STUDY

on the

ASSESSMENT OF PLANTS/HERBS, PLANT/HERB EXTRACTS AND THEIR NATURALLY OR SYNTHETICALLY PRODUCED COMPONENTS AS "ADDITIVES" FOR USE IN ANIMAL PRODUCTION

CFT/EFSA/FEEDAP/2005/01

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FOREWORD

In September 2003 the European Parliament and the Council of the European Union adopted the Regulation No. 1831/2003 on additives for use in animal nutrition. The purpose of this regulation is to establish a Community procedure for authorising the placing on the market and use of feed additives and to lay down rules for the supervision and labelling of feed additives and premixtures in order to provide the basis for the assurance of a high level of protection of human health, animal health and welfare, environment, and "users and consumers" interests in relation to feed additives, whilst ensuring the effective functioning of the internal market.

Any person seeking an authorisation for a feed additive or for a new use should submit an application to the European Commission and the European Food Safety Authority (EFSA), as provided by Article 7 of Regulation No. 1831/2003 at least one year before the expiry date of the previous authorisation for additives with a limited authorisation period, and within a maximum of seven years after the 18th October 2004, date of entry into force of this Regulation for additives authorised without a time limit or pursuant Directive 82/471/EEC.

The guidelines for the applicants to prepare the dossier for the authorisation of additives in animal nutrition are provided according to Directive 2001/79/EC, until specific guidelines following Regulation No. 1831/2003 will be established by the Panel on Additives and Products of Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA).

Under the current regulation detailed guidelines for some family of products are completely missing, that it is the case of the plants/herbs, plant/herb extracts and their naturally or synthetically produced components. For this reason it is one of the priorities of the FEEDAP Panel to implement guidelines/guidance for these types of compounds.

It has been observed that plant/herbs and their extracts are increasingly being used (and informally marketed) not only as sensory additives, but also for other purposes not covered by the legislation, notably better growth or feed conversion, improved meat quality, and for prophylactic purposes. There are strong indications that such use will further increase as replacement additives for the antibiotic growth promoters whose use will be prohibited by the end of 2005.

Nowadays, a compiled list of these compounds or extracts does not exists although the European Federation of Animal Feed Additive Manufacturers (FEFANA) notes that some 280 plant extracts and a further 1700 chemically defined compounds have been used within Member States as sensory additives in animal feeds.

The results of this study will be used by EFSA, in consultation with the European Commission, Member States and other stakeholders, to provide input for the development of guidance/guidelines documents for the authorisation of plants/herbs, plant/herb extracts and their naturally or synthetically produced components for use in animal nutrition.

Claudia Roncancio Peña

FEEDAP Panel

ASSESSMENT OF PLANTS/HERBS, PLANT/HERB EXTRACTS AND THEIR NATURALLY OR SYNTHETICALLY PRODUCED COMPONENTS AS "ADDITIVES" FOR USE IN ANIMAL NUTRITION

CFT/EFSA/FEEDAP/2005/01

INTRODUCTION

The last two decades have seen a substantial increase in use of herbs/botanicals and their products not only as herbal medicinal products and for food supplements, but also in the field of animal nutrition. The use of herbal feed additives, which include essential oils and (exotic) herbal mixtures, is extensively promoted by the producers, but the scientific background underpinning their use often is limited. Consequently an adequate knowledge of quality control (content of active substance(s) stability), efficacy and safety is often lacking. A critical examination of bioactive plant products has to cover analytical aspects, absorption, bioavailability and molecular functionality in addition to feeding experiments and technology development. Finally, quality assurance management (analytical methods) is considered an absolute prerequisite.

Presently herbs/botanicals are used by the feed industry only as sensory additives, functional group "flavouring compounds". These are defined by Annex I of Regulation (EC) No 1831/2003 as "substances the inclusion of which in feedingstuffs increases smell or palatability". This regulation foresees the evaluation of all herbal products and their derived extracts currently on the market and notified to the European Commission. This is to be completed by 2010.

BACKGROUND

The challenge that food suppliers face is to accept the consequences of consumers' demands and aspirations and to become more proactive in developing scientific approaches to meet these demands. In this context, sustainable farming must be guided by the following principles:

- *animal friendly* - scientifically proven to respect the welfare of livestock and to enhance their physiological condition through nutrition
- *consumer friendly* – 100% natural, traceable, no risk of pathogenic cross-resistance, non-GM products, no harmful effects on humans
- *environmentally friendly* – products that actively reduce the environmental impact of intensive livestock production through the reduction of both atmospheric emissions and non-organic effluents.

In-feed antibiotics have been used for growth promotion and prophylaxis against enteric pathogens for the last 50 years. However, recognition that the continuing use of antibiotics for growth promotion contributed to a growing resistance among human pathogens has resulted in these additives have been withdrawn stepwise since 1994. This withdrawal was completed in 2006 and the use of antibiotics for growth promotion is now prohibited in the EU member states.

Proposed alternatives to in-feed antibiotics are extremely diverse and include organic acids, pre- and pro-biotics, enzymes and especially herbs and herbal products/botanicals. For most of the ever-evolving area of feed additives, the *flavour and appetizing substances* based on plants/plant extracts represent a real alternative both for the European feed industry and the livestock production. Although the understanding of their modes of action is a prerequisite for their optimal application and use, in terms of efficacy, at the moment, a full understanding of these aspects in animals is not achieved yet. For example, phytocompounds act along the animal's digestive tract to improve appetite, bacterial modulation, and are able to induce a number of very positive benefits on well being (Kamel, 2001). The antimicrobial properties of

plant extracts and herbs can be dose-dependently bacteriostatic and/or bactericidal. In addition, several investigations have been performed concerning the changes in digestive physiology and digestion at weaning (Zabielski *et al*., 1999), the microbiology of the gut (Jensen, 1998) and the implementation of test models in poultry (Hess, 2002).

Herbs and herbal products/botanicals represent a large array of nutraceuticals, which are defined as "any non-toxic feed component that has scientifically proven health benefits including disease prevention or treatment" (Boothe, 1997). Many classes of plant products have been shown to have antimicrobial activity including phenolics, quinones, flavones, tannins, coumarins, several compounds of essential oils (mono- and sesquiterpenes, phenylpropanoids) and isothiocyanates. One perceived advantage is that many of these plant products occur in complex mixtures and not only as single compounds, hence resistance is less likely to become a problem than with conventional synthetic compounds. An often investigated group is represented by essential oils, especially those containing the phenolic compounds thymol and carvacrol. A review made by Gollnisch *et al.* (2001) has shown, however, that the main parameter studied is the weight gain and less attention has been given to the influence of these compounds on the microflora and particularly the pathogenous microflora and their elimination. The distribution of multi-resistant strains is however decisive for the health status of the animal population.

Another interesting group of secondary plant products are tannins which can confer benefits on animal production and animal health (Caygill and Mueller-Harvey, 1999). The assessment of tannins is however complicated and the data available are partly contradictory due to the fact that this class of compounds comprise many different molecules, some of which have detrimental toxic effects.

Because of the need to asses the safety of all herbal and related products seen under Regulation (EC) No 1831/2003 and because of the potential for herbs and herbal products/botanicals to contribute as zootechnical additives in animal nutrition, the European Food Safety Authority (EFSA) identified the need to collect further information on the efficacy and safety of these products. The immediate aim is to use this data to develop guidelines for their evaluation, following the Regulation (EC) No 1831/2003. A secondary objective is to assess the scientific evidence for effects in livestock other than that driven by their sensory properties in anticipation of herbal-based additives seeking authorisation for use for purposes other than flavouring of feed.

In a first step, a background document has been prepared dealing with the most crucial points of botanicals and botanical preparations identified for being used in animal nutrition. For a selected group of herbs/botanicals model monographs have been elaborated compiling the most recent information on the following issues (Table 1).

Table 1. **Structure and areas of the monographs**

No	Chapter	Section
1	Introduction	a Background experience with the plant b Historical use of the plant
2	Description of the plant	a Systematic b Occurrence c Plant description d Used plant parts and products
3	Phyto-chemical constituents	a Chemistry b Variation / Diversity c Analysis d Specifications, Standardisation
4	Pharmacology	a In vitro b Human studies c Animal studies: ADME
5	Antimicrobial effects	
6	Efficacy / Quality of the animal product	
7	Toxicology / Safety	

The reasons for selecting particular plants/herbs (Table 2) were specific but the final selection was made to illustrate the diversity of the notified sensory additives, taking into account particularly:

- indigenous (European) *vs.* exotic species (some of them introduced since long as culinary herbs or herbal teas; some of them rather 'new');
- plants with clear taxonomic identification *vs.* genera with a number of closely related species showing a high chemical variation;
- cultivated plants as well as plants collected from the wild;
- plants commonly/frequently used as dietary (food) supplements (herbal teas) *vs.* very specifically used species;
- herbs containing typical flavouring agents (e.g. essential oils or bitter substances) *vs.* such of unclear 'sensory' properties;
- species of different pharmacological/toxicological profile.

Inevitably the monographs vary in size and completeness reflecting the availability of published data: some species are well known and documented due to their traditional human (medicinal) use; some other, used since ancient times as food, food additives or fodder crops, are generally recognized as safe, but in some case needing a revised risk assessment due to recent data. Finally, there are relatively 'new' (exotic) species coming from traditional use in other parts of the world which partly still lacking relevant data.

Table. 2: Selected Group of Plants used as Sensory Additives in Animal Feeds (according to Annex I to Part III . 2 of the Contract)

Number	English Name	Latin Name	Page number
1	Yarrow, Milfoil	*Achillea millefolium L.*	*9*
2	Onion	*Allium cepa L.*	*18*
3	Leek	*Allium porrum L.*	*18*
4	Garlic	*Allium sativum L.*	*18*
5	Aloe	*Aloe vera L., A. barbadensis, A. capensis*	*43*
6	Tragant	*Astragalus gummifer Labill.*	*53*
7	Borage	*Borago officinalis L.*	*61*
8	Marigold, Goldbloom	*Calendula officinalis L.*	*74*
9	Common celandine	*Chelidonium majus L.*	*140*
10	Oregano, Spanish (see also 37)	*Coridothymus capitatus, Thymus capitatus*	*182*
11	Echinacea	*Echinacea sp.*	*88*
12	Eucalyptus	*Eucalyptus globulus Labill.*	*97*
13	Fennel, bitter	*Foeniculum vulgare Mill. ssp. vulgare*	*107*
14	Wintergreen	*Gaultheria procumbens L.*	*116*
15	Gentian	*Gentiana lutea L.*	*123*
16	Lavender	*Lavandula angustifolia Mill.*	*133*
17	Lavandin	*Lavandula hybrida (L. angustifolia x L. latifolia)*	*133*
18	Lavandin	*Lavandula hybrida (L.officinalis x L. latifolia)*	*133*
19	Spike lavender	*Lavandula latifolia L.*	*133*
20	Lavender	*Lavandula officinalis Chaich.*	*133*
21	Macleaya, Plume poppy	*Macleaya cordata (Willd.) R. Br.*	*140*
22	Camomile, German	*Matricaria recutita (L.)Rauschert*	*155*
23	Japanese mint	*Mentha arvensis L.*	*170*
24	Pennyroyal	*Mentha pulegium L.*	*170*
25	Spearmint	*Mentha spicata L. var. crispa*	*170*
26	Peppermint	*Mentha x piperita L.*	*170*
27	Oregano	*Origanum dictamnus L.*	*182*
28	Marjoram	*Origanum majorana L. (Majorana hortensis Moench)*	*182*
29	Oregano	*Origanum vulgare L. spp.*	*182*
30	Parsley	*Petroselinum crispum Mill.*	*194*
31	Parsley	*Petroselinum sativum Hoffm.*	*194*
32	Anise, Aniseed	*Pimpinella anisum L.*	*201*
33	Black currant	*Ribes nigrum L.*	*210*
34	Rosemary	*Rosmarinus officinalis L.*	*218*
35	Sage	*Salvia officinalis L.*	*230*
36	Milk thistle	*Silybum marianum L.*	*237*
37	Thyme	*Thymus capitatus. Hoffm. & Link*	*250*
38	Thyme	*Thymus vulgaris L.*	*250*
39	Thyme Spanish	*Thymus zygis L.*	*250*
40	Cats claw	*Uncaria tomentosa Roxb.*	*259*
41	Stinging nettle	*Urtica dioica L., Urtica urens L.*	*269*
42	Valerian root	*Valeriana officinalis L.*	*277*

ACHILLEA MILLEFOLIUM

Achillea millefolium L.

