional rural culture of the inland Marches has quite disappeared. Information collected in this study basically represents the last traces of a rural world in the 20th Century. Most of the young people in the study areas are no longer dedicated to agricultural or pastoral activities, and new migrant waves from Eastern Europe are producing interesting and complex cross-cultural "hybridisations", "creolisations", "syncretisms", "collages" and "bricolages" phenomena (Greverius, 2002). In the Marches, the most rele-

vant new migrant groups are from Albania, Morocco, Macedonia, Romania, Tunisia, Greece, Senegal, Poland, and Germany (Caritas, 2002) (the last "alternative" migration is not obviously due to economic reasons). The very few remaining pastoral activities are carried out by Kossova and Albanian shepherds and Eastern European women have brought their knowledge about food, medicinal and cosmetic plants into the region. The diverse home-made cosmetic preparations used by migrant women are reported in Table 1 with asterisks.

Among the ingredients quoted by migrants, it is interesting to see how *kohl* (antimony and lead sulphide), despite of its toxicity, is used among northern African women. The use of these materials has a long history: Egyptian women were using to ground antimony trisulfide to darken their eye-lids, and the same practice is nowadays spread among many Arabic countries and in the Near East (Bellakhdar, 1997; Hardy et al., 1998; Lev and Amar, 2000, 2002; Lev, 2000).

It is reasonable to assume that the influx of this new TK due to migration flows will continue to spread amongst the women in autochthonous Italian communities. Future studies should aim to gain a better understanding of how the newly introduced traditional knowledge of migrant groups merges with that of the autochthonous populations.

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# Antidepressant effect of three traditional Chinese medicines in the learned helplessness model

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**Abstract**

*Plantago asiatica, Scrophularia ningpoensis* and *Ilex pubescens* are among the traditional Chinese medicines which are more frequently prescribed for treating depression-like ailments in the past and present traditional Chinese medical practice. The present work was therefore conducted to evaluate the presumable antidepressant effects of the extracts derived from the three remedies in mice using the learned helplessness model being used for screening for antidepressant compounds in modern medicinal researches. As a result, the petroleum extracts of *Plantago asiatica* and *Ilex pubescens* as well as the EtOAc extract of *Scrophularia ningpoensis* and the petroleum-soluble fraction of the acidic hydrolysate of the water extract of *Ilex pubescens* (after petroleum extraction) decreased significantly the number of escape failures relative to the control. The finding rationalized the clinical prescription of the herbs for the treatment of depression, and shined a clue for the characterization of the antidepressant phytochemical(s).
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*Keywords: Plantago asiatica*; *Scrophularia ningpoensis*; *Ilex pubescens*; Antidepressant effect; Learned helplessness model

## 1. Introduction

Depression, one of the present major mental disorders (Bland, 1997), is receiving growing attention as the incidence of depression-related ailments is increasing significantly owing to many reasons. The typical symptom of depression includes the lowered mood and lack of interest or pleasure, which usually deprive the patient of the capabilities for work and logical communication. These could even lead eventually to suicide without the timely therapy of depression. Since the discovery of the first antidepressant in mid-1950s, the field has been intensively warmed up. Several new classes of compounds emerged and a few hypotheses on the mechanism of their actions were proposed (Vetulani and Nalepa, 2000). The novel antidepressants are either selective and reversible monoamine oxidase inhibitor (e.g., moclobemide), or selective serotonin reuptake inhibitors (e.g., citalopram) or serotonin and noradrenaline reuptake inhibitors (e.g., venlafaxine). However, most of the drugs are synthetic nitrogen-bearing compounds which have inevitably some serious adverse-effects such as cardiovascular disease, the narrow scope of remedial spectrum and shortage of $t_{1/2}$. As a matter of fact, the clinical application and the efficacy of these drugs were quite limited, and the treatment with some synthetic antidepressant drugs has to be paused owing to certain unbearable side-effects. Accordingly, there is an urgent need for the research and development of more effective antidepressants without any (or with lower) adverse-effect.

In the traditional Chinese medicine, the incidence of depression and/or associated disorders has been attributed to *liver qi* stagnation indicating in a comprehensive manner the state of the symptom including mental stress, hypochondriac and hernial pain, or lumps in the breasts, irregular menstruation, etc. Prior to the application of synthetic antidepressant drugs, many traditional Chinese medicines and empirical formula were successfully used to treat the depression-like disorder by dispersing stagnant *liver qi* underlining the possibility that folk remedies could be an important source of new antidepressant drugs (Almeida et al., 1998; Luo et al., 2000). As a follow-up to our previous investigation of Banxia Houpu decoction, an ancient Chinese empirical formula (Luo et al., 2000), we expended our attention to *Plantago asiatica* L. (Plantaginaceae), *Scrophularia ningpoensis* Hemsl. (Scrophulariaceae) and *Ilex pubescens* Hook. et Arn. (Aquifoliaceae), all having been frequently

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prescribed for treating *liver qi* stagnation in the traditional Chinese medicine since ancient times (Jiangsu New Medical College, 1977). Previously, the *Plantago asiatica* extract has been shown to have antioxidant, antibacterial, anti-inflammatory, hepaprotective and anti-hyperlipidemic effects (Samuelsen, 2000). And quite recently the extract of *Ilex pubescens* has been reported to be antioxidant and choleretic (Filip and Ferraro, 2003) while that of *Scrophularia ningpoensis* has been ascertained to be antibacterial, anti-inflammatory and antioxidant (Schinella et al., 2002). However, little is reported in any modern scientific journal concerning their usages and efficacies in treating depression-like ailments although well documented in many traditional Chinese medicinal monographs. We therefore reinvestigated the three plants to ascertain the presumable antidepressant action using the learned helplessness model, which is now accepted as a reliable behavioral assessment for the management of depression-associated disorders (Besson et al., 1999; Steciuk et al., 1999). The results are presented in this communication.

## 2. Material and methods

### 2.1. Plant material

*Plantago asiatica, Scrophularia ningpoensis* and *Ilex pubescens* were purchased from the Jiangsu Medical Material Company with each sorted according to the morphological characteristic. The voucher specimen under numbers 00912, 00913 and 00914, respectively were identified by Associate Prof. L.X. Zhang, and preserved in the Herbarium of Nanjing University, Nanjing, P.R. China.

### 2.2. Preparation of extracts

The air-dried whole herb of *Plantago asiatica* (200 g) were pulverized and extracted thrice (500 ml each) with petroleum by refluxing at 60–70 °C for 2 h. After filtration, the extract was concentrated *in vacuo* into a residue (I, 10 g). The air-dried root of *Scrophularia ningpoensis* (300 g) was chopped roughly followed by three-time extraction (500 ml each) with EtOAc at 80 °C for 2 h. *In vacuo* evaporation of the solvent from the extract afforded a gum (II, 23 g). The air-dried stem of *Ilex pubescens* (200 g) was powdered and extracted thrice (500 ml each) with petroleum by refluxing at 60–70 °C for 2 h. Removal of the solvent from the extract *in vacuo* gave the residue (III, 8 g). The material after petroleum extraction was again air-dried, and subsequently re-extracted thrice with a fivefold volume of water (1000 ml) by refluxing for 1 h. After filtration, the filtrate was adjusted to pH = 1 with 1 M HCl, and the acidified liquid was heated at 80 °C for 1 h. Then the reaction mixture was neutralized with 37% $NH_3$–$H_2O$ to pH = 7 followed by extraction with 250 ml petroleum for four times. The petroleum layer was evaporated *in vacuo* to a dryness (IV, 2.3 g).

### 2.3. Animals

Male ICR mice (18–22 g) were kept on a 12-h light-dark cycle at a constant temperature (22 ± 2 °C) in groups of 12 in macrolon cages (21 cm × 11 cm × 11 cm) with free accesses to food and water throughout the experiment.

### 2.4. Drug administration

All the test samples were either dissolved in water or, if insoluble, dispersed in an aqueous suspension of Tween 80 (0.5% w/v). Vehicles and fluoxetine given at a dose of 10 mg/kg served as negative and positive controls, respectively. After doses ranges were chosen according to our preliminary screening tests, randomly grouped animals were administered separately via gastric intubation with I, III and IV at 2.5, 5 and 10 mg/kg as well as II at 5, 10, 15 and 20 mg/kg. The administration volume was 1 ml/100 g (bodyweight).

### 2.5. Apparatus and experimental procedure

The following experiment was performed as outlined earlier (Martin et al., 1987). On the first day, every mouse was individually placed in a plexiglass chamber (20 cm in height and 10 cm in diameter) with a stainless steel grid floor which connected to a electric-generator, and the mice were exposed to inescapable electric footshocks (0.3 mA DC, 15 s duration) on the electrified grid floor for 1 h. Control mice (viz. 'con1' in figures) were placed for 1 h in same conditions without shocks. Forty-eight hours after the inescapable shocks, all the mice were subjected to an avoidance-escape test in a shuttle-box (20 cm × 40 cm × 20 cm) with two compartments of equal size with a route between them, and equipped with a grid floor made of stainless steel bars. Every mouse was placed singly in the shuttle-box. A 3-min environmental adaptation period was set before the trials. The test consisted of 30 trials at an interval of 30 s, in which the mice were required to cross from one side to another to escape the shock. For each trail, a light signal was present for the first 3 s, during which mice were allowed to move from one side to another to avoid the shock. If no response occurred within this period, an electric shock was delivered with the light on. If it did not escape during the shock application, the shock and light were turned off automatically after 3 s, and this is called an escape failure. Subsequently, another trial began and the numbers of escape failure were recorded. The avoidance-escape test was repeated on 4th and 5th days, but without giving the adaptation time of 3 min.

The tested samples were administered via gastric intubation to mice repeatedly on five consecutive days. The first administration was administered 6 h after the footshock on day 1, and then once daily. Different doses were set for each test samples (2.5, 5, 10 and 20 mg/kg per day). The behavioral tests were performed in mice 30 min after the administration.

### 2.6. Statistical analysis

The number of escape failures recorded over the 30 trials of the shuttle-box test was expressed as mean ± S.E.M. The data obtained were evaluated by one-way analysis of variance (ANOVA) following by Duncan's test.