1. INTRODUCTION

Achillea millefolium L. has a long history of use as traditional herb medicine even in veterinary medicine (Ludwig, 1996; Moder, 1997; Hirtl, 2000). Preparations in the form of infusions, decoctions or fresh juices have been applied against anorexia, stomach cramps, flatulence, gastritis, enteritis, internal and external bleeding (coughing blood, nosebleed, hemorrhoidal and menstrual bleeding, bloody urine), wounds, sores, skin rash as well as dog and snake bites. Its name is derived from Achilles, a mythical Greek character, who carried it with his army to heal the wounds of his fellow soldiers. This medicinal utilisation is also reflected in some of its common name: Woundwort, nosebleed, blood wort, sanguinary, herb militaris, and knight's milfoil [Chandler *et al*., 1982].

2. DESCRIPTION OF THE PLANT

The perennial herb grows to a height up to 30-60 cm. Due to the numerous small hairs covering it, the plant appears grey-green. The stem is angular with lanceolate, dissected leaves ranging from 3-20 cm in length and 1-6 cm in width. The dissection is giving the leaves a feathery appearance. The flowers are arranged in flattened, terminal, loose heads or cymes and are most often white, although pink, magenta, and red also occur. Yarrow is flowering throughout the whole summer from May to October. The odour is highly aromatic and somewhat resembling chamomile [Chandler *et al*., 1982].

2.1 SYSTEMATICS

Achillea millefolium L. belongs to the genus *Achillea* with more than 120 species, divided into 5 sections: Sectio Millefolium (ADANS.) W.D.J. KOCH, Sectio Ptamica (MILL.) W.D.J. KOCH, Sectio Achillea, Sectio Babounya (DC.) O. HOFFM. and Sectio Arthrolepis (BOISS.) BOISS..
Solely plants of the *Achillea millefolium* group belonging to the Sectio Millefolium (ADANS.) W.D.J. KOCH are used as medicinal plant. This group consists of several species difficult to distinguish which again are polymorphic, e.g. *A. asplenifolia* VENT. and *A. setacea* WALDST. et KIT. (diploid), *A. collina* BECKER ex REICHENB. (tetraploid), *A. millefolium* L. sensu stricto and *A. distans* WALDST. et KIT. (hexaploid), and *A. pannonica* SCHEELE (octaploid). Due to the existence of several chemotypes the content and chemistry of the essential oil is highly complex [Mokute et al., 2003; Hofmann et al., 1992]. For pharmaceutical preparations at least 2% of essential oil with 0.02% proazulene are demanded (Ph. Eur.). Therefore, hexaploid and octaploid species considered as azulene-free should be avoided [Chandler *et al*., 1982].

2.2 OCCURRENCE

Yarrow is a common herb found in fields and urban waste places throughout the temperate and boreal zones of the northern hemisphere and, to a lesser extent, of the southern hemisphere [Tutin et al. 1976; Ohwi, 1965; Hultén, 1968; Holm et al., 1979; Fernald, 1950; Angier, 1978]. *Achillea millefolium* L. sensu stricto (*A. millefolium* L. s.str.) is the most widespread variety, found sparsely in southern Europe, abundantly throughout central and northern Europe, and has been widely introduced into North America. There *Achillea lanulosa* NUTT., a tetraploid species, is distributed. *Achillea borealis* BONG. is popular on the west coast from California to Alaska, and in the Arctic and Subarctic [Mulligan and Bassett, 1959]. In India it grows up to an altitude of 1.050-3.600 m in the temperate Himalaya [Agnihotri et al., 2005].

2.3 PLANT PARTS AND PRODUCTS

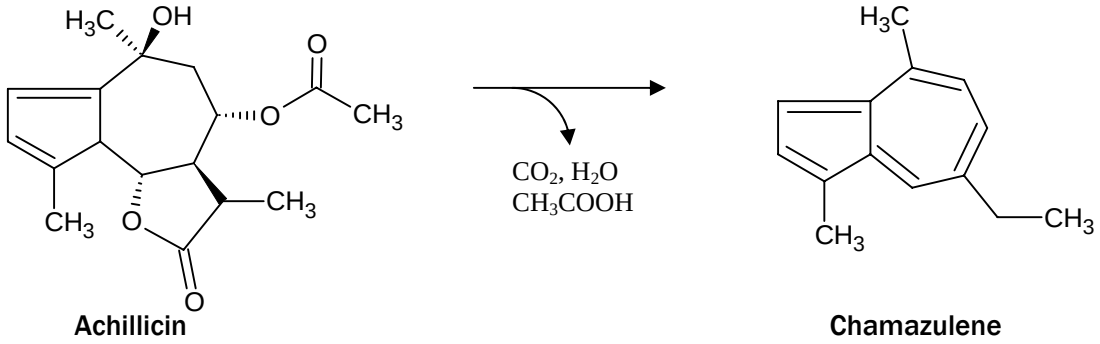
The plant parts used are the fresh or dried aerial parts or the whole fresh plant including the rhizomes of *Achillea millefolium* L.. The best quality crude drug is harvested from tetraploid (sub)species at time of full flowering and beginning of maximum development, where plants from native habitat show higher azulene content than from cultivated fields. Therefore, the drug in the European market is usually collected in the south of Europe and only few originates from cultivated plants. Thus, mixtures of the *A. millefolium* group with related species occur [Marchart and Kopp, 2003]. Furthermore, flower materials are best since stem content lowers azulene yield. The largest amount of proazulene has been found in buds or inflorescences at the beginning of flowering. After harvesting, shade drying at ordinary temperatures proved to be suitable, with azulene contents approximately 20% less than from the fresh plant. Alternatively, infrared drying can be used, however losses of 50% have to be accepted [Kucera, 1956]. The plants are processed to teas, juices, extracts (1:1 with 25% ethanol) and tinctures (1:5 with 45% ethanol) [Hänsel et al., 1992].

3. INGREDIENTS/CONSTITUENTS:

Yarrow contains a large number of therapeutically interesting active compounds. For the most medicinal effects the volatile components in the essential oil, including the sesquiterpene lactones, seem to be relevant. The oil content and composition are influenced by environmental conditions [Kowal and Pic, 1980; Verzar-Petri and Shalaby, 1977], the age of the plant [Ruminska, 1970], the part of the plant used [Racz-Kotilla and Racz, 1969; Yaskonis, 1971], and the season of harvest [Kowal and Pic, 1980; Racz-Kotilla and Racz, 1969; Verzar-Petri and Shalaby, 1977; Yaskonis, 1971]. Thus, the oil content changes during ontogenesis from 0.13% in the vegetative stage to 0.34% in the stage of full bloom. At the same time increasing amounts of monoterpenes in relation to the sesquiterpenes, and a decrease of azulene could be observed [Rohloff et al., 2000; Figueiredo et al. 1992a; Popescu and Katz, 1978; Popescu and Winand, 1977; Racz-Kotilla and Racz, 1969].

In numerous studies over 100 compounds of the essential oil isolated from the aerial parts of *A. millefolium* and roots have been identified [Figueiredo et al., 1992a; 1992b; Hofmann et al., 1992; Laurenco et al., 1999]. Relating to the chromosome number, azulene-containing (tetraploid) and azulene-free (hexaploid and octaploid) plants and difference in the proportion of mono- and sesquiterpenes exist. The major compounds of an oil obtained from the tetraploid species *A. collina* with a comparatively low proportion of only 34 % monoterpenes were chamazulene (25%), β-pinene (23%), caryophyllene (10%), and α-pinene (5%). The artefact chamazulene is formed from precursor compounds like achillicin (8 α-acetoxy-10-epi-artabsin), a sesquiterpene lactone, by the elimination of water and acetic acid and following decarboxylation during steam distillation. The structures of achillicin and chamazulene are given in Figure 3.1.. Further proazulenes are the sesquiterpene lactones 8 α-angelicoyloxy-10-epi-artabsin, 8 α-tigloxy-10-epi-artabsin, rupicolin A and rupicolin B [Kastner et al., 1991; Kastner et al., 1992; Zitterl-Eglseer et al., 1991]. In contrast, the oil of hexaploid *A. millefolium* contained 80% monoterpenes and no chamazulene. The main components of this oil were camphor (18%), sabinene (12%), 1,8-cineole (10%), α-pinene (9%), and isoartemisia ketone (9%)

[Hofmann et al. 1992]. The differences in the chemical compositions of the volatile oils emphasise the high importance of chromosome numbers.



Achillicin Chamazulene

Fig. 3.1: Structure of achillicin and chamazulene

The aerial parts of *Achillea* ssp. contain a range of flavonoid glycosides responsible for the spasmolytic activity of yarrow. Apart from the aglycones apigenin and luteolin, mainly their 7-*O*-glycosides could be identified. In addition, quercetin, rutin, isorhamnetin, and the methoxylated aglyca artemetin, casticin were found. The highest amounts of flavonoids (0.028%-0.186%) were present during anthesis [Falk et al., 1975; Guedin et al., 1993; Ivancheva et al., 2000; Ivancheva et al., 2002; Glasl et al., 2002]. Furthermore, the occurrence of *C*-glycosylflavones based either on apigenin or luteolin in the genus *Achillea* is described [Valant-Vetschera, 1982; 1985]. However, within the *A. millefolium* complex, only the three taxa *A. setacea*, *A. cusipedata* and *A. asiatica* produced vitexin, isovitexin, swertisin, orientin, isoorientin, swertiajaponon and di-*C*-glycosylapigenins. Therefore, the *C*-glycosylflavones have been shown to be of chemotaxonomic importance for the difficult differentiation between species of the genus *Achillea*. Especially *A. nobilis* showing morphological similarities can be distinguished from the most species of the *Achillea millefolium* group by its flavonoid pattern [Valant, 1978; Valant-Vetschera, 1982; 1984; 1985; 1987; 1994; Kastner et al., 1995; Marchart and Kopp, 2003].
Other constituents reported include phenolic acids, triterpene, sterols, and a range of alkamides in the subterranean parts [Ulubelen et al., 1990; Greger and Hofer, 1989].

3.1 Chemical Analysis

The constituents of the essential oil were analysed by GC and GC-MS after steam distillation of the dried plant [Mockute and Judzentiene, 2003; Agnihotri et al., 2005, Dokhani et al., 2005].
Sesquiterpene lactones were identified from a dichloromethane extracts of flower heads of *Achillea pannonica* SCHEELE by GC-MS, GC-IR and NMR [Werner et al., 2003].
Characterisation of guajanolides, e.g. desacetylmatricarin, leucodin, achillin and their 8 alpha-angeloxy-derivatives, were performed by TLC on silica gel and HPLC using isocratic and gradient systems with different methanol-water mixtures as mobile phases [Glasl et al., 2003].
Flavonoids were separated and quantified using capillary electrophoresis after extraction of the dried material with methanol water (40:60, v/v) [Marchart and Kopp, 2003]. Furthermore, flavonoid glycosides and free aglycones from flower heads and leaf exudates were investigated [Valant-Vetschera and Wollenweber, 1988; Gherase et al., 2004].