## 3. Results

As shown in Figs. 1–3, the mouse given vehicle only (without pre-footshocks, designated as "control 1") exhibited the lower numbers of escape failures whereas animals exposed to inescapable footshocks followed by the administration of vehicle ("control 2") showed relatively high numbers of escape failures ($P < 0.005$). The increment in the number of escape failures in the learned helpless animals (control 2) was indicative of a behavioral deficiency caused by exposure to an uncontrollable aversive situation, which is considered to be inductive of depression.

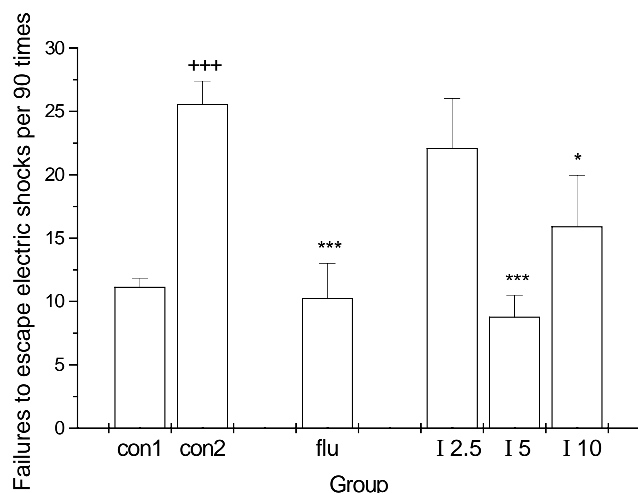


Fig. 1. Effect of *Plantago asiatica* extract (I) on failure numbers to escape electric shocks during the learned helplessness test. Con1: unshocked animals with vehicle; con2, flu and I: shocked animals administered with vehicle, fluoxetine and I. The values are mean ± S.E.M. Comparison con2 vs. con1: $^{+}P < 0.05$, $^{++}P < 0.01$ and $^{+++}P < 0.005$; comparison flu and I vs. con2: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.005$.

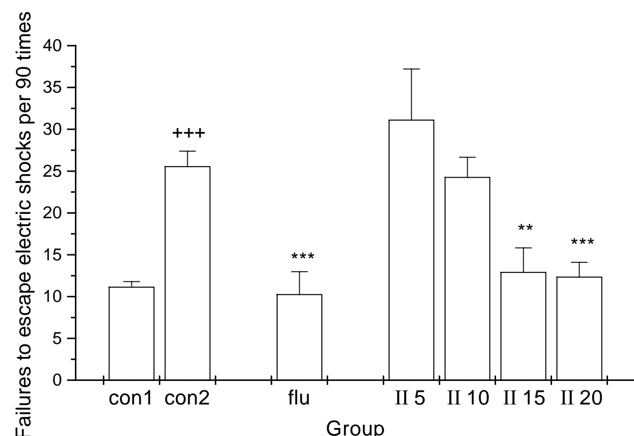


Fig. 2. Effect of *Scrophularia ningpoensis* extract (II) on failure numbers to escape electric shocks during the learned helplessness test. Con1: unshocked animals administered with vehicle; con2, flu and II: shocked animals administered with vehicle, fluoxetine and II. The values are mean ± S.E.M. Comparison Con2 vs. Con1: $^{+}P < 0.05$, $^{++}P < 0.01$ and $^{+++}P < 0.005$; comparison flu and II vs. Con2: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.005$.

The extracts of *Plantago asiatica* (I), *Scrophularia ningpoensis* (II) and *Ilex pubescens* (III) along with the petroleum-soluble fraction (IV) of the acidic hydrolysate of the water extract of *Ilex pubescens* after petroleum extraction decreased the number of escape failure in the learned helplessness test. Specifically, the extract I at 5 mg/kg showed a stronger effect ($P < 0.005$) than those discerned at doses of 2.5 and 10 mg/kg (Fig. 1). The extract II, at the highest dose (20 mg/kg), decreased remarkably the number of escape failures ($P < 0.005$). And the effect faded away with the dose decreased (Fig. 2). The extract III at 5 mg/kg and the fraction IV at 10 mg/kg have significant 'failure-reducing' activity ($P < 0.005$). However, no effect could be discerned with the extract III if the dose was lower than 2.5 mg/kg or higher than 10 mg/kg (Fig. 3). Furthermore, a lower effect was discerned with IV at 5 mg/kg. Surprisingly, a better effect was found with IV at 2.5 mg/kg ($P < 0.05$) (Fig. 3).

As to the positive control, the mice receiving the inescapable footshocks followed by administration of fluoxetine at the dosage of 10 mg/kg reduced the escape failure number ($P < 0.005$), which was close to that observed with the normal (control 1).

## 4. Discussion

The results from the learned helplessness test showed that the extracts could reverse the learning deficits caused by uncontrollable and unpredictable shocks with some discernible effects similar to that of fluoxetine, a clinically using antidepressant. The learned helplessness test meets the different criteria to be qualified as a reliable animal model of depression (Sherman et al., 1982; Willner, 1986; Qian et al., 1992; Besson et al., 1999; Steciuk et al., 1999). And this ascertained as well the presence of antidepressant substances in the three investigated herbs under the guidance of folk remedy through a believable behavioral model. The action of II is in a dose-dependent manner, but those of rest three extracts not. This could be due to a complex combination of multiple factors including bioavailability and presumable synergy of the active principles.

As to the phytochemical studies, the petroleum extract (I) of *Plantago asiatica* contained mainly essential oils and other lipophilic principles including iridoid and terpenoids (Samuelsen, 2000; Ringbom et al., 1998), and the EtOAc extract (II) of *Scrophularia ningpoensis* consisted mainly constituents of moderate polarity such as oxygenated iridoid, phenylpropanoid and triterpenoid (Bhandari et al.,

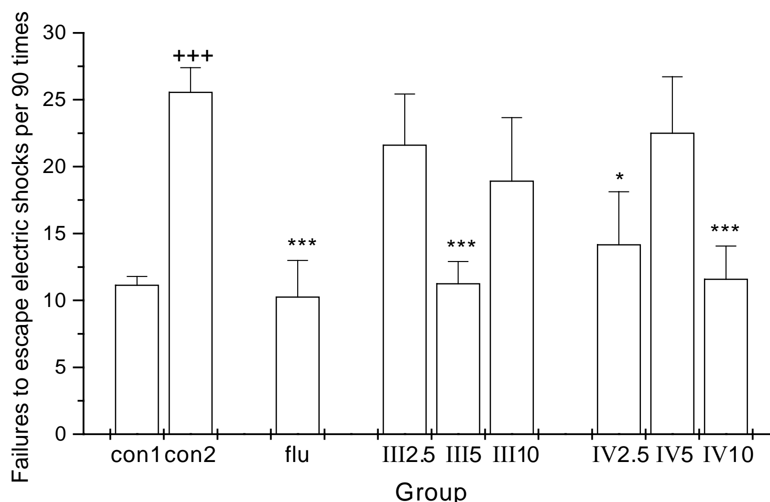


Fig. 3. Effect of *Ilex pubescens* extract (III) and the petroleum-extractable fraction (IV) of the acidic hydrolysate of the water extract of *Ilex pubescens* after petroleum extraction on failures to escape electric shocks during the learned helplessness test. Con1: unshocked animals administered with vehicle; con2, flu, III and IV: shocked animals administered with vehicle, fluoxetine, III and IV. The values are mean ± S.E.M. Comparison Con2 vs. Con1: $^{+}P < 0.05$, $^{++}P < 0.01$ and $^{+++}P < 0.005$; comparison flu, III and IV vs. Con2: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.005$.

1997; Li et al., 1999, 2000). Previous phytochemical investigations of *Ilex* species, including *Ilex pubescens*, have found many classes of chemical constituents like triterpenoid saponins, flavone glycoside, alkaloids, amino acids, fatty acids (Alikaridis, 1987). Therefore, the petroleum extract (III) of *Ilex pubescens* most probably contain lipophilic principles like free or non-glycosylated triterpenoid and flavone (Hidaka et al., 1987), and the petroleum-soluble fraction (IV) derived from the water extract of *Ilex pubescens* through hydrolysation in acidic condition should contain sapogenins of triterpenoid (Ouyang et al., 1996). It is noteworthy that the hydrolysis of the water extract of *Ilex pubescens* is desired as the polar glycosides therein are usually not easy to pass the blood–brain barrier. This may explain why lipophilic sapogenins in *Ilex pubescens* could remarkably decrease the escape failures of the learned helpless animals.

The present results indicated that the extracts of the three herbs prescribed traditionally for the treatment of depression have a pronounced antidepressant effect in the animal model of depression rationalizing their application in the traditional Chinese medical practice. Further investigation aiming at the characterization of the antidepressant constituents and at understanding the mode of action is highly desired.

## Acknowledgements

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# Inhibition of MAO A and B by some plant-derived alkaloids, phenols and anthraquinones

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**Abstract**

A total of seventeen phytochemicals including seven alkaloids (piperine, strychnine, brucine, stachydrine, tetrandrine, frangchinoline and sinomenine), four phenols (paeonol, honokiol, magnolol and eugenol) and six anthraquinones (emodin, rhein, chrysorphanol, aloe-emodin, physcion and 1,8-dihydroxyanthraquinone) was examined for inhibitory activity of monoamine oxidase (MAO) A and B from rat brain mitochondrial. Among these compounds, piperine and paeonol were found to be inhibitory against MAO A in a dose-dependent manner with $IC_{50}$ values of 49.3 and 54.6 μM, respectively. Piperine, paeonol and emodin were shown to inhibit MAO B in a dose-dependent manner with the $IC_{50}$ data of 91.3, 42.5 and 35.4 μM, respectively. Lineweaver–Burk transformation of the inhibition data indicated that the inhibitory action of piperine on MAO A was of mixed type, and that of paeonol on the same type of the enzyme was of non-competitive type. For piperine, the $K_i$ and $K_I$ were determined to be 35.8 and 25.7 μM, respectively. For paeonol, the $K_i$ was estimated to be 51.1 μM. The inhibition of piperine and paeonol on MAO B was of competitive type with $K_i$ values of 79.9 and 38.2 μM, respectively. The inhibition of emodin on MAO B was of mixed type with the $K_i$ and $K_I$ data of 15.1 and 22.9 μM, respectively. The present investigation showed that the phytochemicals piperine, paeonol and emodin are potent MAO inhibitors whereas other compounds were inactive against any type of MAO at 100 μM in the present assay.
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*Keywords:* Piperine; Paeonol; Emodin; Monoamine oxidase

## 1. Introduction

Monoamine oxidase (MAO, EC.1.4.3.4) is an important enzyme in the metabolism of a wide range of endogenous monoamine neurotrasmitters such as noradrenaline, dopamine, and serotonin (5-HT). This enzyme catalyzes as well the removal of exogenous amines. Some MAO A inhibitors are efficacious for treating anxiety and depression while the inhibition of MAO B appears to be effective to prevent and treat Parkinson's disease (Silverman et al., 1993; Kanazawa, 1994). However, severe adverse effects such as cytotoxic (Kohda et al., 1998), hyperpyrexia, disseminated intravascular coagulation, convulsions, coma and muscle rigidity (Power et al., 1995) have been observed with some classical MAO-A and/or -B inhibitors mainly owing to the interactions with other drugs and foodstuffs (Dingemanse, 1993). Thus, there is an urgent need to find new MAO inhibitors devoid desirably of these severe adverse effects. As a follow-up to our previous investigation of plant-derived inhibitors of both types of MAO (Kong et al., 2000, 2001; Pan et al., 2000; Zhou et al., 2001), we here with wish to report the pharmacological results with the inhibition on MAO A and B (from rat brain mitochondrial) of seventeen phytochemicals originated from the traditional Chinese medicine, which have long been used for the treatment of some mental diseases and anti-aging (Jiangsu College of New Medicine, 1977).

## 2. Materials and methods

### 2.1. Reagents

The phytochemicals (seven alkaloids piperine (Dwuma-Badu et al., 1976), strychnine (Akopian and Shcherbina, 1970), brucine (Yang and Yan, 1993), stachydrine (Singh et al., 1975), tetrandrine (Lin et al., 1993), frangchinoline and sinomenine (Yamasaki, 1976), four phenols paeonol

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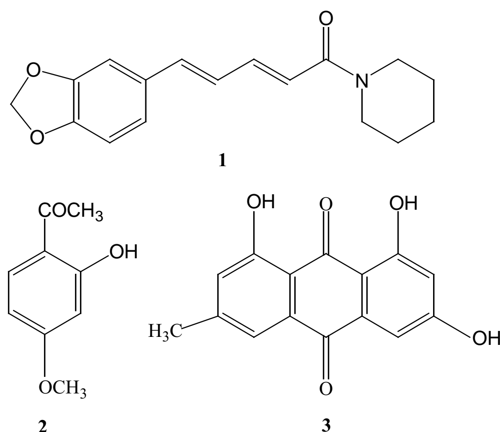


Fig. 1. Structures of piperine (1), paeonol (2) and emodin (3).

(Di et al., 1998), honokiol, magnolol (Maruyama et al., 1998) and eugenol (Zheng et al., 1992), and six anthraquinones emodin (Huang et al., 1991), rhein, chrysorphanol, aloe-emodin, physcion (Min et al., 1998; Agarwal et al., 2000) and 1,8-dihydroxyanthraquinone (Eckardt et al., 1985) were provided by the National Institute for Control of Pharmaceutical and Biological Products, Beijing, China. And the purity of each product was ascertained by TLC and HPLC analyses. The structures of bioactive compounds were given in Fig. 1. [$^{14}$C]-serotonin (5-HT) and [$^{14}$C]β-phenylethylamine (β-PEA) were products of DuPont NEN (USA), and clorgyline, *l*-deprenyl and dimethyl sulfoxide (DMSO) were purchased from Sigma (USA). All other chemicals used in the study were of analytical grade.

### 2.2. MAO A and B assay

Rat brain mitochondrial fraction was prepared as a source of MAO activity following the procedure described previously (Schurr and Livne, 1976). Briefly, the mitochondrial fraction and sodium phosphate buffer (50 mM, pH 7.4) were mingled in a proportion of 1:20 with gentle agitation at 4° for 60 min. The mixture was centrifuged immediately at 16,000 × *g* for 30 min at 0° and the pellets were resuspended in the same buffer containing additional sucrose at a concentration of 250 mM. MAO activity was assessed radiochemically by slightly modifying the procedure outlined previously (Fowler et al., 1979; Pizzinat et al., 1999). Thus, the assay mixtures contained 50 μM [$^{14}$C]5-HT or 10 μM [$^{14}$C]β-PEA as specific substrates for MAO A and B, respectively, 10 μl solution of isolated compounds in DMSO at different concentrations, and 100 mM sodium phosphate buffer (pH 7.4) up to a final volume of 200 μl. After a 20 min preincubation at 37 °C, the reaction was started by adding 50 μg of the mitochondrial fraction. The reaction was allowed to proceed at 37 °C for 20 min, and terminated by addition of 2 M HCl (1 ml), the radioactive product was extracted with 2 ml of toluene/ethyl acetate (v/v, 1:1). The radioactivity of the organic phase was counted in a liquid scintillation spectrometer. Blank samples were prepared by adding 2 M HCl (1 ml) prior to reaction, and worked up subsequently in the same manner. Enzyme activity was expressed as nmol product formed per mg protein per min. In the kinetic analyses, the reaction mixture consisting of different concentrations of [$^{14}$C]5-HT (20–200 μM) or [$^{14}$C]β-PEA (3.3–20 μM) were used as MAO A or B substrates, respectively, in the absence and presence of inhibitors.

### 2.3. Estimation of protein

Protein concentration was estimated by the Lowry method (Lowry et al., 1951) using bovine serum albumin as the standard.

### 2.4. Data analysis

The data were presented as $\bar{x} \pm s$. The $IC_{50}$ value was calculated using computer software 'GraphPad InPlot'. The $K_i$ and $K_I$ values were determined by consulting Lineweaver–Burk's plot using linear regression analysis. Specifically, $K_i$ was calculated from the slope of the inhibition curve by the equation (slope $= K_m/V_{max}(1+[I_0]/K_i)$) ($[I_0]$, $K_m$ and $V_{max}$ representing inhibitor's initial concentration, Michaelis constant and maximum initial velocity, respectively), and $K_I$ was calculated from the *y*-intercept of the inhibition curve using the equation $y$-intercept $= 1/V_{max}(1 + [I_0]/K_I)$.

## 3. Results

### 3.1. Inhibition of phytochemicals on MAO A

Among the seventeen test compounds, piperine and paeonol (Fig. 1) inhibited the activity of MAO A in a dose-dependent manner with $IC_{50}$ values of 49.3 and 54.6 μM, respectively (Fig. 2). However, others exhibited no inhibition on this type of MAO ($IC_{50}$ value > 100 μM). In the study, the $IC_{50}$ value of clorgyline, a MAO A inhibitor used as a positive control, was estimated to be 0.2 μM. The Lineweaver–Burk plots of piperine and paeonol for 5-HT (as a substrate) were shown in Figs. 3 and 4. The mode of inhibition of MAO A by piperine was shown to be of mixed type with $K_i$ and $K_I$ data of 35.8 and 25.7 μM, respectively. The mode of inhibition of MAO A by paeonol was non-competitive with the $K_i$ value of 51.8 μM.

### 3.2. Inhibition of phytochemicals on MAO B

Piperine, paeonol and emodin (Fig. 1) among the assayed compounds inhibited the activity of MAO B in a dose-dependent manner with $IC_{50}$ values of 91.3, 42.5 and 35.4 μM, respectively (Fig. 5). In our study, the $IC_{50}$ value of deprenyl, a MAO B inhibitor used as a positive control, was 0.3 μM. The modes of inhibition towards β-PEA as a

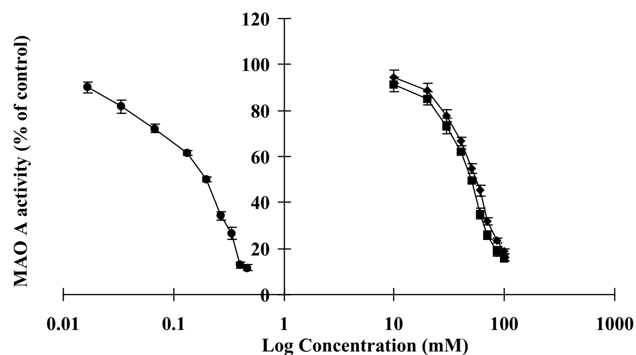


Fig. 2. Dose-dependent inhibitory actions of piperine and paeonol on MAO A. MAO A assays were performed as described in Section 2. Different concentrations of piperine (■), paeonol (◆) and clorgyline (●)were incorporated in the assays. Results are expressed as percentage of control where no inhibitor was added. Data are the average of five independent experiments and error bars indicate standard deviations.

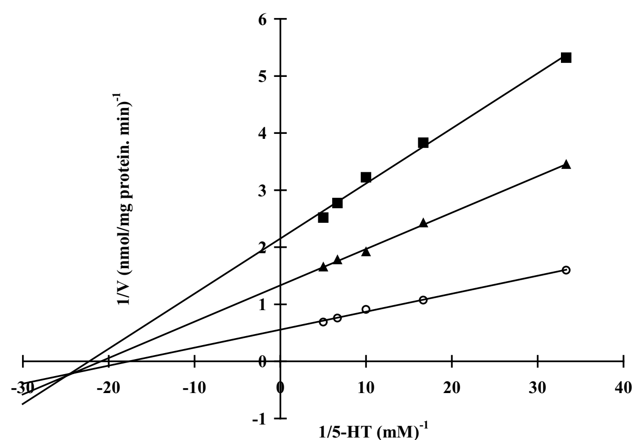


Fig. 3. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO A by piperine. MAO assay was performed at different concentrations of the substrate [$^{14}$C]5-HT. Control without any inhibitor (○), in the presence of 25 (▲) and 50 μM (■) piperine. The values are expressed as the average of triplicates.