3.2 Standardisation, Stability

According to DAB 10 drugs containing at least 0.2% of volatile oil and not less than 0.02% azulene are required. Determination of the volatile oil content after steam distillation is conducted volumetrically (ÖAB 90) or photometrically at 610 nm (ÖAB 90, DAC 86), respectively. A test method for pro-azulenes was developed by Verzar-Petri and Hanh (1977). After extraction of the powdered drug with chloroform and addition of 4-dimethyl-aminobenzaldehyde reagent, the solution was extracted with petrolether. Drugs containing pro-azulenes show a distinctly blue colour of the lower phase.
Saberi (1990) developed a procedure for the qualitative estimate of the chamazulene content. For this, 100 mg of flowers were moistened with a few drops of 1% sulphuric acid, followed by

sublimation at 150°C *in vacuo*. Material poor in azulene furnishes a reddish rather than an intense blue to blue-green sublimate. Additionally, capillary gas chromatography of the sublimate allowed quantification.
The identity of the volatile oil is proved by TLC, using caryophyllene and guaiazulene as reference compounds, as described by the DAC 1986.
Studies regarding the stability of *Achillea millefolium* drugs are scarce. Kucera (1956) observed a loss of 20% of azulene compared to the fresh plant after shade drying, which increased to 50% using infrared drying. Data concerning the stability of pharmaceutical preparations of yarrow were not available.

4. PHARMACOLOGY

The internal and external use of yarrow is very similar to that of Matricaria flos. Fresh plant juice, infusions and other galenic preparations are applied for their antiphlogistic, spasmolytic, stomachic, carminative, and cholagogue effects. The main applications are in gastrointestinal complaints, e.g. inflammation, diarrhoea, flatulence and cramps. In addition, it is also employed as a bitter aromatic to stimulate the appetite and the secretion of bile. Externally, yarrow is used in the form of poultices, lotions, and baths, but mostly in the form of preparation with alcohol, e.g. percolates, fluid extracts, for inflammation of the skin and mucous membranes, as well as for healing wounds. In folk medicine, the drug is often employed as a haemostyptic, e.g. in bleeding from haemorrhoids, and in problems of menstruation [Chandler et al., 1981; De La Puerta and Herrera, 1995].

4.1 ANIMAL STUDIES

Anti-inflammatory activity was found for 11,13-dehydrodeacetylmatricarin and rupicolin B in an aqueous extract of the aerial parts of *Achillea setacea* in the croton oil ear test. An inhibition of oedema development for 11,13-dehydrodeacetylmatricarin of 45.5% and for rupicolin B of 14.3 % was observed [Zitterl-Eglseer et al., 1991]. Sosa et al. (2001) investigated the anti-inflammatory activity of the sesquiterpene 1,4-dihydroxy-germacra-5E-10(14) [DHGD] isolated from *Achillea pannonica* SCHEELE. As references, hydrocortisone and the non steroidal anti-inflammatory drug (NSAID) indomethacin were used. DHCD inhibited ear odema in a dose-dependent manner (61%) and was more effective than NSAID (43%). For comparision, the reduction by hydrocortision was 68%.
A protein-carbohydrate complex isolated from the aqueous extract of *Achillea millefolium* flowers and subcutaneously applied to mice (40 mg/kg) exhibited systemic and antiphlogistic activity [Goldberg et al., 1969].
In mice and rats with alloxan diabetes yarrow showed marked hypoglycaemic and glycogen sparing properties [Molokovskii et al., 1989].
Tozyo et al. 1994 isolated and characterised three sesquiterpenoids form *Achillea millefolium*, achimillic acids A, B and C, which were found to be active against mouse p-388 leukemia cells *in vivo*. A pronounced cytotoxic effect against HeLa cancer cells (IC_{50} = 25.92 ± 4.96µg/ml), and lower cytotoxicity against K562 leukemia cells (IC_{50} = 48.59 ± 18.31 µg/ml) were observed for a combined chloroform and ethylacetate herb extract of *Achillea alexandri-regis*. In contrast, the methanol extract was found to be a moderately cytotoxic *in vitro* agent against HeLa and K562 cells [Kundakovic et al., 2005].

4.2 HUMAN STUDIES

In a double-blind placebo controlled clinical trial the antihyperlipidemic and antihypertensive effects of a hydroalcoholic extract of *Achillea wilhelmsii* C. KOCH were investigated. The results showed a significant decrease in triglycerides, total cholesterol and LDL-cholesterol after 4 months, and a significantly increased HDL-cholesterol after 6 months treatment, respectively [Asgary et al., 2000]. Sibel and Canan (2005) studied the protective effects of an infusion prepared from 15 *Achillea* species against hydrogen peroxide induced oxidative damage in human erythrocytes and leucocytes. The results indicated that infusions of all *Achillea* species

investigated are a potential source of natural antioxidants for treatment and prevention of diseases in which lipidperoxidase is involved.
The efficacy of a herbal medicine containing an *Achillea millefolium* extract on liver cirrhosis was probed in a randomized, double-blind, placebo-controlled study. After 6 months significantly better child-pugh score, decreased ascites, decreased serum alanine and aspartate aminotransferase activities compared to the placebo were observed. The hepatoprotective effect in cirrhotic patients was attributed to the diuretic, anti-inflammatory, anti-oxidative, and immunomodulating properties of the herbs [Huseini et al., 2005].

4.3 ADME (ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION)

At the date of write no information available.

5. MICROBIOLOGY

In a disk diffusion assay the extract of aerial parts of yarrow (hexane:ether:methanol, 1:1:1) possessed a broad spectrum of antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteritis, Aspergillus niger* and *Candida albicans* [Stojanovic et al., 2005]. The essential oil of *Achillea millefolium* ssp. *millefolium* AFAN. showed antimicrobial activity against *Streptococcus pneumoniae, Clostridium perfringens, Candida albicans, Mycobacterium smegmatis, Acinetobacter lwoffi* and *Candida krusei* [Candan et al., 2003]. Against respiratory tract pathogens like *Klebsiella pneumoniae* and penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* the essential oil of *Achillea clavennae* showed maximum antibacterial activity. The oil also exhibited strong activity against Gram-negative *Haemophilus influenzae* and *Pseudomonas aeruginosa*, whereas Gram-positive *Streptococcus pyogenes* was the most resistant [Skocibusic et al., 2004].
Furthermore, a water extract of *Achillea millefolium* possessed strong activity against *Plasmodium falciparum* and *Babesia gibsoni,* inducing malaria and anemia in dogs, respectively [Murnigsih et al., 2005].

6. EFFICACY

The digestive and appetising properties of Yarrow attributed to bitter substances and essential oils are used in different animal species (ruminant, horse, pig, dog, cat, chicken) (Reichling et al., 2005). Special prescriptions are available for calves suffering from diet-related gastroenteritis (Gachnian and Assenov, 1990; Rabinovich, 1981).

7. TOXICOLOGY

To evaluate the possible toxicitiy of aqueous extracts from leaves of *Achillea millefolium* on reproductive endpoints in Wistar rats, adult male rats were treated daily with yarrow extract (0.3, 0.6, 1.2 g/kg/day) administrated orally during 90 days. Over the treatment period, no clinical signs of toxicity or estrogenic/antiestrogenic activity were detected. Furthermore, the body weight gain was similar in all groups [Dalsenter et al., 2004].
Applications of Millefolii herba are allowed for administration to food-producing animals in the EU [VO (EWG) Nr. 2377/90]. No experiences are available about the application of Calendulae flos to pregnant and lactating animals [Reichling et al., 2005].
Rarely allergic reactions (pruritus, exanthem) and in long term applications gastritis could be observed [Danilenko and Rodionov, 1982; Manolov, 1987; Jurenitsch, 2003].

7.1 SAFETY

Dosage/Administration

According to the German Commission E monographs: Unless otherwise prescribed an average daily dosage of 4.5 g of yarrow, 3 teaspoonfuls of juice pressed from the fresh plant or 3 g of yarrow flowers and equivalent preparations, respectively, for internal use. For external use, e.g. hip baths, 100 g of yarrow to 20 L of water are recommended.

Side effects

Yarrow may cause contact dermatitis with itching, inflammatory reactions, and the formation of vesicles. The guaianolide peroxides α-peroxyachifolide is believed to be responsible for the allergic contact dermatitis [Hausen et al., 1991].

Interactios with other drugs

None known.

KS

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ALLIUM SP.

Allium sativum L. (garlic)

Allium cepa L. (onion)

Allium porrum L. (leek)

1. INTRODUCTION

Among *Allium* species *Allium sativum L.*, *Allium cepa* L. and *Allium porrum* L. have been cultivated since ancient times as vegetables and characteristic pungent flavouring agents for food but also for their pharmaceutical properties. The use of garlic, onion and leek is well documented by the Egyptians, Greeks and Romans. In the Middle ages Constantinus Africanus, Hildegard von Bingen, Albertus Magnus and many others mentioned the plants in their medicinal books [Sendl, 1995].

However, scientific investigations started only 200 years ago. One of the earliest studies was performed in 1844 by the German chemist Wertheim, who obtained some strongly smelling substances named as allyl derivatives (from *Allium*) and sulphurlallyl derivatives [Wertheim, 1844]. During the 19th and the 20th century scientific work on garlic extraction and identification of many constituents has been performed. In recent years most investigations aimed at standardisation of the active principles in pharmaceutical preparations. Further research work is

still needed to elucidate and quantify the active principles including pharmacokinetics and metabolism [Sendl, 1995].

2. DESCRIPTION OF THE PLANTS

Allium sativum L.
Garlic is a bulbous perennial plant. A wild species of garlic is probably *A. longicuspis* REGEL, which grows wild in southwestern Asia. The bulb, which is commonly used for food flavouring, has 5-8 cm in diameter and is composed of several single bulblets (6-20) also known as cloves. The bulb varies slightly in shape, colour and flavour depending on variety and growing conditions. The foliage comprises a central stem up to 100 cm with erected flat or keeled leaves. The white, pink or purple flowers are arranged at the top of the stem.

Allium cepa L.
The onion bulb commonly used as a vegetable or seasoning in foods has been cultivated for over 4000 years and has never been found as wild species. *Allium cepa* is classified in two infraspecific groups (Hanlet, 1990): The common 'onion' group constitutes the vast majority of modern highly adapted cultivars. One characteristic feature of this group is large, single bulbs. The second group, called 'Aggregatum', contains rapidly dividing varieties forming laterals and as a consequence thereof bulb clusters. The shallot and the multiplier onion belong to this group. Their propagation is typically vegetative and flowers are infertile to members of the 'common' onion-group, which is indicative of a shared species. *Allium cepa* is a biennial crop that forms a large bulb in the first year of growth. The bulb is composed of fleshy enlarged leaf bases. The outermost layers do not swell but become thin, dry, and discoloured, forming a covering, which is generally removed prior to use. The bulbs vary in colour (white, yellow, red) and pungency from mild to very strong. After vernalisation by winter cold, rapid re-growth of foliage in spring is followed by the production of several tubular flower stalks (60-120 cm), each producing a single spherical umbel of around 200 flowers.

Allium porrum L.
Leek is a vegetable valuable in the fall and winter. Cultivated forms are known as the garden leek and the Egyptian leek, which is grown for its leaves in Egypt and elsewhere in the Middle East. It is supposed to go back to *A. ampeloprasum* L. originating from the Mediterranean region. Wild leek (*A. tricoccum* AIT.), grown in America, is a plant with a cluster of ovoid bulbs and large oblong elliptical leaves.
Rather than forming a tight bulb such as onion, leek develops a long cylinder of bundled leaf sheaths up to 0.9 m. They are milder than onions and are grown mainly for their long, thick, tender white, yellow and greenish stems.

2.1 SYSTEMATICS

According to more recent taxonomic concepts, *Allium* and its close relatives are recognized as distinct family termed Alliaceae. The derivation of the botanical name is still uncertain and the classification has been discussed for a long time. The genus *Allium* comprises more than 500 species, but garlic, onion, leek and chives constitute the major *Allium* foods consumed by humans [Dahlgren et al., 1985; Sendl, 1995]. Rich in species, the genus is divided into three subgenera *Amerallium* TRAUB, *Nectaroscordum* (LINDL.) TRAUB and *Allium* L., 18 sections and several subsections.