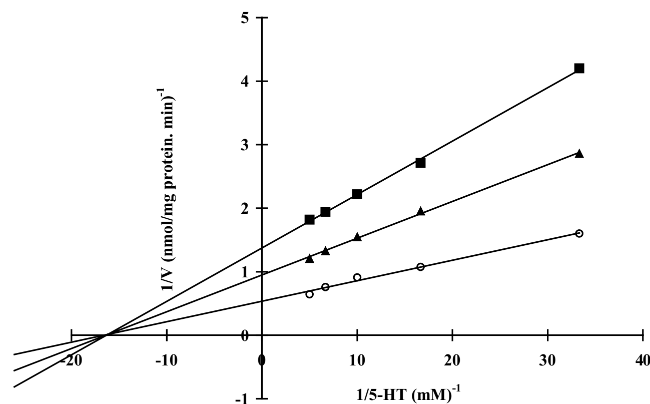


Fig. 4. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO A by paeonol. MAO assay was performed at different concentrations of the substrate [$^{14}$C]5-HT. Control without any inhibitor (○), in the presence of 27 (▲) and 54 μM (■) paeonol. The values are expressed as the average of triplicates.

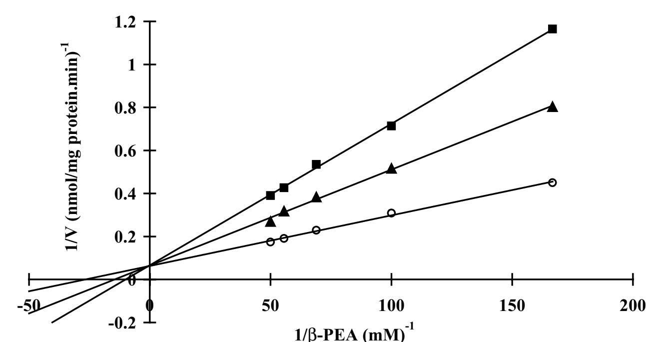


Fig. 6. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO B by piperine. MAO assay was performed at different concentrations of the substrate [$^{14}$C]β-PEA. Control without any inhibitor (○), in the presence of 45 (▲) and 90 μM (■) piperine. The values are expressed as the average of triplicates.

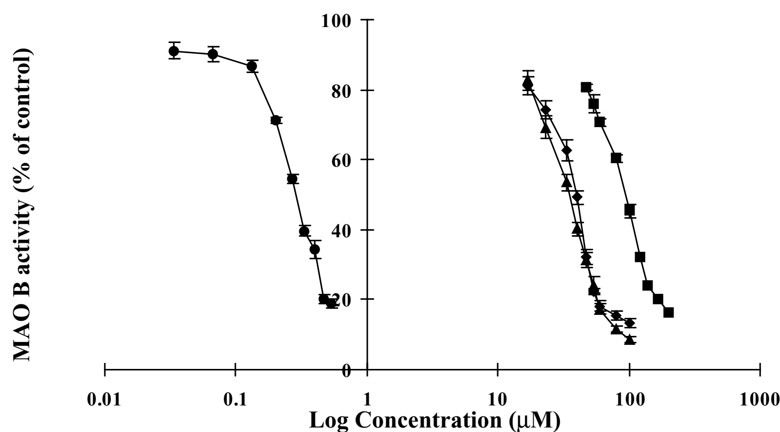


Fig. 5. Dose-dependent inhibitory actions of piperine, paeonol and emodin on MAO B. MAO B assays were performed as described in Section 2. Different concentrations of piperine (■), paeonol (◆), emodin (▲) and deprenyl (●) were incorporated in the assays. Results are expressed as percentage of control where no inhibitor was added. Data are the average of five independent experiments and error bars indicate standard deviations.

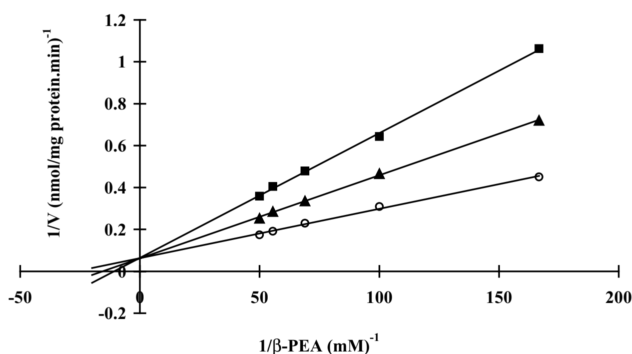


Fig. 7. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO B by paeonol. MAO assay was performed at different concentrations of the substrate [$^{14}$C]β-PEA. Control without any inhibitor (○), in the presence of 21 (▲) and 42 μM (■) paeonol. The values are expressed as the average of triplicates.

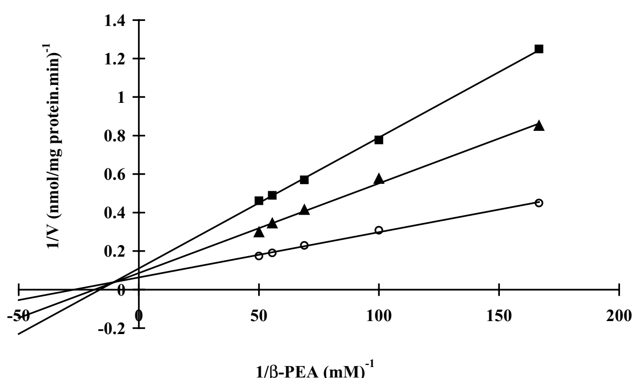


Fig. 8. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO B by emodin. MAO assay was performed at different concentrations of the substrate [$^{14}$C]β-PEA. Control without any inhibitor (○), in the presence of 17 (▲) and 35 μM (■) emodin. The values are expressed as the average of triplicates.

substrate by both piperine and paeonol were of competitive type with $K_i$ values of 79.9 and 38.2 μM, respectively (Figs. 6–8). However, emodin was of mixed type for β-PEA as a substrate with the $K_i$ and $K_I$ values of 15.1 and 22.9 μM.

## 4. Discussion

Among seven alkaloids, only the piperidine derivative piperine (1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine) showed inhibitory activities towards MAO A and B. And, inhibition of piperine on MAO A was more potent than that on MAO B. Others such as the benzylisoquinoline alkaloids sinomenine and fangchinoline, the pyrroline derivative stachydrine and monoterpenic indole base strychnine had no inhibitory activities towards any type of MAO in the present assay. In our previous communication (Kong et al., 2001), we mentioned that jatrorrhizine with a phenolic hydroxyl have a stronger inhibitory activity against MAO A and B than that of berberine of the type but without phenolic hydroxyl which could be necessary for the initiation of the enzyme inhibitory activity. Regarding piperine which is free of phenolic hydroxyl, we postulated that the discerned inhibition could be presumably initiated by the hydrogen bonding of its naked amide with active protons such as –NH–, –OH and –SH in the active sites of both type of MAO. Piperine is best known as the pungent principle of the black pepper (*Piper nigrum*) and found to have a wide spectrum of pharmacological activities such as being anticonvulsant and stimulating serotonin biosynthesis in the rat brain (Eldershaw et al., 1994). The present investigation ascertained for the first time that piperine is also an MAO inhibitor, and the finding may be of importance to obtain a better understanding of the traditional application of *Piper nigrum*.

Among the four assayed plant phenols, paeonol (2-hydroxy-4-methoxyacetophenone) showed exclusively inhibitory activities towards MAO A and B. However, its inhibition on MAO A is a bit less than that on type B of the enzyme. This observation could rationalize to some extend the traditional application of root bark of *Paeonia suffruticosa* (the main source plant of paeonol) as a sedative agent to treat central stress (Jiangsu College of New Medicine, 1977). Surprisingly, the other three phenols eugenol, honokiol and magnolol exhibited no inhibition on any type of MAO in the study. The striking difference in the enzyme inhibition among these plant phenols could be due to the deviation of the structure type, and of the feasibility for the functions (say, phenolic hydroxyl and ketone) to interact with the active site of MAO via hydrogen bonding. However, the anxiolytic effect of honokiol and magnolol, which also were the main principals of a famous formula Banxia Houpu Decoction, often used to treat depression and anxiety (Maruyama et al., 1998; Luo et al., 2000), is most probably based on other mechanism(s).

Among six anthraquinones, only emodin (3-methyl-1,6,8-trihydroxyanthraquinone) showed an inhibition on MAO B. Structurally, emodin is closely related to 1,8-dihydroxyanthraquinone, physcion (3-*O*-methyl ether of emodin) chrysophanol (3-dehydroxy-emodin). Both hydroxyls on C-1 and C-8 are equally hydrogen-bonded with the 9-carbonyl group limiting presumably their interaction with the active sites of MAO B. Furthermore, the quinones with 3-hydroxymethyl group and H-6 as in aloe-emodin and rhein, or without any substituent on $C_3$ and $C_6$ as in 1,8-dihydroanthraquinone, did not show any inhibition on both type of MAO. The observation indicated that the 'free phenolic hydroxyl', as emodin bears, is necessary for inhibiting MAO B. Phytochemically, emodin happens to be the main constituent of rhizomes of *Polygonum multiflorum* that has been used for anti-aging purpose in China since ancient times. Previously, the extract of the plant was also found to be inhibitory against MAO B without ascertaining the corresponding active constituents (Jiangsu College of New Medicine, 1977; Cheng et al., 1991). Our findings indicate that emodin could be the main MAO B inhibitory principle in the herb, and presumably in the extract as well.