2.2 OCCURRENCE

Garlic
Garlic used in Europe comes from Spain, Hungary, Czech Republic, Slovakia, Italy and France. Large amounts are cultivated in China, India, Egypt and Japan. In America the main producers of garlic are Mexico, Argentina and the United States, especially California.

It requires fertile, sandy, clay soil. The climate should be warm, sunny, not to windy or rainy. Garlic is vegetatively propagated because of its sterility which precludes plant breeding in order to obtain higher amounts of constituents [Sendl, 1999].

Onion and leek

Their origin is believed to be located in central Asia as the highest diversity of *Allium* species can be found in the irano-turanian floristic region (Hanelt, 1990). Onions and leek are native to Eurasia, and now grow all over the world, especially in moderate climates.

For common dry bulb production a crop culture can be established either from seeds, sets or transplants. Onions are classified according to day length they require to induce bulbing. As a result of the long history of cultivation and modern methods of selection and breeding, onions, technically long-day plants (>14 h day length), have been adapted to intermediate (12-14 h) and short (10-12 h) day length. The choice of cultivars depends first on the photoperiodical conditions in the growing area and on the desired method of planting, since suitability for set production is not given for every variety. The planting methods and the impact of the type of preceding crop within a crop rotation have shown to be significant factors affecting quality traits of onions [Resemann et al., 2004a]

Leek is often sold as small seedlings in flats which are started early in greenhouses, to be set out as the weather permits. Once established in the garden, leeks are hardy. Many varieties can be left in the ground during the winter to be harvested as needed.

2.3 Plant Parts and Products

Allium plants attracted interest as spice, herb and vegetable, but also for pharmaceutical. With the objective of seasoning garlic, onion and leek are freshly harvested or dried and used for consumption. The plants are also utilized for the production of volatile oils, juices, extracts in liquid and dried forms and macerates [Koch, 1988a].

Raw *Allium*

Raw *Allium* species possess the natural flavour and provide textural and water-retaining properties when incorporated into food products, although the major constituent of the plants is water. However, the unpretentious production conditions and inadequate instructions of the farmers may cause considerable hygienic problems. As a consequence, high microbial loads are found in the raw material. Bacterial contamination poses a tremendous risk, which has been tragically borne out in the U.S. where sautéed onions in hot sandwiches were responsible for a large outbreak of botulism [Solomon and Kautter, 1986].

Dehydrated *Allium*

Onions for dehydration are generally white or yellow varieties, grown for strong pungency and high solid contents. The root bases of the bulbs are washed thoroughly. The tops and the outer contaminated layers are removed. The peeled onions and garlic, respectively, are sliced or chopped and dehydrated in hot air tunnels. Since the microbial load is not entirely eliminated, additional decontamination treatments are required. Finally, the onion or garlic slices may be ground, powdered or granulated [Fenwick and Hanley, 1990a]. 12 different types of dehydrated onion products have been known [Farell, 1985].

Allium oil

The garlic oil comprising 0.1-0.2% of the fresh weight of garlic is obtained by steam distillation of the minced raw material. One gram of the reddish-brown liquid is equivalent in flavouring terms to 900 g of fresh garlic or 200 g dehydrated powder. The pungency of the product makes it difficult to use directly, and it is therefore diluted in vegetable oil or encapsulated [Fenwick and Hanley, 1990a].

The onion oil is derived from distillation of the fresh raw material standing for a period of hours prior to distillation. In contrast to garlic, the dark amber liquid is obtained in low yields (< 0.1%) depending on source, sulphur fertilisation and processing conditions [Fenwick and Hanley, 1990a; Resemann et al., 2004a].

Leek oil results from the distillation of freshly crushed whole leeks ranging from 0.005% to 0.2% of fresh weight depending on the maturity and physiological character of the plants [Fenwick and Hanley, 1985].

Allium juice

After inspection and washing, the raw material is disintegrated by means of a grinding mill affording a medium structured mash. The mash is pumped into a stirred buffer tank, where the macerate typically rests between 15 to 60 minutes to complete enzymatic reactions. Juice extraction is accomplished by pressing or decantation. Before entering the preheater the juice is adjusted to the desired pH. After being preheated the juice is sterilised for a few seconds [Resemann, 2004]. Addition of pectinolytic enzyme preparations, acetic acid and NaCl diminish the viscosity and avoid discoloration, respectively, which derives from non enzymatic Maillard reactions between sugars and amino acids [Fenwick and Hanley, 1990a; Koch, 1988a].

Allium extracts

Allium extracts are obtained by extraction of the raw material with aqueous alcohols. The addition of methanol and ethanol, respectively, inactivates enzyme systems such as alliinase and peroxidase isoenzymes. Sometimes the liquid extracts are thoroughly evaporated, spray dried or lyophilised [Duziuban, 1979].

Allium macerates

The raw material is minced and blended with vegetable oils dissolving the lipophilic agents.

3. INGREDIENTS/CONSTITUENTS

The proximate composition of garlic, onions and leek is shown in Table 1, but can vary considerably dependent on individual varieties [Ketiku, 1976;].

Table 1 Proximate composition (%) and energy values (cal 100 g^{-1}) of garlic, onions and leek [Fenwick and Hanley, 1990b]

	Moisture	Protein	Fat	Carbohydrate	Ash	Energy
Garlic	61.3	6.2	0.2	30.8	1.5	137
Leaves, stems	86.4	2.6	0.5	9.5	1.0	44
Shoots	77.7	1.2	0.3	20.1	0.7	76
Flowers	88.4	1.4	0.2	9.4	0.6	39
Onion	89.1	1.5	0.1	8.7	0.6	38
Immature bulbs/tops	91.6	1.6	0.4	5.8	0.6	28
Green onion	86.8	0.8	tr.	8.5		35
Bulb + top	89.4	1.5	0.2	8.2	0.7	36
Bulb + white top	87.6	1.1	0.2	10.5	0.6	45
White top	91.8	1.6	0.4	5.5	0.7	34
Leek	85.4	2.2	0.3	6.0	0.9	52
Flowers	83.1	5.5	0.5	10.5	0.4	55

Besides the compounds listed in Table 1, *Allium* species also contain numerous trace elements, vitamins, amino acids, anthocyanins (red onions: cyanidin and peonidin glycoside), flavonols (mainly quercetin glycosides), phenolics (e.g. ferulic acid, protocatechuic acid and phloroglucinol), sterols and saponins.

Plants of the genus *Allium* represent a natural, rich source of organosulphur compounds with different amounts in the three species described. The most important classes are the amino acids cysteine and methionine, the γ-glutamyl peptides and especially the S-substituted cysteine sulphoxides.

γ-Glutamyl-S-alkylcysteines, alliin and other S-alk(en)yl-L-cysteine-sulphoxides:

Allium plants selectively contain two or three kinds of S-alk(en)yl-L-cysteine sulphoxides ('ACSOs') and γ-glutamyl-S-alkylcysteines (Figure 1A,B) with varying amounts and proportions, corresponding to their flavours.

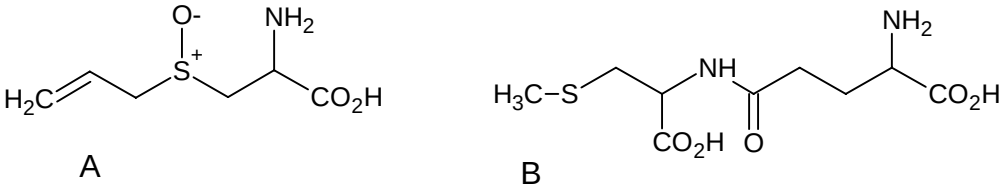

Figure 1 Structures of S-allyl-L-cysteine-(+)-sulphoxide (alliin) (A) and γ - glutamyl-S-methyl-cysteine (B)

Overall, the primary sulphur-containing constituents ranging from 0.5% to 1.3% on a fresh weight basis containing alliin (Figure 1) as the major component [Kubec et al., 1999]. Alliin concentrations can increase during storage as a result of the transformation of γ–glutamyl-cysteines [Milner, 2001]. Alliin itself is odourless and the characteristic odour arises from allicin and lipophilic sulphur compounds formed when the bulb is crushed or damaged. In the intact cells the sulphoxides as flavour precursor are compartmentalised in the cytoplasm and the hydrolytic enzyme alliinase in the vacuole. Disruption of cell tissue results in the release of alliinase, which rapidly hydrolyses the sulphoxides to volatile sulphides. The intermediate sulphenic acid is very reactive and gives rise to a large number of volatile sulphur-containing products [Sendl, 1995].
Additionally, other cysteines and cysteine sulphoxides such as (+)-S-methyl-L-cysteine sulphoxides, (+)-S-(trans-1-propenyl)-L-cysteine sulphoxide, γ–glutamyl-S-allylcysteines were found in smaller concentrations [Fenwick and Hanley, 1985]. In contrast to garlic, onions and leek mainly contain S-propenyl, S-propyl- and S-methylcysteine sulphoxides ranging from 0.2-0.5% on fresh weight [Sun and Pike, 1998] and smaller amounts of γ–glutamyl-S-alk(en)yl-L-cysteines.
Freeman and Whenham (1975) divided the *Allium* species into three groups: *A. sativum* and *A. ursinum* containing mainly ACSOs, *A. cepa* and *A. schoenoprasum* including (+)-S-(trans-1-propenyl)-L-cysteine sulphoxide as major component, and finally *A. flavum* and *A. siculum* being dominated by derivatives of S-methyl-L-cysteine.

The lachrymatory factor (LF)

The sulphenic acid exhibits a unique flavour development in that it gives rise to the lachrymatory factor [Virtanen and Spare, 1961]. Based on works of Wilkens (1964), Brodnitz and Pascale (1971) and Block et al. (1979, 1980) the responsible compound was finally identified as thiopropanal S-oxide possessing a *E:Z* ratio of 5:95. In a recent study, Imai et al. (2002) gave evidence for this isomerisation to be catalysed by the action of the enzyme LF-synthetase. Thiopropanal S-oxide is highly volatile and responsible for the tear producing effect and the pungent, biting flavour perception of particularly cut onion.

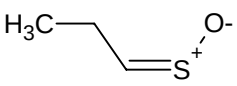

Figure 2 Structure of (Z)-thiopropanal S-oxide (LF)

Allicin and other thiosulphinates:

Allicin (Figure 3), the odoriferous substance of *Allium* species, is the predominant thiosulphinate in garlic generated by the action of the enzyme alliinase as mentioned above.

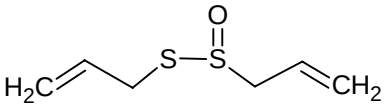

Figure 3 Structure of diallyl-thiosulphinate (allicin)

Symmetrically or asymmetrically substituted thiosulphinates, responsible for the characteristic smell of freshly prepared *Allium* extracts, are formed by the condensation of sulphenic acids. The amounts of thiosulphinates vary in the different *Allium* species as can be seen from Table 3.