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# Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice

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**Abstract**

The anti-amoebic effects of crude methanol extracts of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall against *Entamoeba histolytica* infecting the caecum of mice were studied. Caecal amoebiasis in mice was induced by injection of *Entamoeba histolytica* trophozoites directly into the caecum. The mice were then treated orally with the extract, a standard drug (metronidazole), or vehicle p.o. for five consecutive days, beginning 24 h after the infection and were examined on the sixth day. At a dose of 1000 mg/kg per day, the extracts of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall had a curative rate of 100, 40 and 26%, respectively. At a concentration of 500 and 250 mg/kg/day, extract from *Piper longum* fruit was still effective in 93 and 46% of the cases, respectively, while extract from *Piper sarmentosum* root at a dose of less than 1000 mg/kg per day did not cure any mice from amoebiasis. Extract of *Quercus infectoria* nut gall at a concentration of 500 and of 250 mg/kg per day cured 26 and 13% of mice, respectively. Metronidazole at a concentration of 125 and of 62.5 mg/kg per day had a curative rate of 100 and 60%, respectively. The severity of caecal wall ulceration was reduced in mice which received the extract and metronidazole as compared to the control animals.
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*Keywords:* Amoebiasis; *Entamoeba histolytica*; *Piper longum*; *Piper sarmentosum*; *Quercus infectoria*

## 1. Introduction

Among parasitic infections, amoebiasis ranks third worldwide in lethal infection, after malaria and schistosomiasis (Walsh, 1988; Petri and Mann, 1993). Although it is asymptomatic in 90% of cases, about 50 million people are estimated to suffer from the symptoms of amoebiasis such as haemorrhagic colitis and amoebic liver abscess (Ravdin, 1995). These infections result in 50 000–100 000 deaths annually. In South Africa and India, the disease is rather common (Walsh, 1986).

During 1987–1997, in Thailand, there were more than 50 000 cases of dysentery reported each year and approximately 400 people died. For about 90% of the cases, the cause of dysentery is unknown; however, *Entamoeba histolytica* was detected in 2–3% of cases (Anon, 1997). Among children under five years who were admitted with acute diarrhea in a hospital, *Entamoeba histolytica* was confirmed in 7.8% of the cases (Suwatana, 1997). The estimated number of infected cases may be much higher due to the lack of a sensitive and specific diagnostic test (Petri et al., 2000).

The most effective and commonly used drug for treatment of intestinal protozoa infection is metronidazole (Tracy and Webster, 1996). However, this drug has been reported to cause mutagenicity in bacteria (Legator et al., 1975) and is carcinogenic in rodents (Rustia and Shubik, 1972; Shubik, 1972). It has been reported that the human pathogenic bacterium, *Helicobacter pylori*, becomes resistant to metronidazole in vitro (Zwet et al., 1994). Moreover, it seems to act as an immunosuppressive agent in experimental rats, both in cell-mediated and humoral immune responses (Saxena et al., 1985). These are the main reason why there is a need to develop a safe and effective alternative antiamoebic agent.

For people in developing countries, medicinal plants are popular because their products are safe and widely available at low cost. Some compounds extracted from medicinal plants already play an important role against infectious diseases e.g. quinine from *Cinchona* sp., and artemisinin from *Artemisia annua*; both are effective against malaria. In the present study, *Piper longum* (Linn.) fruit (Plf), *Piper sarmentosum* (Roxb.) root (Psr) and *Quercus infectoria* (Oliv.)

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nut gall (Qin) were selected because these species are routinely used to cure bloody diarrhea in Thai traditional medical practice. It is, therefore, of interest to scientifically evaluate their effect on amoebiasis for potential antiamoebic activity in vivo. The selected plants were extracted with methanol and tested against caecal amoebiasis in mice. The anti-amoebic effect of the extract was compared with the standard drug metronidazole.

## 2. Materials and methods

### 2.1. Isolation and cultivation of Entamoeba histolytica

The culture of *Entamoeba histolytica* used in this experiment was isolated from the bloody stool diarrhea of a patient from Maharaj hospital, Nakorn Srithamarat, Thailand. Briefly, the untreated fecal samples were collected after diagnosis as *Entamoeba histolytica* infection and cultured in Boeck & Drbohlav medium with some modification as described elsewhere (Sawangjaroen et al., 1993). Calf bovine donor serum (10%) was used instead of horse serum. The culture was incubated at 37 °C and *Entamoeba histolytica* trophozoites along with their associated bacteria were subcultured every 24/48 h.

### 2.2. Preparation of extracts from medicinal plants

Plf and Qin were purchased from the medicinal plant store while Psr was collected from the area around Hatyai, Songkhla, Thailand. Voucher specimens for Plf, Qin and Psr have been deposited at The Prince of Songkla University Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand under voucher specimen numbers K. SAWANGJAROEN 1 (PSU), K. SAWANGJAROEN 2 (PSU) and K. SAWANGJAROEN 3 (PSU), respectively. The plants (or parts) were washed, cut into small pieces and dried in sunlight or in an oven at 50 °C maximal. Each plant material was subsequently pulverized and macerated in absolute methanol, at the ratio of 1 kg of plants per 3 l of methanol. The supernatants were collected after 7 days and the remaining plant was macerated again. This procedure was repeated twice. Whole methanol extract from each plant was filtered and evaporated to dryness under a low pressure with a rotary evaporator, at 55 °C. The extracts were then stored at 4 °C until use. Methanol extraction of Plf, Psr and Qin gave 22.8, 4.45 and 46.7% yield, respectively.

### 2.3. Inoculation procedure

Female Swiss albino mice, weighing between 25 and 35 g, aged 1–1.5 months were used throughout the experiment. The mice were prepared for *Entamoeba histolytica* infection according to the method of Ray and Chatterjee (1981) with a slight modification. Briefly, 24 h before the commencement of the surgery, the mice (25–35 g) were starved, and, in the morning and evening, the mice were pretreated orally with 0.5 ml of 25% $MgSO_4$ in distilled water. On the next day, the mice were anesthetized by an intraperitonial injection of pentobarbital sodium 40 mg/kg. Laparotomy was performed to expose the caecum. The suspension of actively motile *Entamoeba histolytica* at the volume of 0.2–0.3 ml containing $2.0 \times 10^4$–$2.5 \times 10^4$ trophozoites was injected directly into the caecum. The caecum was then returned into the peritoneal cavity, the abdominal muscle was closed and the skin sutured. Rat pellets and drinking water were provided ad libitum. The mice were randomly selected for the treatment and control groups.

### 2.4. Effects of crude extracts and metronidazole on amoebiasis in mice

Extract of Plf, of Psr and of the standard drug, metronidazloe in tablet form, were suspended in a 20% gum acacia solution in distilled water. The extract of Qin was suspended in distilled water. All treatment were administered daily p.o. using a feeding tube, for five consecutive days, beginning 24 h after infection with *Entamoeba histolytica*. The doses of plant extract used were 1000, 500, 250 and 125 mg/kg body weight per day and for metronidazole 125 and 62.5 mg/kg per day. The control animals were treated with 20% gum acacia solution in distilled water (for Plf, Psr and metronidazole) and with distilled water (for Qin). Fifteen animals were used for each treatment. On the sixth day, the animals were sacrificed by cervical dislocation and the caecum was carefully examined macroscopically for lesions and the content structure. The severity of infection was scored according to the method of Neal ranging from 0 for normal to 4 for severe structure destruction (Neal, 1951). The presence of *Entamoeba histolytica* trophozoites in the caecum was observed under light microscope. In the absence of *Entamoeba histolytica* trophozoites, a small amount of caecum content was transferred into a fresh medium and cultured for 24–48 h and this was then examined for trophozoites under light microscope.

## 3. Results and discussion

The effects of extracts from Plf, Psr and Qin against experimental caecal amoebiasis in mice are shown in Table 1. The results from the present study demonstrate that methanol extracts from selected medicinal plants are effective against *Entamoeba histolytica* in mice as evaluated by the number of mice cured and the reduction of severity of the mice caecal content and caecal wall lesions in comparison to the untreated mice. The anti-amoebic effects of all extracts are clearly dose-dependent. Most of the published data on medicinal plants against *Entamoeba histolytica* in vivo is based on the rat model (Sohni et al., 1995; Ghoshal et al., 1996). Our study shows for the first time that the selected

Table 1
Effect of crude methanol extract of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice

| Test materials | Dose (mg/kg per day) | Number of mice cleared/treated (% cured) | Average caecal score[a] (range) | |
|---|---|---|---|---|
| | | | Contents | Walls |
| *Piper longum* fruit | 125 | 0/15 (0) | 0.4 (0–1) | 0.4 (0–1) |
| | 250 | 7/15 (46) | 0 (0–0) | 0 (0–0) |
| | 500 | 14/15 (93) | 0 (0–0) | 0 (0–0) |
| | 1000 | 15/15 (100) | 0 (0–0) | 0 (0–0) |
| *Piper sarmentosum* root | 125 | 0/15 (0) | 2 (2–2) | 2 (2–2) |
| | 250 | 0/15 (0) | 2 (2–2) | 2 (2–2) |
| | 500 | 0/15 (0) | 1.4 (0–2) | 1.26 (0–2) |
| | 1000 | 6/15 (40) | 0.2 (0–1) | 0.2 (0–1) |
| *Quercus infectoria* nut gall | 125 | 0/15 (0) | 1.06 (0–2) | 0.93 (0–3) |
| | 250 | 2/15 (13) | 0.62 (0–2) | 0.62 (0–2) |
| | 500 | 4/15 (26) | 0.25 (0–2) | 0.25 (0–2) |
| | 1000 | 4/15 (26) | 0.01 (0–2) | 0.01 (0–2) |
| Metronidazole | 62.5 | 9/15 (60) | 0.06 (0–1) | 0.06 (0–1) |
| | 125 | 15/15 (100) | 0 (0–0) | 0 (0–0) |
| Untreated control | – | 0/20 (0) | 2.55 (2–3) | 2.4 (2–3) |

[a] Caecal scores were graded upon the following criteria (Neal, 1951).
Wall: normal, 0; slight thickening, 1; marked local thickening and contraction, 2; extensive thickening and contraction, 3; caecum shapeless (extensive ulceration with abscess formation), 4.
Contents: normal, 0; slightly less solid than normal, 1; slightly mucoid, 2; mucoid (some solid matter present), 3; no solid matter (white or yellow mucus only), 4.

medicinal plant also reduces the severity of caecum due to *Entamoeba histolytica* infection in mice.