Table 3 Thiosulphinates and related compounds from extracts of *Allium* species determined by HPLC (mol% based on total thiosulphinates) [Block et al., 1992, 1993]

Compound	Garlic	Yellow Onion	Leek
AllS(O)SAll	89	-	-
AllS(O)SCH=CHMe-(Z,E)	5.3	-	-
AllSS(O)CH=CHMe-(E)	1.6	-	-
n-PrSS(O)Propenyl-(E)	10	12	8
n-PrS(O)SPropenyl-(Z,E)	17	10	15
n-PrS(O)SPr-n	14	13	25
MeSS(O)Propenyl-(E)	24	24	12
AllS(O)SMe	-	-	-
MeS(O)SPropenyl-(Z,E)	33	25	27
MeSS(O)Pr	1	1	5
MeS(O)SPr	1	1	-5
AllSS(O)Me	-	-	-
MeS(O)SMe	1	14	3

Abbreviations All: Allyl-; Me: Methyl-; Pr: Propyl-

Ajoene/Cepaenes

Ajoene (Figure 4), an oily liquid in garlic extracts, was found to be a very potent inhibitor of thrombocyte aggregation. The *cis*- and *trans*-isomers of ajoene are major secondary degradation products of allicin which are formed under certain conditions via allylsulphenic acid. Besides ajoene, methyl and dimethyl homologues of ajoene have been proposed, since the corresponding cysteine sulphoxides and thiosulphinates have been found in garlic [Sendl, 1995].

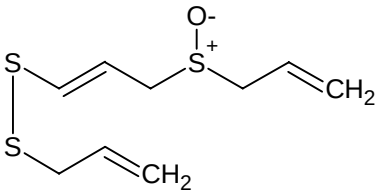

Figure 4 Structure of 4,5,9-trithiododeca-1,6,11-triene-9-oxide (ajoene)

Condensation of the LF with sulphenic acids leads to a new class of biologically active compounds named cepaenes (Bayer et al., 1989a; Kawakishi and Morimitsu, 1988). Cepaenes are reported to impart a "fruity, fresh onion, sulphur, green, melon-like" flavour [Block et al., 1997] to the sensory perception of freshly cut onion.

Vinyldithiins/Zwiebelanes

2-Vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin (Figure 5) first described by Brodnitz et al. (1971) are cyclic compounds which are also degradation products of allicin. Commercially available oily extracts of garlic contain mainly vinyldithiins [Sendl, 1995].

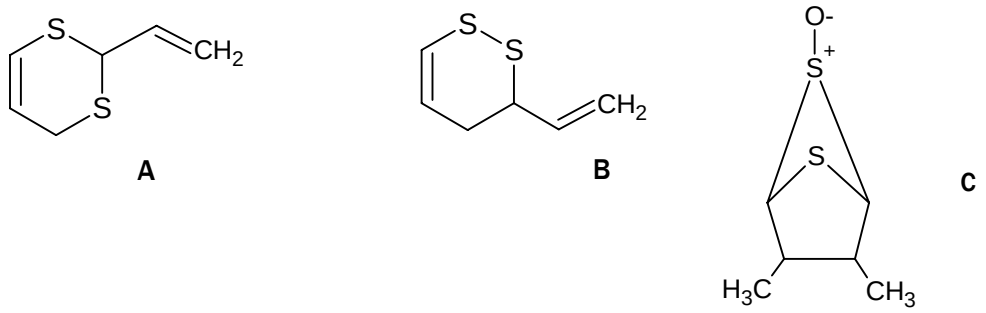

Figure 5 Structures of 2-Vinyl-4H-1,3-dithiin (A), 3-vinyl-4H-1,2-dithiin (B) and 2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexan-5-oxide (C)

In freshly prepared chloroform extract of onions two bicyclic sulphur compounds called zwiebelanes were found [Bayer et al., 1989b]. Their structures were elucidated as *cis*- and *trans*-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexan-5-oxides. Besides onion, which contains about 14% zwiebelanes based on total thiosulphinates, about 7.5% were also found in leek [Block et al., 1993].

Sulphides

Sulphides are mainly found in distilled garlic oil and after storage of aqueous extracts for several days. Sulphides are lipophilic, volatile, quite stable liquids, which are responsible for the typical smell and taste after ingesting garlic. One major compound of garlic oil is diallyldisulphide accounting for 60% of the oil. During the last years, several sulphides have been identified and isolated from *Allium* products [Horie et al., 1925; Lawson et al., 1991a,b]. Commercially available steam distilled volatile oils consist almost exclusively of diallyl-methyl-, allyl- and dimethyl- sulphides containing one to six sulphur atoms [Sendl, 1995].

The components of flavours e.g. flavour precursors, alliinase and peptides were first isolated from onion and garlic bulbs, but have also been reported in the leaf blades, base plate and roots

of onion, garlic and leek [Freeman, 1975; Lancaster et al., 1986; McCallion and Lancaster, 1984]. Flavour precursors are not uniformly distributed within the bulb scales and leaf blades [Freeman, 1975; Lancaster et al., 1986]. In the onion bulb flavour precursor concentration was highest in the base plate and innermost bulb scales, progressively decreasing to the outermost scales which were dried and almost free from flavour. Young leaf blades were higher in flavour precursor concentration than old leaf blades. The seeds of onions did not contain flavour precursors, although γ-glutamyl peptides and alliinase are present [Freeman, 1979]. A similar pattern was found in leek [Lancaster and Boland, 1990].

Non sulphur compounds – enzyme systems

As already mentioned garlic and other *Allium* species contain enzyme systems namely alliinase (E.C. 4.4.1.4), which is a pyridoxal phosphate enzyme first described by Stoll and Seebeck (1948), γ-glutamyltransferase, cleaving the γ-glutamyl peptides in sprouting onion bulbs [Schwimmer and Austin, 1971]. Additionally, peroxidase and other common enzymes were also found [Croci et al., 1991; Koch, 1988b].

Non sulphur-compounds – carbohydrates

The non structural carbohydrates in *Allium* species include glucose, fructose and sucrose together with a series of oligosaccharides, the fructans [Bose and Shrivastava, 1961; Darbyshire and Henry, 1978; Steer and Darbyshire, 1979]. The three different trisaccharides 1^F-fructosylsucrose, 6^F-fructosylsucrose and 6^G-fructosylsucrose are potentially possible in plants. 1^F- and 6^G-fructosylsucrose have been identified in onion and have been reported in leek [Bacon, 1959; Darbyshire and Henry, 1978]. The addition of further fructosyl groups leads to the formation of tetrasaccharides and higher polymers.

3.1 Chemical Analysis

Many attempts have been made to determine the active principles and the flavour components in fresh plant material and preparations thereof by means of colorimetric measurements. Nowadays, GC and HPLC show the sensitivity and specificity required for an exact determination of the active principles. In most cases, the sulphur constituents are extracted with petroleum ether, dichlormethan or supercritical carbon dioxide at room temperature. Allicin and other allyl thiosulphinates can be isolated by extracting with alcohols at room temperature or by co-distillation with water from the crushed raw material followed by extraction of the distillate with ether [Sendl, 1995]. Besides HPLC and GC, the extracts are used for TLC and SFC-MS (supercritical fluid chromatography – mass spectrometry) analysis [Calvey et al., 1994; Jansen et al., 1987; Kappenberg and Glasl, 1990; Miething, 1985; Resemann and Carle, 2003; Saito et al., 1989].

Ajoenes and vinyldithiins were determined by HPLC and GC, respectively [Brodnitz et al., 1971; Iberl et al., 1990; Koch and Jaeger, 1989]. Furthermore, allyl sulphides were extracted with aqueous acetonitril and also identified by GC, GC-MS, HPLC and HPLC-MS respectively [Jirovetz, 1992; Miething, 1988; Laakso et al., 1989; Lawson and Gardner, 2005; Resemann et al. 2004b Vernin et al., 1986; Yan et al., 1992, 1993;].

3.2 Standardisation, Stability

Allium products, mainly based on garlic, have become popular in recent years and a variety of culinary and pharmaceutical preparations are available on the market. Analytical control of these products is necessary, since there are different types of applications having different chemical composition and dosage of the active principles. The determination of the stable precursors along with methods for the assay of allicin would be a reasonable evaluation method also in terms of testing the intact alliin-alliinase system which is a prerequisite for the formation of all pharmacologically active secondary products [Sendl, 1995].

Besides Helv VI and EB 6 requiring at least 0.3% allicin and 0.2% volatile oils in *A. sativi* bulbus, minimum requirements regarding the concentration of active constituents of *Allium* products were not found. According to Helv VI the quantitative determination of allicin depends on the specific reaction of pyruvic acid with 2,4-dinitrophenylhydrazine, which, together with ammonia and allicin, arises from the enzymatic cleavage of alliin. Since colorimetric methods are not selective, the determination nowadays is performed by HPLC.

When fresh garlic is crushed or blended, allicin formation is completed in approximately 6 s. Allicin itself is rather unstable and decomposes within 20 hours at room temperature yielding diallyl disulphide as the major products and diallyltrisulphide, diallyl sulphide, sulphur oxide, and trace amounts of ajoene and dithiins [Block, 1985; Lawson et al., 1991b; Tamaki and Sonoki, 1999].
Generally, the stability of the thiosulphates depends on their environment, particularly in the presence of oils and proteins. Compounds reacting with thiosulphinates like cysteine or other thiol-containing components rapidly decrease the thiosulphinate content. Additionally, alkaline media (pH ≥ 10) cause rapid transformation of allicin to diallyl sulphide [Kice and Rogers, 1974; Mueller, 1989]. Lawson and Gardner (2005) investigated the stability of thiosulphinates in different condiments and observed a loss of allyl thiosulphinates accompanied by a nearly quantitative increase in diallyl disulphide and further compounds of unknown identity that were less polar than diallyl trisulphide. Considering crushed fresh garlic no significant decrease of the thiosulphinates could be determined after storage at 4 °C for 12 days.

4. PHARMACOLOGY

Plants of the genus *Allium* exert antiatherogenic, lipid-lowering, antihypertensive, antiaggregatory, vasodilatory and antioxidant effects, which are primarily due to allicin and its transformation products. The data given below are based on the ESCOP monograph (2003).

4.1 IN VITRO EXPERIMENTS

4.1.1 ANTIATHEROGENIC AND LIPID-LOWERING EFFECTS

Inhibitory effects of extracts and isolated fractions from garlic on cholesterol biosynthesis were observed in chicken hepatocytes and monkey livers. At equivalent doses, both the drug and its lipophilic and hydrophilic fractions caused 50-75% inhibition of cholesterol biosynthesis [Quereshi et al., 1983]. In particular, the active principles ajoene and allicin were evaluated in cultivated rat hepatocytes and human liver cell line HepG2. In rat hepatocytes, ajoene inhibited sterol biosynthesis with an IC_{50} of 15 µM, but allicin being almost ineffective in contrast to other studies observing an inhibition of cholesterol biosynthesis of standardized garlic powder (1.3% alliin) with an IC_{50} of 90 µg/mL [Gebhardt, 1991, 1993]. In HepG2 cells both compounds inhibited sterol biosynthesis with IC_{50} values of 7 and 9 µM, respectively [Gebhardt et al., 1994].
Besides fresh garlic and garlic powder, petroleum ether, methanol and water extracts of garlic and garlic preparations showed inhibition of cholesterol biosynthesis [Yeh and Yeh, 1994].
During a 24 hour incubation period garlic powder significantly reduced the level of cholesteryl esters by 26% and free cholesterol by 32% in cells of atherosclerotic plaques of human aorta and inhibited their proliferative activity by 55% at 1 mg/mL [Orekhov et al., 1995].

4.1.2 VASCULAR RESISTANCE, FIBRINOLYSIS AND PLATELET AGGREGATION

In isolated arterial strips from dogs, garlic at 0.2-200 g powder/L and ajoene at 10^{-6} to 10^{-3} M hyperpolarised membrane potential by 9.3 mV and 4.2 mV [Siegel et al., 1991, 1992]. Similarly, in human coronary artery smooth muscle garlic at concentrations of 0.2-200 mg/L caused dose-dependent hyperpolarisation (2.7 mV at maximum) and relaxation (20% at maximum). Allicin and Ajoene produced hyperpolarisation of up to 5.1 mV and 4.4 mV and diminished vascular tone by 24% and 11%, respectively [Siegel, 1998]. In isolated rat aorta lyophilised garlic powder

inhibited noradrenaline-induced contractions. The EC_{50} values were 5.28 x 10^{-3} g/mL in aortas with endothelia and 5.81 x 10^{-3} g/mL in aortas without endothelia [Oetztuerk et al., 1994].
Garlic preparations and organosulphur compounds from garlic and onion inhibited collagen-stimulated platelet aggregation in platelet-rich plasma with IC_{50} values of 460 µg/mL of aqueous garlic extract, 190 µg/mL of total polar constituents, 205 µg/mL of alliin, 14.0 µg/mL of allicin and 13.2 µg/mL of total thiosulphinates [Lawson et al., 1992; Srivastava, 1989]. Diallyldisulphide (40-200 µg/mL) and diallyltrisulphide (5-100 µg/mL) showed dose-dependent inhibition of platelet aggregation and thromboxane B_2 secretion induced by arachidonic acid, adrenaline and collagen [Bordia et al., 1998]. Additionally, some flavonoids (astragaline, kaempherol, kaempherol-3-O-neohesperioside) isolated from leek markedly inhibited platelet aggregation and ATP release induced by arachidonic acid or collagen [Fattorusso et al., 2001; Tzeng et al., 1991].

4.1.3 ANTIOXIDANT EFFECTS

Allicin inhibited lipid peroxidation induced by an ascorbic acid/Fe^{2+} system at a concentration of 0.18 mM [Rekka and Kourounakis, 1994]. The antioxidant effect of an aqueous garlic extract obtained from 1 mg of garlic powder was comparable to that of 30 nM of ascorbic acid or 3.6 nM of α-tocopherol [Lewin and Popov, 1994]. Radical scavenging activity of preparations containing ACSOs has also been demonstrated [Imai et al., 1990].
Garlic powder strongly inhibited superoxide production in phorbol ester-activated granulocytes with an IC_{50} of 390 µg/mL, whereas an alliin-enriched but alliinase-inactivated garlic extract did not inhibit superoxide production at concentrations up to 1 g/mL [Siegers et al., 1999].
An aqueous solution of garlic powder (1.3% alliin) reduced radicals generated by the Fenton reaction and liberated by cigarette smokers [Toeroek et al., 1994].

4.1.4 OTHER EFFECTS

An aqueous extract from fresh garlic (100-500 µg/mL) increased the proliferation and improved the morphological characteristics of cultured human fibroblasts [Sevendson et al., 1994]. A similar extract non-competitively and irreversibly inhibited cyclooxygenase activity in rabbit platelets and in lung and vascular aortic tissues with IC_{50} values of 0.35, 1.10 and 0.9 mg, respectively [Ali and Thompson, 1995].
Sapongenins isolated from leek were tested for cytotoxic activity on two different cell lines in vitro. The results indicate an activity for almost all of the compounds [Fattorusso et al., 2000].

4.2 ANIMAL STUDIES

4.2.1 ANTIATHEROGENIC AND LIPID-LOWERING EFFECTS

Garlic powder (0.6% allicin) fed to rats for 11 days at 0.25, 0.5 and 1 g/kg body weight, respectively, protected against isoprenaline-induced myocardial damage as shown by histological examination and biochemical analysis [Ciplea and Richter, 1988]. In isolated perfused hearts from rats which had been given 1% of garlic powder (1.3% allicin) in their diet for 10 weeks, the size of the ischaemic zone, incidence of ventricular tachycardia and ventricular fibrillation were significantly reduced in comparison with untreated controls [Isensee et al., 1993; Jacob et al., 1991]. A petroleum ether extract of garlic (corresponding to dried garlic at 1 g/kg body weight) fed to rats for 5 days in combination with an atherogenic diet, significantly prevented elevation of serum cholesterol and triglyceride levels (92.4 and 93 mg/mL, respectively, compared to 320 and 370 mg/dL in control animals), and reduced the incidence and grade of atherosclerotic lesions compared to animals fed on the atherogenic diet alone [Lata et al., 1991]. Rabbits were given cholesterol in their diet at 0.5 g/kg body weight daily for 2 months, then fed for 3 months on a normal diet with or without the addition of fresh garlic at 1 g/kg daily. Compared to rabbits receiving a normal diet over the 3 month period, those given garlic had reduced plaque areas with half as many lesions [Mand et al., 1985].
Few studies were undertaken to determine the effects of two brown onion varieties on blood lipid and oxidative status using pigs as a model [Gabler et al., 2005; Ostrowska et al, 2004]

leading to similar results. Supplementation resulted in a 21% reduction in the averaged fasted and postprandial plasma triacylglyceride measurements taken over a 6 weeks period in comparison with the control pigs. The cholesterol concentrations were significantly reduced by 5.5 and 12.4% in pigs that consumed low (10 g onion/MJ) and high (25 g onion/MJ) doses of onion, respectively. Inhibition in the rate of serum lipoprotein oxidation was increased by 23%. The data confirmed that onion consumption provide a dietary means of diminishing some of the risk indices associated with heart diseases, but the responses varied with type and dose of onion [Gabler et al., 2005].
Additionally, hydroalcoholic extracts of leek have been investigated on plasma lipid levels in rabbits divided into control, hypercholesteremic control and three treatment groups (hypercholesterolemic diet + 250 mg, 500 mg, 1000 mg /kg per body weight of extract). During the 12 weeks feeding, plasma total cholesterol decreased in all groups treated with *A. porrum* extract in a dose dependent fashion. Changes in the distribution of cholesterol in HDL or LDL were found indicating that this plant may be useful for the treatment of hyperlipidemia [Movahedian et al., 2004].

4.2.2. ANTIHYPERTENSIVE EFFECTS

Garlic powder was given to spontaneously hypertensive juvenile rats at 1 g/kg body weight for several weeks. It could be shown that the development of hypertension was inhibited and myocardial hypertrophy reduced. In spontaneously hypertensive adult rats (6-7 month of age) garlic lowered blood pressure and myocardial hypertrophy [Jacob et al., 1993]. Garlic powder (1.3% alliin) added at 0.5% to the diet of hypertensive rats significantly prolonged their life span by 22 days compared to a control group of hypertensive rats given the normal diet. During the course of the study systolic blood pressure was significantly lower in the garlic group [Braendle et al., 1997]. The antihypertensive effect was confirmed by another study [Al-Qattan et al., 1999].

4.2.3 OTHER EFFECTS

In an animal model using guinea pigs a high protective effect following oral treatment with lyophilised onion extract against allergen- and platelet activating factor-induced bronchoconstriction could be demonstrated, identifying thiosulphinates and cepaenes as active compounds. Considering the isolated thiosulphinates both *in vivo* and *in vitro*, allicin was devoid of activity [Breu and Dorsch, 1994].
Effects of green leek juice were investigated on liver damage induced by CCl_4 in male rats. After 6 weeks, daily administration of 8 mL/kg body weight leek juice resulted in significant decreases in activities of serum glutamic oxaloacetic and pyruvic transaminase, respectively, hepatic glutathione peroxidase and hepatic superoxide dismutase confirming the assumption that green leek juice exerts a protective effect against CCl_4-induced hepatotoxicity in rats [Lee, 2001].

4.3 HUMAN STUDIES

4.3.1 RHEOLOGICAL AND ANTIATHEROSCLEROTIC EFFECTS

In a randomised, double-blind, placebo-controlled, crossover study, the influence on cutaneous microcirculation of a single oral dose of 900 mg of garlic powder (1.3% alliin) was evaluated in 10 healthy volunteers. After 5 hours, a significant increase of 55% was observed in erythrocyte velocity in skin capillaries resulting from vasodilatation of precapillary arterioles, which increased in diameter by 8.6% [Jung et al., 1991]. Another study has shown that a daily ingestion of 600 mg of a standardised garlic powder preparation (1.3% alliin) lowered susceptibility to lipoprotein oxidation by 34% [Phelps and Harris, 1993].
In an open study involving 101 healthy volunteers, the effects of taking 300-900 mg of standardised garlic powder daily on elastic properties of aorta were investigated for at least 2 years. Compared to results from an untreated control group, garlic intake attenuated age-related increases in aortic stiffness. Significant differences were observed in pulse wave velocity

and pressure-standardised elastic vascular resistance [Breithaupt-Kroegler et al., 1997]. Another open study involving eight volunteers took one clove of garlic (approximately 3 g) daily for 16 weeks. Serum thromboxane B_2 was reduced from 243 to 24 ng/mL [Ali and Thompson, 1995].
Healthy volunteers (n = 30) took 10 g of fresh garlic daily for 2 months. Clotting time increased from 4.15 to 5.02 min and fibrinolytic activity decreased from 4.17 to 3.26 hours. There were no changes in the control group [Gadkari and Joshi, 1991].
In a randomised, double-blind study 60 young patients (average age 24 years) with constantly elevated spontaneous platelet aggregation and increased risk of ischaemic attack were treated with 800 mg of standardised garlic powder (1.3% alliin) or placebo for 4 weeks. In the garlic group the ratio of circulating platelet aggregation decreased by 10.3% and spontaneous platelet aggregation by 56.3%, whereas the placebo group remained unchanged [Kiesewetter et al., 1993b; Kiesewetter et al., 1991]. The same amount of garlic powder was given to 80 patients with peripheral arterial occlusive disease daily for 12 weeks. In the garlic group plasma viscosity decreased by 3.6% and platelet aggregation dropped by 59% while these parameters were unchanged in the placebo group [Kiesewetter et al., 1993a].

4.3.2 Lipid-lowering effects

In a randomised, double-blind, placebo-controlled study, significant reductions of up to 30% in fasting and postprandial plasma triglycerides were observed in 24 volunteers after 6 weeks of daily treatment with 900 mg of garlic powder [Rotzsch et al., 1992]. The effects of 600 mg of standardised garlic powder daily taken by 68 volunteers for 15 weeks total cholesterol and triglycerides showed a tendency to decrease in the verum group [Saradeth et al., 1994]. In an open study already mentioned above eight healthy volunteers (initial total cholesterol 6.1 mM) took one clove of garlic (approximately 3 g) daily for 16 weeks. Total cholesterol decreased by 21% [Ali and Thompson, 1995] and by 15.5% in 15 volunteers taking 10 g of garlic daily for 2 months. No change was observed in the control group [Gadkari and Joshi, 1991].
Furthermore, a large number of comparative, placebo-controlled and open clinical studies were conducted regarding the lipid lowering effects of garlic and preparations thereof confirming the reduction of total cholesterol and triglyceride levels as well as an increase in HDL-cholesterol [Beck and Gruenwald, 1993; De A Santos and Johns, 1995; Harenberg et al., 1988; Holzgartner et al., 1992; Kandziora, 1988; Kiesewetter et al., 1993a; Mader, 1990; Orekhov et al., 1996; Vorberg and Schneider, 1990; Walper, 1994]. Intergroup differences could not be observed [Neil et al., 1996; Simons et al., 1995; Superko et al., 2000].

4.3.4 Antihypertensive effects

In a randomised, double-blind, placebo-controlled study, 52 patients with hypercholesterolaemia were treated with 900 mg of standardised garlic powder (1.3% alliin) daily for 6 months. Systolic and diastolic blood pressure were reduced by 17% and 10%, respectively in the verum group, but remained unchanged in the placebo group [De A Santos and Gruenwald, 1993]. Another randomised study compared the effects of daily treatment with 600 mg of standardised garlic powder (1.3% alliin, 0.6% allicin release) and those of 1.98 mg garlic oil in 80 patients with mild hypercholesterolaemia. By the end of 4 months of treatment, initial systolic and diastolic blood pressures of 151 and 96 mmHg had decreased by 19 and 17%, respectively, in the garlic powder group, whereas no effects were observed in the garlic oil group [De A Santos and Johns, 1995]. Auer et al. (1990) evaluated the effects of 600 mg of standardised garlic powder (1.3% alliin) in 47 patients with mild hypertension for 12 weeks. In the verum group, initial supine and standing diastolic blood pressures of 102 and 101 mmHg decreased by 13 and 11%, respectively, while in the placebo group initial values of 97 and 94 mmHg decreased by 4% and 0.5%, respectively. Initial systolic blood pressures of 171 and 161 mmHg in the verum and placebo groups, respectively, decreased by 12% and 6%. Intergroup comparison was not reported. Further randomised, double-blind studies were conducted evaluating the effect of garlic on blood pressures in patients with hypertension and hypercholestolaemia [Holzgartner et al., 1992; Kandziora, 1988; Kiesewetter et al., 1993a; Vorberg and Schneider, 1990]. With the

exception of the study of Jain et al. (1993) garlic supplementation resulted in a significant decrease of systolic and diastolic blood pressures.