The pooled controls of 20 mice were all positive for amoebae at the time of sacrifice. This amoebic infection generally produced score of caecal content and caecal wall ranging between 2 and 3 with the average of 2.55 and 2.40, respectively. This indicates the virulence of the strain of *Entamoeba histolytica* used in this study. Although, this strain was originally isolated from human bloody stool diarrhea, it was still infective in mice. It is generally known that axenic strain of *Entamoeba histolytica* becomes non-invasive after prolonged cultivation in vitro (Phillips et al., 1972; Phillips, 1973). We found that the amoebae isolated from the control mice infected with this strain of *Entamoeba histolytica* was still virulence and could be used subsequently.

In the present study, mice treated with metronidazole at a concentration of 125 mg/kg per day for 5 days were successfully cured from amoebiasis, confirming that this strain of *Entamoeba histolytica* was still sensitive to this drug. Our results on efficacy of metronidazole were similar to the studies of several investigators whose studies on caecal amoebiasis were performed, both in rats and mice models (Bhopale et al., 1995; Sohni et al., 1995; Ghoshal et al., 1996).

The extract from Plf appeared to be the most effective at a concentration of 1000 mg/kg per day, as this dose cleared all *Entamoeba histolytica* from the intestine of mice on the day of examination. This is comparable to metronidazole at the dose 125 mg/kg per day. Although treatment with extract from Plf at a concentration of less than 1000 mg/kg per day did not cure all animals, the caecal content and caecal wall of these mice appeared normal indicating the effectiveness of the extract against the parasites. The use of this extract to treat amoebiasis may at least help in reducing severity occurred in the intestine. Our finding from this study on the effect of Plf on *Entamoeba histolytica* are consistent with those previously reported that an ethanol extract of Plf at a concentration of 1000 mg/ml per day can cure 90% of rats infected with *Entamoeba histolytica* (Ghoshal et al., 1996). Although Plf is effective for the treatment of amoebiasis in rodents, the mode of actions of Plf extract against *Entamoeba histolytica* is unknown. An in vitro study showed that allicin from freshly crushed garlic inhibited the activity of cysteine proteinases, an important contributor to amoebic virulence (Ankri et al., 1997). In addition, piperine which is widely known to be a major constituent of Plf is not effective as an amoebicide either in vitro or in vivo (Ghoshal et al., 1996). Further investigations are therefore needed to identify an active compound of this extract and to determine whether the alteration of the enzyme activity is the target mode of action of this extract.

The methanol extracts from Psr and Qin appeared to be effective against caecal amoebiasis in mice in this study. However, the effect of these extracts on the amoebiasis seem to be much less potent than that of Plf. It is unlikely therefore that their antidysenteric activity occur solely from the antiamoebic activity. Their mode of actions on the treatment of dysentery have yet to be determined.

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# Memory enhancing activity of *Glycyrrhiza glabra* in mice

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**Abstract**

In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* (family: Leguminosae) have been employed clinically for centuries for their anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities. The present study was undertaken to investigate the effects of *Glycyrrhiza glabra* (popularly known as liquorice) on learning and memory in mice. Elevated plus-maze and passive avoidance paradigm were employed to test learning and memory. Three doses (75, 150 and 300 mg/kg p.o.) of aqueous extract of *Glycyrrhiza glabra* were administered for 7 successive days in separate groups of animals. The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice. Furthermore, this dose significantly reversed the amnesia induced by diazepam (1 mg/kg i.p.) and scopolamine (0.4 mg/kg i.p.). Anti-inflammatory and antioxidant properties of liquorice may be contributing favorably to the memory enhancement effect. Since scopolamine-induced amnesia was reversed by liquorice, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission in mouse brain. However, further studies are necessitated to identify the exact mechanism of action. In the present investigation, *Glycyrrhiza glabra* has shown promise as a memory enhancing agent in all the laboratory models employed.
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*Keywords:* Liquorice; Amnesia; Learning; Memory

## 1. Introduction

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Dementia is of several types and it invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning and memory processes (Nabeshima, 1993). Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals (Higashida and Ogawa, 1987) and human beings (Sitaram et al., 1978). Epidemiological studies of Indian population reveal that dementia is largely a hidden problem (Shaji et al., 2002). Prevalence rates for dementia increase exponentially with advancing age (Kawas et al., 2000; Vas et al., 2001). Since allopathic system of medicine is yet to provide a radical cure, it is worthwhile to look for new directions, which would minimize the memory loss seen in elderly patients.

In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* (family: Leguminosae) have been in clinical use for centuries. The roots have antiulcer, expectorant, diuretic, laxative, sedative (Hikino, 1985), antipyretic (Lata et al., 1999), antimicrobial and anxiolytic activities (Ambawade et al., 2001). The main constituent of *Glycyrrhiza glabra* is glycyrrhizin which has antiviral (Ceremelli et al., 1996) anti-inflammatory (Yokota et al., 1998) and antioxidant action (Ju et al., 1989). In Ayurveda, it is used extensively to relieve "Vata" and "Kapha" inflammations. Ayurvedic system of medicine is based on three fundamental principles or doshas called Vata, Pitta and Kapha. These doshas govern all cellular processes responsible for healthy life. Vata governs all movements/activities, Pitta governs heat/energy levels and regulates various transformations whereas, Kapha controls growth, structural modifications and lubrication. When these principles, which guide the processes of our body/mind get disturbed in an individual due to bad environment or poor diet the individual starts suffering from some disease. For instance, when, Vata gets out of balance, the consequences are hyper-active mind, circulatory disorders, poor neurotransmission, irreg-

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ular elimination and uncomfortable menses. If Pitta is disturbed, we observe excessive acidity resulting in heartburn, peptic ulcers, hot temper and inflammations. Whereas, if Kapha gets out of balance, the result is chronic congestion, weight gain, high cholesterol levels and acne.

### 1.1. Objective

The present study was undertaken to investigate the effects of *Glycyrrhiza glabra*, popularly known as liquorice on learning and memory in mice.

## 2. Materials and methods

### 2.1. Animals

Male Swiss albino mice (3 months old), weighing around 25 g and procured from disease free small animal house, CCS Haryana Agriculture University, Hisar (Haryana) were used in the present study. They had free access to food and water, and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. Food given to mice consisted of wheat flour kneaded with water and mixed with small amount of refined vegetable oil. The animals were acclimatized for at least 5 days before behavioral experiments. Experiments were carried out between 09:00 and 14:00 h. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) and care of laboratory animals was taken as per CPCSEA guidelines (Reg. No. 0436).

### 2.2. Drugs

Liquorice powder (Himalaya Drug Company, Bangalore, India), scopolamine hydrobromide (Sigma-Aldrich, USA) and diazepam (Ranbaxy, India) were used in the present study.

### 2.3. Laboratory models for testing learning and memory

(i) Scopolamine-induced amnesia (Interoceptive Behavior Model).
(ii) Diazepam-induced amnesia (Interoceptive Behavior Model).
(iii) Elevated plus-maze (Exteroceptive Behavior Model).

Elevated plus-maze served as the exteroceptive behavior model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory was followed as per the parameters described by the investigators working in the area of neuropsychopharmacology (Itoh et al., 1990; Reddy and Kulkarni, 1998; Dhingra et al., 2003; Parle and Dhingra, 2003). The apparatus consisted of two open arms (16 cm × 5 cm) and two enclosed arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was the time taken by mouse with all its four legs to move into one of the enclosed arms. TL was recorded on the first day. If the animal did not enter into one of the enclosed arms within 90 s, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for another 10 s and then returned to its home cage. Retention of this learned-task was examined 24 h after the first day trial. Another laboratory model, viz. passive avoidance apparatus was employed to substantiate the findings and overcome the limitations of elevated plus-maze.

(iv) Passive avoidance paradigm (Exteroceptive Behavior Model).

Passive avoidance behaviour based on negative reinforcement was used to examine the long-term memory (Reddy and Kulkarni, 1998; Parle and Dhingra, 2003). The apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V ac) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 s and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from wood platform to grid floor with all its paws on the grid floor. Animals showing SDL in the range (2–15 s) during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. When the animals stepped down before 60 s, electric shocks were delivered for 15 s. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 s. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 s.

### 2.4. Preparation of liquorice extract

Liquorice extract was prepared (Indian Pharmacopoeia, 1966) by extracting liquorice powder with chloroform water (0.1%) in the ratio of 1:8 by double maceration (each maceration for 24 h). The aqueous extract obtained was passed through muslin cloth and the filtrate was boiled for 5 min. This extract was then set aside for 18 h and was filtered again using filter paper. The extract was concentrated until the density of the liquid extract became 1.06 g/ml. The solid contents of the aqueous extract were 0.03 g/ml. The yield of

the extract was 34.6%. The extract was administered orally to separate groups of mice in three different doses 75, 150 and 300 mg/kg (equivalent to 2.6, 5.19 and 10.38 g, respectively, of dried plant material). The response of animals to these doses was observed after 90 min. These doses were selected on the basis of our pilot study and earlier reports (Al-Qarawi et al., 2002).

### 2.5. Vehicle

Liquorice extract was diluted in distilled water. Scopolamine hydrobromide was dissolved in normal saline. Injection of diazepam (Calmpose®) was diluted in normal saline. Volume of oral administration and i.p. injection was 1 ml/100 g of mouse.

### 2.6. Drug protocol

Animals were divided into 14 groups and each group comprised of a minimum of five animals. Groups I to X represent observations on elevated plus-maze and groups XI to XIV represent observations using passive avoidance paradigm.

#### 2.6.1. Using elevated plus-maze

Group I: control group for elevated plus-maze ($n = 6$): distilled water (1 ml/100 g) was administered p.o. for 7 days. After 90 min of administration on 7th day, transfer latency was recorded. Retention of learned task was examined after 24 h.