4.3.5 OTHER EFFECTS

The effects of the active principles of *Allium* species, particularly garlic, indicate the importance of the drugs regarding immunocompetence, prevention of cardiovascular diseases and antiasthmatic and antiinflammatory reactions [Milner, 2001]. The latter was described by Dorsch and coworkers (1989a,b, 1987) investigating the anti-inflammatory potential of fresh onion juice, particularly the thiosulphinates and cepaenes, often recommended in the folk medicine of various countries for pain and swelling after bee or wasp stings. Onion treatment depressed the cutaneous inflammation after allergen or anti-IgE-testing and oedema formation was particularly reduced. Additionally, a reduction of immediate and late bronchial reactions of human patients after allergen inhalation and subsequent treatment with onion juice has been observed [Breu and Dorsch, 1994].
Furthermore, there is some evidence that garlic has a precise role in the cancer process, although major limitations still exist. Laboratory based studies with model cancers designate garlic and its related sulphur components suppressing the incidence of breast, colon, skin, uterine, esophagus, and lung cancers and alter the biological behaviour of tumours. This protection may arise from several mechanisms, including blockage of N-nitroso compounds formation, suppression of the bioactivation of several carcinogens, enhanced DNA repair, reduced cell proliferation, and/or induction of apoptosis. It is possible and quite probable that several of these cellular events are modified simultaneously.

4.4 ADME (ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION)

Several studies investigating the absorption behaviour of flavonols have shown that quercetin and isorhamnetin glucosides were significantly elevated and accumulated in plasma following ingestion of onions [Aziz et al., 1998; Boyle, et al., 2000; McAnlis et al., 1999]. The effective jejunal permeability (P_{eff}) and percentage absorbed were 8.9 x 10^{-4} cm/s and 60%, respectively, for quercetin-3,4-glycoside. Furthermore, a portion approximately 0.5% of the amount absorbed, was excreted back into the lumen as quercetin-3´-glucuronide and finally eliminated in urine [Hollman et al., 1995; Petri et al., 2003]
The pharmacokinetic behaviour of ACSOs and vinyldithiin was investigated after oral administration to rats, mice, and dogs. ACSOs were rapidly and easily absorbed in the gastrointestinal tract and distributed mainly in plasma, liver, and kidney. The bioavailability was 98.2, 103.0, and 87.2% in rats, mice, and dogs, respectively. ACSOs are subsequently N-acetylated by N-acetyltransferase in to S-allyl mercapturic acid and other metabolites and excreted both into urine and breath [De Rooij 1996a,b; Laakso et al., 1989; Minami et al., 1989; Verhagen et al., 2001]. However, mice excreted both ACSOs and the N-acetyl form. The half-life of ACSOs was longer in dogs than in rats and mice [Nagae et al., 1994]. 1,3-Vinyldithiin appeared to be less lipophilic and is rapidly eliminated from serum kidney and fat tissue, whereas 1,2-vinyldithiin was more lipophilic and showed a tendency to accumulate in fat tissue [Egen-Schwind et al., 1992].
The metabolism of fresh garlic and its purified components was researched in rats by extracting the metabolites from hepatocytes and identifying the components using GC-MS [Sheen et al., 1999]. Little is known about the pharmacokinetic of allyl sulphur compounds *in vivo*. It is hypothesised that allicin would be rapidly transformed in the liver to diallyl disulphide and allyl mercaptan already mentioned above [Egen-Schwind et al., 1992b; Sheen et al. 1999]. Diallyl disulphide is transformed to allyl mercaptan, allyl methyl sulphide, allyl methyl sulphoxide and allyl methyl sulphone [Germain et al., 2002; Sheen et al., 1999]. Recently, it is concluded that diallyl disulphide might be reconverted to allicin in tissues principally by oxidation due to cytochrome P450 monooxygenases and flavin-containing monooxygenases [Teyssier, et al., 1999]. However, it is quite unclear how much allicin might be formed under physiological conditions. Brady et al. (1991) have shown the conversion of diallyl sulphide to diallyl sulphoxide and diallyl sulphone both *in vivo* and *in vitro*.

5. MICROBIOLOGY

The findings in scientific works support the historical use of garlic, onion and leek in the treatment of varieties of infections. Considerable evidence indicates that garlic extracts and leek juice can inhibit a range of Gram-negative and Gram-positive bacteria and serve as an antifungal agent [Arora and Kaur, 1999; Yin and Tsao, 1999], whilst onion and shallot juice showed no effect upon Gram-negative bacteria [Dankert et al., 1979]. Besides its antibacterial and antifungal actions, garlic shows also antiviral and antiprotozoal effects [Harris et al., 2001].

5.1 ANTIPROTOZOAL PROPERTIES

The extract of garlic is effective against a host of protozoa including *Opalina ranarum, O. dimidicita, Balantidium entozoon, Entamoeba histolytica, Trypanosomes, Leishmania, Leptomonas* and *Crithidia* [Reuter et al, 1996]. Its possible use as an antiprotozoal against *Entamoeba histolytica* was observed by Mirelman et al. (1987) who found an inhibitory activity with crude extracts at 25 µg/mL and a lethal dosage at 50 µg/mL. Based on these results further studies were conducted to investigate the antigiardial activity of garlic removing the symptoms of giardiasis from all patients within 24 hours [Soffar and Mokhtar, 1991].
The active principles are allicin, which degrades to diallyl trisulphide exhibiting also antigiardial activity [Lun et al., 1994], ajoene and organosulphides.

5.2 ANTHELMINTIC PROPERTIES

Garlic extracts have been shown to exert anthelmintic activity against common intestinal parasites, including *Ascaris lumbricoides* and hookworms [Bastidas, 1969; Vohora et al., 1973].

5.3 ANTIBACTERIAL PROPERTIES

Garlic has been proven to be effective against a vast number of Gram-positive, Gram-negative and acid-fast bacteria, including *Pseudomonas, Proteus, Staphylococcus aureus, Escherichia coli, Salmonella, Klebsiella, Micrococcus, Bacillus subtilis, Clostridium, Mycobacterium* and *Helicobacter*. It has been documented that garlic exerts a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria [Rees et al., 1993]. The antibacterial activity is mainly attributed to allicin, since the antibacterial effectiveness of garlic extract is greatly reduced.

5.4 ANTIFUNGAL PROPERTIES

Several fungi were shown to be susceptible, including *Candida, Torulopsis, Trichophyton, Cryptococcus, Aspergillus, Trichosporon* and *Rhodotorula*. Again, allicin showed antifungal activity but also diallyl trisulphide and ajoene [Cai, 1991, Yoshida et al., 1987].

5.5 ANTIVIRAL PROPERTIES

Only few studies have been done to investigate antiviral properties against influenza A and B, cytomegalovirus, rhinovirus, HIV, herpes simplex virus 1 and 2, viral pneumonia and rotavirus [Fenwick and Hanley, 1985; Meng et al., 1993; Tsai et al., 1985; Weber et al., 1992].

6. EFFICACY

6.1 HENS

Northern fowl mites (NFM) are external parasites that can lower egg production and cause anaemia and even death in laying hens. The topical application of garlic (10% garlic juice in

water) for 3 weeks significantly reduced NFM infestation in laying hens [Birrenkott et al., 2000]. Furthermore, *in vivo* investigations revealed that garlic treatment at 2% and 4% concentrations in feed protected chicks from experimental candidiasis. The disease induced by oral inoculation of chicks with *Candida albicans* was successfully cured by providing a ration containing 5% garlic [Prasad and Sharma, 1980].

The inclusion of garlic supplement within chick diets and their associated effects on performance were studied. The birds were fed a basal wheat/soybean meal formulated to be low in vitamin E and supplemented with garlic (at 10g/kg) and other herbs. It has been shown that garlic diets resulted in the highest bodyweights and weight gains and lower FCR (feed conversion ratio) compared with the other plants investigated. The beneficial effects of garlic in the diet are evident and may indicate an antimicrobial effect *in vivo*. However, longer periods of dietary garlic inclusion (>10-20d) may affect the meat flavour as a strong odour was detectable in the hens [Cross et al., 2004].

6.3 SHEEP

A diet consisting entirely of onions fed to pregnant ewes produced Heinz bodies hemolytic anaemia in all sheep after 21 days, what could be confirmed by Selim et al. (1999) and Kirk and Bulgin (1979). After 28 days of daily consumption of 20 kg onions/ewe, the anaemia stabilised and for the remaining 74 day the packed cell volume increased in the majority of sheep, although it did not return to normal. Compared to control onion-fed ewes had comparable body condition scores and fleece weights. There was no significant difference in pregnancy or lambing rate. Greater numbers of sulphate reducing bacteria and more ruminal hydrogen sulphide were present in onion-fed sheep. On the basis of this study it appears that pregnant ewes may be fed a pure onion diet with minimal detrimental effects. This adaptation to a pure onion diet is in part likely due to the apparent ability of the sheep's rumen to quickly develop a population of sulphate-reducing bacteria that decrease the toxicity of onion disulphides, namely n-propyl disulphide [Knight et al., 2000]. Lincoln et al. (1992) observed similar effects after feeding a balanced growth diet to cattle as described for sheep.

6.4 CATTLE

Earlier attempts to use onions as a feed for cattle have induced toxicoses caused by dipropyl disulphide [Hutchinson, 1977; Koger, 1956; Verhoeff et al., 1985]. Cattle were more susceptible than other domestic animals to onion toxicosis; dogs and horses were intermediate in susceptibility and sheep and goats were the most resistant [Schalm et al., 1975]. Although toxicity of domestic onions is well documented, certain cattle feeders have successfully fed onions and had satisfactory feeding performance without observing toxic effects. This success in feeding onions appears to be dependent on mixing onions in the diet, which could be confirmed by Lincoln et al. (1992) who applied an onion diet containing up to 25% onions (based on dry matter) without having health problems.

Berard et al. (1936) observed an undesirable odour and taste in milk when the cows consumed garlic. Undesirable flavour of the milk is reduced, if the consumption of such material is 4 hour or more before milking. The authors advise to avoid such feed from the ration and even from the pastureland. Contamination of crops such as wheat with especially wild *Alliums* may cause processing problems, off flavours in grain, and thus lower the quality of the product [Fenwick and Hanley, 1985].

More information regarding the positive and negative effects, respectively, of an *Allium* enriched diet could not be found.

7. TOXICOLOGY

The intravenous LD_{50} of allicin in mice was determined as 60 mg/kg; when administered subcutaneously the LD_{50} was 120 mg/kg [Cavallito and Bailey, 1944]. For the vast majority of individuals garlic, onion and leek were non toxic at the dosages commonly used.