Groups II, III and IV ($n = 5$ each): liquorice aqueous extract (75, 150 and 300 mg/kg, respectively) was administered orally for 7 days. TL was noted after 90 min of administration on 7th day and after 24 h.

Groups V and VI ($n = 5$ each): scopolamine hydrobromide (0.4 mg/kg i.p.) and diazepam (1 mg/kg i.p.), respectively, were injected before training. TL was recorded after 45 min of injection. Retention was examined after 24 h.

Group VII ($n = 5$): TL was recorded on first day. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording TL on second day.

Group VIII ($n = 5$): liquorice extract (150 mg/kg) was administered for 7 days p.o. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. after 90 min of administration of liquorice extract on 7th day. TL was recorded after 45 min of injection and after 24 h.

Group IX ($n = 5$): liquorice extract (150 mg/kg) was administered for 7 days p.o. TL was recorded after 90 min of administration of extract. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording TL on 8th day.

Group X ($n = 5$): liquorice extract (150 mg/kg) was administered orally for 7 days. Diazepam (1 mg/kg) was injected i.p. after 90 min of administration of liquorice extract on 7th day. TL was recorded after 45 min of injection and after 24 h.

#### 2.6.2. Using passive avoidance paradigm

Group XI: control group for passive avoidance paradigm ($n = 6$): distilled water (1 ml/100 g) was administered p.o. for 7 days. After 90 min of administration on 7th day, SDL was recorded during both the sessions of training. Retention of learned task was examined after 24 h.

Group XII ($n = 5$): liquorice extract (150 mg/kg) was administered orally for 7 days. SDL was recorded after 90 min of administration on 7th day and after 24 h.

Group XIII ($n = 5$): animals were trained on first day and SDL was recorded during both sessions of training. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording SDL on second day.

Group XIV ($n = 5$): liquorice extract (150 mg/kg) was administered for 7 days p.o. SDL was recorded after 90 min of administration of liquorice extract. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording SDL on 8th day.

### 2.7. Statistical analysis

All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's '$t$' test. $P < 0.05$ was considered as statistically significant.

## 3. Results

### 3.1. Effect on transfer latency (using elevated plus-maze)

TL of first day reflected learning behavior of animals whereas, TL of second day reflected retention of information or memory. Liquorice extract (75 mg/kg) administered for 7 days orally did not have any significant effect on TL of first day of training and on second day as compared to control. The higher dose (150 mg/kg) of the extract significantly decreased TL on first day as well as on second day, indicating significant improvement of learning and memory (Table 1). Surprisingly, the highest dose (300 mg/kg) of the extract significantly increased TL of first day, indicating significant impairment in learning. The dose-selection of scopolamine hydrobromide and diazepam was based on our earlier studies (Parle and Dhingra, 2003). Scopolamine hydrobromide (0.4 mg/kg) injected before training impaired learning significantly as indicated by increased TL. While scopolamine injected after training impaired memory significantly. Diazepam (1 mg/kg) injected before training impaired learning significantly. Liquorice extract (150 mg/kg) administered orally for 7 days protected the animals from scopolamine- and diazepam-induced impairment in learning and memory.

### 3.2. Effect on step-down latency (SDL)

Liquorice extract (150 mg/kg) administered for 7 days significantly increased SDL (242 ± 39.1) as compared to con-

Table 1
Effect of liquorice extract on transfer latency (TL) of mice using elevated plus-maze paradigm

| Group no. | Treatment | Dose ($kg^{-1}$) | TL on 1st/7th day | TL after 24 h |
|---|---|---|---|---|
| I | Control (vehicle) | 10 ml | 25.5 ± 3.0 | 18.5 ± 2.0 |
| II | Liquorice extract for 7 days p.o. | 75 mg | 23.7 ± 3.5 | 14.8 ± 2.8 |
| III | Liquorice extract for 7 days p.o. | 150 mg | 15.2 ± 1.1[a] | 12.3 ± 1.2[a] |
| IV | Liquorice extract for 7 days p.o. | 300 mg | 34.6 ± 1.8[a] | 24.4 ± 1.8 |
| V | Scopolamine HBr (before training) | 0.4 mg i.p. | 52.2 ± 6.3[a] | 30.4 ± 6.6 |
| VI | Diazepam (before training) | 1 mg i.p. | 49.7 ± 7.0[a] | 30.4 ± 6.8 |
| VII | Scopolamine HBr (after training) | 0.4 mg i.p. | 24.7 ± 8.6 | 44.8 ± 13.2[a] |
| VIII | Liquorice extract for 7 days + scopolamine HBr (before training) | 150 mg, 0.4 mg | 15.6 ± 2.6[b] | 10.7 ± 1.4[b] |
| IX | Liquorice extract for 7 days + scopolamine HBr (after training) | 150 mg, 0.4 mg | 15.1 ± 1.8 | 15.3 ± 3.1[b] |
| X | Liquorice extract for 7 days + diazepam (before training) | 150 mg, 1 mg | 16.7 ± 1.8[c] | 10.5 ± 2.2[c] |

Values are in Mean ± S.E.M.
[a] $P < 0.05$ as compared to control group.
[b] $P < 0.05$ as compared to scopolamine alone (before/after training).
[c] $P < 0.05$ as compared to diazepam alone (before training).

trol group (119.5 ± 35.7) on second day indicating improvement of memory. Scopolamine hydrobromide (0.4 mg/kg) significantly decreased SDL (15.6 ± 2.2) on second day indicating impairment of memory (amnesia). Liquorice extract (150 mg/kg) administered orally for 7 days significantly reversed amnesia (SDL = 23.4 ± 1.8) induced by scopolamine hydrobromide.

## 4. Discussion

In the present study, 150 mg/kg of liquorice extract (equivalent to 5.19 g of dried plant material) administered orally for 7 days improved learning and memory of mice significantly in both the exteroceptive behavioral models employed. The stimulus lie outside the body in exteroceptive behavior models, whereas, it lies within the body in the case of interoceptive models. This is the first research finding showing enhancement of learning and memory by liquorice. Furthermore, pretreatment with liquorice extract (150 mg/kg) for 7 days protected the animals from learning and memory impairment produced by interoceptive stimuli (scopolamine and diazepam). These findings suggested the possible neuroprotective role for liquorice. The impairment of learning due to the highest dose (300 mg/kg) of the extract probably represented the lethal effect of the extract. This paradoxical effect could also be due to the sedative property of the drug (Hikino, 1985). Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain in Alzheimer's disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in Alzheimer's disease patients. Moreover, it has also been observed that elderly patients suffering from Alzheimer's disease showed reduction in symptoms of Alzheimer's disease upon chronic use of anti-inflammatory drugs (McGeer and McGeer, 1999). Indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electroconvulsive shock-induced retrograde amnesia and also against amyloid deposits in the brain (Rao et al., 2002; Stephan et al., 2003). Anti-inflammatory action of liquorice (Yokota et al., 1998) might also be contributing to the observed memory-enhancing activity of liquorice. Oxygen free-radicals are implicated in the process of ageing and may be responsible for the development of Alzheimer's disease in elderly persons (Sinclair et al., 1998). Oxygen-free radicals and other products of oxidative metabolism have been shown to be neurotoxic (Sayre et al., 1997) and antioxidant-rich diets improved cerebellar physiology and motor learning in aged-rats (Bickford et al., 2000). The protective effect of liquorice extract may be attributed to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function, thereby enhancing the memory. Furthermore, liquorice has been found to possess antioxidant property as well (Ju et al., 1989). Thus, a combination of anti-inflammatory, antioxidant and neuroprotective role could all be leading to the net memory-enhancing effect.

## 5. Conclusion

In the present investigation, *Glycyrrhiza glabra* has shown promise as a memory enhancing agent in mice in all the laboratory models employed.

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Short communication

# Anti-inflammatory evaluation of *Ionidium suffruticosam* Ging. in rats

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**Abstract**

The anti-inflammatory activity of *Ionidium suffruticosam* (Violaceae) methanol extract was evaluated on carrageenin, histamine and serotonin-induced rat hind paw oedema acute models. The extract at doses of 200 and 400 mg/kg has been found to possess significant anti-inflammatory activity on the tested experimental models. The extract at the dose level of 400 mg/kg exhibited maximum anti-inflammatory activity in all the animal models. In a chronic test, the extract (400 mg/kg) showed 42.78% reduction in granuloma weight. The effect produced by the extract was comparable to that of phenylbutazone, a proto type of a non-steroidal anti-inflammatory agent.
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*Keywords: Ionidium suffruticosam*; Anti-inflammatory; Carrageenin; Histamine; Serotonin; Chronic test; Phenylbutazone

## 1. Introduction

Inflammation is commonly divided into three phases, acute inflammation, the immune system response and chronic inflammation. Rheumatoid arthritis is an important in pain and destruction of bone and cartilage that can lead to severe disability and in which systemic changes occur that can result in shortening of life (Katzung, 1998). A review of plants exhibiting anti-inflammatory activity showed that different species of 96 genera belonging to five families have such activity (Handa et al., 1992). *Ionidium suffruticosam* Ging. is a plant, which is found abundantly in India, Africa, and Australia (Kirtikar and Basu, 1975). The plant is well known as Ratanpuras (Hindi), Nunbora (Bengal), and Orilaitamarai (Tamil) in Indian traditional medicine. In ayurvedic system of medicine, this plant is used in diuretic, demulcent, and tonic (Nadkarni et al., 1996). It is also useful in the treatment of asthma, epileptic fits, cough, pain and dysentery (Kirtikar and Basu, 1975). The claim that the anti-inflammatory activity of *Ionidium suffruticosam* resides in the plant is speculative and has not yet been documented. In this study, we have attempted to investigate the anti-inflammatory activities of this plant of methanol extract.

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## 2. Materials and methods

### 2.1. Plant material

Whole plants of *Ionidium suffruticosam* Ging. were collected by the traditional herbal collectors Bankura, West Bengal, India during the month of July and August. Taxonomical identification of the plant was done by The Botanical Survey of India, Shibpur, Howrah, and West Bengal. The voucher specimen DJU/SCM-IS11 was preserved in our laboratory for future reference. The plants were collected freshly and dried under shade, pulverised by a mechanical grinder then passed through a 40 mesh sieve and stored in a closed vessel for further use.

### 2.2. Preparation of extract

The shade dried powdered plant were extracted by using methanol as a solvent in a soxhlet extraction apparatus. The solvent was removed under vacuo, a semisolid mass (9.8% w/w with respect to the dry starting material) and was stored in a desiccator. For pharmacological experiments, a weighed amount of the dried extract was suspended in a 2% (v/v) aqueous Tween 80 solution.

### 2.3. Phytochemical screening

On preliminary screening, the extract showed the positive Liebermann–Burchard reaction for steroids and posi-

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Table 1
Effect of *Ionidium suffruticosam* on carrageenin-induced paw oedema in rats

| Treatment | Dose | Oedema rate percentage (mean ± S.E.M.) | | | | |
|---|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h | 5 h |
| Control (ml) | 10 | 29.8 ± 1.1 | 37.6 ± 1.3 | 41.1 ± 1.2 | 43.4 ± 1.1 | 46.4 ± 1.4 |
| Extract (mg/kg) | 200 | 27.7 ± 1.0 (7.0) | 32.4 ± 1.1** (13.9) | 34.6 ± 1.1** (15.9) | 37.2 ± 1.0** (14.3) | 38.6 ± 1.1** (16.9) |
| Extract (mg/kg) | 400 | 26.2 ± 1.2 (12.08) | 27.8 ± 1.5* (26.0) | 27.9 ± 1.3* (32.1) | 32.4 ± 1.3* (25.3) | 34.2 ± 1.4* (26.3) |
| Phenylbutazone (mg/kg) | 100 | 23.3 ± 3.4 (21.9) | 24.6 ± 1.3* (34.6) | 25.1 ± 1.4* (38.9) | 28.6 ± 1.6* (34.1) | 31.3 ± 1.2* (32.5) |

Figures in parenthesis indicate oedema inhibition percentage. $n = 6$ animals each group.
* $P < 0.001$ compared with control.
** $P < 0.01$ compared with control.

tive Noller test for triterpenoids (Noller et al., 1942), which were confirmed by thin layer chromatography study (Stahl, 1969). Further separation of the specific phytochemical is in progress.

### 2.4. Test animals

Male albino Wistar rats weighing 200–250 g supplied by M/s B.N. Ghosh & Co., Calcutta, India, were used for this experiment. The animals were deprived of food for 24 h before experimentation. All studies were carried out by using six rats in each group.

### 2.5. Carrageenin, serotonin and histamine-induced rat paw oedema models

Oedema was induced by subplantaer injection of 0.1 ml of 1% freshly prepared suspension of carrageenin (Sigma, USA) into the right hind paws of the rats of four groups of six animals each. The volume of the injected and contra-lateral paws were measured 1, 2, 3, 4 and 5 h after induction of inflammation using a plethysmometer according to the method described by Winter et al. (1962). The test groups received the extract (200 and 400 mg/kg), the standard group received phenylbutazone (100 mg/kg) and the control animals received the vehicle only. All the treatments were given orally 30 min prior to the injection of carrageenin. In a similar manner, the other models serotonins, histamine were also performed (Maity et al., 1998).

In all the above cases, the degree of hind paw volume plethysmographically before and 1, 2, 3, 4 and 5 h after carrageenin, serotonin and histamine injection. The oedema and inhibition rates were calculated as follows (Lin et al., 1994)

$$\text{oedema rate } (E)\ \% = \frac{V_1}{V_c} \times 100$$

$$\text{inhibition rate } (I)\ \% = \frac{E_c - E_t}{E_c} \times 100$$

where $V_c$ is the contra-lateral paw volume of the rats (left hind paw without stimulus infection) at $t$ (h), $V_t$ is the right hind paw volume of the rat (with stimulus injection) at $t$ (h), $E_c$ is the rate of the control group and $E_t$ is the oedema rate of the treated group.

### 2.6. Chronic test

The rats were divided into four groups with six animals in each group. After shaving off the fur, the animals were anaesthetised. Through a singly needle incision, sterile preweighed cotton pellets (20 mg) were implanted in the axilla region of each rat as described D'art et al. (1960) with slight modification. Methanol extracts (200 and 400 mg/kg), phenylbutazone (100 mg/kg) and control vehicle were administered orally to the respective group of animals for 7 consecutive days from the day of cotton-pellet implantation. On the eighth day, the animals were anaesthetised again, the cotton pellets were removed surgically and made free from extraneous tissues; incubated at 37 °C for 24 h and dried at 60 °C to constant weight. The increment in the dry weight of the pellets was taken as measure of granuloma formation (Winter and Porter, 1975).

Table 2
Effect of *Ionidium suffruticosam* on serotonin-induced paw oedema in rats

| Treatment | Dose | Oedema rate percentage (mean ± S.E.M.) | | | | |
|---|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h | 5 h |
| Control (ml) | 10 | 28.2 ± 1.3 | 36.1 ± 1.2 | 42.1 ± 1.0 | 44.6 ± 1.1 | 48.7 ± 1.4 |
| Extract (mg/kg) | 200 | 26.1 ± 1.1 (7.4) | 30.5 ± 1.0** (15.3) | 35.3 ± 1.1* (16.1) | 36.5 ± 1.3* (18.2) | 37.4 ± 1.1** (23.2) |
| Extract (mg/kg) | 400 | 24.5 ± 1.0 (13.1) | 26.8 ± 1.3* (25.8) | 28.3 ± 1.3* (32.8) | 32.6 ± 1.4* (26.90) | 34.9 ± 1.4* (28.3) |
| Phenylbutazone (mg/kg) | 100 | 23.5 ± 1.0 (16.7) | 24.1 ± 1.4* (33.2) | 25.2 ± 1.4* (40.1) | 27.4 ± 1.5* (38.6) | 28.9 ± 1.2* (38.6) |

Figures in parenthesis indicate oedema inhibition percentage. $n = 6$ animals each group.
* $P < 0.001$ compared with control.
** $P < 0.01$ compared with control.

Table 3
Effect of *Ionidium suffruticosam* on histamine-induced paw oedema in rats

| Treatment | Dose | Oedema rate percentage (mean ± S.E.M.) | | | | |
|---|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h | 5 h |
| Control (ml) | 10 | 29.9 ± 1.2 | 35.6 ± 1.1 | 41.6 ± 1.5 | 45.4 ± 1.5 | 49.4 ± 1.4 |
| Extract (mg/kg) | 200 | 27.2 ± 1.0 (9.0) | 30.2 ± 1.0** (15.1) | 35.3 ± 1.2** (15.14) | 37.1 ± 1.5** (18.3) | 38.6 ± 1.3* (21.9) |
| Extract (mg/kg) | 400 | 24.1 ± 1.4 (19.4) | 26.3 ± 1.2* (26.1) | 27.3 ± 1.3* (34.4) | 32.4 ± 1.1* (28.2) | 34.9 ± 1.4* (23.3) |
| Phenylbutazone (mg/kg) | 100 | 23.3 ± 1.2 (22.0) | 23.0 ± 1.4* (35.4) | 23.9 ± 1.3* (42.5) | 28.2 ± 1.3* (37.7) | 30.9 ± 1.2* (37.4) |

Figures in parenthesis indicate oedema inhibition percentage. $n = 6$ animals each group.
* $P < 0.001$ compared with control.
** $P < 0.01$ compared with control.

Table 4
Effect of *Ionidium suffruticosam* plant extract on weight of granuloma in rats

| Treatment | Dose | Weight of granuloma (mean ± S.E.M.) | Inhibition (%) |
|---|---|---|---|
| Control (ml) | 10 | 42.3 ± 1.3 | – |
| Extract (mg/kg) | 200 | 32.4 ± 1.5** | 23.4 |
| Extract (mg/kg) | 400 | 24.2 ± 1.3* | 42.8 |
| Phenylbutazone (mg/kg) | 100 | 19.8 ± 1.6* | 53.2 |

$n = 6$ animals each group.
* $P < 0.001$ compared with control.
** $P < 0.01$ compared with control.

### 2.7. Statistical analysis

The results were expressed as mean ± S.E.M. The significance was evaluated by Student's *t*-test compared with control. $P < 0.05$ was considered significant (Woodson, 1987).

## 3. Results

The anti-inflammatory activity of the extract of *Ionidium suffruticosam* against pedal oedema has been shown in Tables 1–3, which showed significant anti-inflammatory activity and the results were comparable to that of phenylbutazone, a prototype of a non-steroidal anti-inflammatory drug. The extract (400 mg/kg, p.o.) showed a maximum 32.11% inhibition in carrageenin, 32.17% inhibition on serotonin, 34.37% inhibition in serotonin-induced rat paw oedema. In chronic tests, the extract (400 mg/kg) showed 42.78% reduction in granuloma weight (Table 4).

## 4. Discussion

The present study establishes the anti-inflammatory activity of the methanol extract of the plant of *Ionidium suffruticosam* in the models used. Carrageenin-induced oedema is commonly used as an experimental model for evaluation the anti-inflammatory potential of natural products (Winter et al., 1962; Della Loggia et al., 1986) and is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after the administration of carrageenin; amore pronounced second phase is attributed to release of bardykinin protease, prostaglandin and lysosome. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents (Castro et al., 1968). The extract effectively suppressed the inflammation produced by histamine and serotonin. So it may be suggested that its anti-inflammatory activity is possibly backed by its anti-serotonin activity, which is responsible for the same. The extract exhibited significant anti-inflammatory activity in the cotton pellet test. This reflected its efficacy to a high extent to reduce an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation (Arrigoni-Martellie, 1977). The effect of the extract in the inflammation process induced by stimulus injection indicates that they act by affecting a time-delayed system in a similar fashion to glucocorticoids.

This suggests that the active principle (steroid and triterpenoid) of the extract have some degree of affinity for the glucorticoid receptors. The methanol extract of *Ionidium suffruticosam* exhibited significant anti-inflammatory potential at the dose levels examined. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

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