7.1 SAFETY

7.1.1 DOSAGE/ADMINISTRATION

Garlic: Unless otherwise prescribed an average daily dosage of 4 g of fresh garlic (one medium clove) and equivalent preparations, respectively for internal use [Blumenthal et al., 1998a,b].
Onion: Unless otherwise prescribed an average daily dosage of 50 g of fresh bulb or 20 g of cut dried bulb (Commission E), pressed juice from fresh onions used as infusion (5-10 mL in 120 mL water [Lust, 1974]) and tincture (5 mL 3 to 4 times daily [Lust, 1974; Fleming et al., 1998]), respectively.
Note: If onion preparations are used over several months, the daily maximum amount for diphenylamine is 0.035 g.
Leek: At the date of write no information available

7.1.2 SIDE EFFECTS/ADVERSE REACTIONS/CONTRADICTIONS/PRECAUTIONS

Garlic: Garlic is classified as GRAS including during pregnancy and lactation and in children, when ingested in amounts commonly found in foods. Serious toxicity has not been reported, but in rare instances gastrointestinal symptoms such as irritations and distress, changes of the intestinal flora or allergic reactions may occur. Chronical application of large amounts of raw garlic to rats resulted in anemia, weight loss and failure to grow. Genotoxicity and mutagenicity were not found in the micronucleus and Ames test, respectively [Abraham and Kesavan, 1984; Yoshida et al., 1984]
Note: The odour of garlic may pervade the breath and skin.
Onion: None known.
Leek: At the date of write no information available

7.1.3 INTERACTIONS WITH OTHER DRUGS

Garlic: None known
Onion: None known
Leek: At the date of write no information available

US

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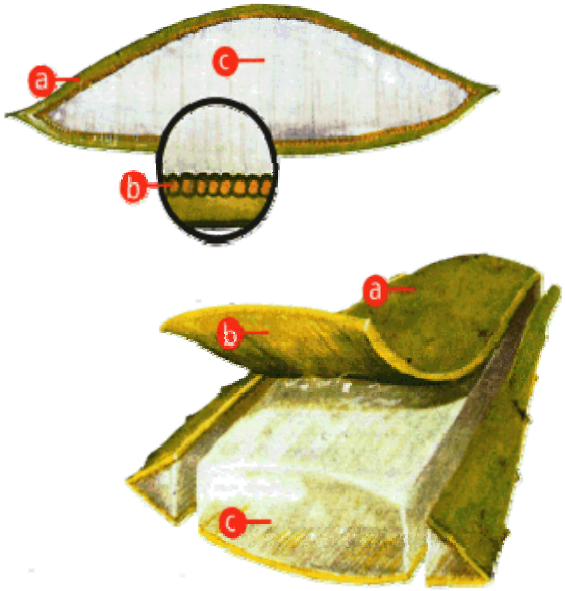
ALOE SP

Aloe vera



a: leaf epidermis, b: latex ducts, c: mesophyll

1. INTRODUCTION

Aloe is native to southern and eastern Africa, and subsequently introduced into northern Africa, the Arabian peninsula, China, Gibraltar, the Mediterranean countries and the West Indies. It is commercially cultivated in Aruba, Bonaire, Haiti, India, South Africa, the United States of America, Central America and Venezuela. [WHO]

Aloe has been called the "plant of immortality" because it can live and bloom without soil. It was given as an offering at the funerals of pharaohs and used in the baths of Egyptian queens. Today, Egyptians still hang an aloe plant over the door of a new house to provide a long and fruitful life for its occupants. According to the Roman scholar, Pliny, the plant was also used for embalming. The Greek physician Dioscorides used aloe gel for mouth infections, sores, wounds, hair loss, genital ulcers, hemorrhoids, boils, inflammation, and as a laxative. In India, the whole leaves and the fresh gel have been used as a laxative, to improve appetite and digestion, to promote menstrual flow, and to destroy and expel intestinal worms. In the seventh century, aloe gel was used in Asia for inflammatory skin conditions and sinusitis. Aloe latex (taken from the bundle sheath cells inside the leaf) has been used for its laxative effect; however, in 2002, the FDA banned the use of aloe latex in over-the-counter drug products in the United States.

Three distinct preparations of the aloe plant are used in a medicinal capacity: aloe latex, aloe gel, and aloe whole leaf extract. The constituents of aloe are purported to have anti-inflammatory, antibacterial, antitumor, antiallergic, and immunostimulatory properties. Topically, aloe gel is used to treat burns, abrasions, bruises, cuts, and may be effective in treating herpes simplex and psoriasis. Internally, aloe gel is beneficial for healing gastric and mouth ulcers, and may be beneficial in lowering blood glucose in diabetic patients and reducing blood lipid levels in patients with hyperlipidemia (high levels of fats in the blood). The extract is beneficial in the treatment of asthma, and potentially useful in treating cancer and AIDS. Clinical trials are in progress to obtain conclusive evidence for the use of aloe in the treatment of arthritis, cancer, AIDS, gastric ulcer, and colitis. Aloe is shown to increase collagen and elastin formation, which may reduce wrinkling, aid in wound healing, and protect against damage from ultraviolet rays.

Aloe vera is a very common household plant due to wound and burn healing abilities. Aloe gel is found in various personal care products such as moisturizers, cleansers, sun lotions, toothpastes, mouthwashes, deodorants, and shampoos. Aloe is processed into various flavored

and unflavored drinks that are ingested with the belief that this bitter liquid will stimulate the appetite and digestion. Aloe is also used for its bitter taste to discourage nail biting.

History of use (WHO):

Aloe drug
It was used as treatment of seborrhoeic dermatitis, peptic ulcers, tuberculosis, fungal infections and for reduction of blood sugar (glucose) levels. (WHO)

Aloe Vera Gel
It is widely used for the external treatment of minor wounds and inflammatory skin disorders. The gel is used in the treatment of minor skin irritations, including burns, bruises, and abrasions. The gel is further used in the cosmetics industry as a hydrating ingredient in liquids, creams, sun lotions, shaving creams, lip balms, healing ointments, and face packs for humans.
The treatment of acne, haemorrhoids, psoriasis, anaemia, glaucoma, peptic ulcers, tuberculosis, blindness, seborrhoeic dermatitis, and fungal infections. (WHO)

2. DESCRIPTION OF THE PLANT

The genus contains laut Crosswhite and Crosswhite (1984) at least 324 species of herbs, shrubs, and trees, primarily African, with some in Madagascar and the Arabian Peninsula.

***Aloe vera* (L.) Burm. f.**
It is a succulent, almost sessile perennial herb. The leaves are 30-50 cm long and 10 cm broad at the base; the colour is pea-green (when the leaf is young it´s spotted with white). It has bright yellow tubular flowers, 25-35 cm in length, arranged in a slender loose spike. The stamens are frequently project beyond the perianth tube. (WHO)

***Aloe ferox* Mill.**
It is an arborescent perennial shrub with a single stem of 2-3 m in height, crowned by a large rosette of numerous leaves which are glaucous, oval-lanceolate, 40-60 cm in length, thorny on the ridge and the edges; inflorescence an erect raceme 60 cm in height. The flowers have a perianth of 2,5 cm in length and are red, yellow or orange. (WHO)

2.1 SYSTEMATICS:

***Aloe vera* (L.) Burm. f.:**
Aloe barbadensis Mill., *Aloe chinensis* Bak., *A elongata* Murray, *A. indica* Royle, *A. officinalis* Forsk., *A. perfoliata* L., *A. rubescens* DC, *A. vera* L. var. *littoralis* König ex Bak., *A. vera* L. var. *chinensis* Berger, *A. vulgaris* Lam.
In most reference books, *Aloe barbadensis* Mill. is regarded as the correct species name, and *Aloe vera* (L.) Burm. f. is considered a synonym. However, according to the International Rules of Botanical Nomenclature, *Aloe vera* (L.) Buem. f. is the legitimate name for this species. The genus *Aloe* has been placed taxonomically in a family called Aloeaceae. [WHO]

***Aloe ferox* Mill.:**
Aloe horrida Haw., *A. perfoliata* Thunberg., *A. pseudoferox* Salm. Dyck, *A. socotrina* Masson., *A. supralaevis* Haw., *Pachydendron ferox* Humb. & Bonpl., *P. supralaeve* Haw. (WHO)

2.2 PLANT MATERIAL USED:

Aloe drug
It is a solidified juice originating in the cells of the pericycle and adjacent leaf parenchyma, and flowing spontaneously from the cut leaf, which is allowed to dry with or without the aid of heat.

Aloe vera Gel

Aloe vera Gel is not to be confused with the juice, which is the bitter yellow exsudate originating from the bundle sheath cells of the leaf. *Aloe Vera* Gel is the colourless mucilaginous gel obtained from the parenchymatous cells in the fresh leaves of *Aloe vera* (L.) Burm. f. (Liliaceae). (WHO)

The Preparation of fresh gel: harvest the leaves and wash them with water and a mild chlorine solution. Remove the outer layers of the leaf including the pericyclic cells, leaving a "fillet" of gel. Care should be taken not to tear the green rind which can contaminate the fillet with leaf exudate. (WHO)

3. INGREDIENTS / CONSTITUENTS

Aloe drug

The main active constituents are aloins A and B and 5-hydroxyaloin A, which are aloe-emodin anthrone-C-glycosides. Aloinosides A and B, which are anthrone-C- and O-glycosides, are also considered as active constituents contributing to the sum of hydroxy-anthracene glycosides. Other constituents include 2-acetonyl-5-methyl-chromones (also known as aloeresins), small quantities of 1,8-dihydroxy-anthraquinones (e.g. aloe-emodin) cinnamic acid and 1-methyl-tetralin derivatives. (ESCOP)

It contains not less than 18.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) and calculated with reference to the dried drug. (ESCOP)

The main active constituents of the latex are anthraquinones, including the hydroxyanthracene derivatives 25-40 percent aloins A and B, barbaloin, isobarbaloin, and emodin (Bradley, 1992; Newall et al., 1996; Wichtl and Bisset, 1994). Aloe perryi contains approximately 25 to 28 percent aloin and A. ferox approximately 10 percent (Duke, 1985). Also included are 3 to 4 percent 7-hydroxyaloins A and B, aloe-emodin, resins, aloesin and its aglycone aloesone, chromone derivatives and chrysophanol (Wichtl and Bisset, 1994; Wren, 1988).

Aloe Vera Gel

Aloe Vera Gel consists primarily of water and polysaccharides (pectins, hemicelluloses, glucomannan, acemannan, and mannose derivates). It also contains amino acids, lipids, sterols (lupeol, campesterol, and beta-sitosterol), tannins, and enzymes. Mannose 6-phosphate is a major sugar component. (WHO)

Acemannan, a fraction obtained from the gel, may be related to the reported wound healing, antiviral (Womble and Helderman, 1988) and immune-stimulating activities of aloe (Zhang and Tizard, 1996).

In the United States the internal use of the gel is approved by the FDA only as a “dietary supplement.” Manufacturers cannot claim that their products can cure, treat, or prevent any disease. The external use of the gel is approved by the FDA only as a cosmetic ingredient.

3.1. CHEMICAL ANALYSIS

Chemical assays:

Aloe drug

Thin-layer chromatography and microchemical analyses are employed for the qualitative analysis for the presence of anthracene glycosides. Quantitative analysis of total anthracene glycosides, calculated as barbaloin, is performed by spectrophotometry.

***Curacao* or *Barbados Aloe*, derived from *Aloe vera* (L.) Burm. f.**

Contains not less than 28% of hydroxyanthracene derivatives, expressed as barbaloin.

***Cape Aloe,* derived from A. ferox Miller and its hybrids with A. africana Mill. and A. spicata Baker**

Contains not less than 18% of hydroxyanthracene derivatives, expressed as barbaloin.
(WHO)

Aloe Vera Gel
Carbohydrates (0,3%), water (98,5%). Polysaccharides composition analysis by gas-liquid chromatography.

3.2. STANDARDISATION

Stability:

Aloe Vera Gel
At present no commercial preparation has been proved to be stable. Because many of the active ingredients of Aloe Vera Gel appear to deteriorate on storage, the use of fresh gel is recommended. The gel may be stabilized by pasteurization at 75-80°C for less than 3 minutes. Higher temperatures held for longer times may alter the chemical composition of the gel. (WHO)

4. PHARMACOLOGY

Aloe drug

Experimental pharmacology
*Aloe´*s mechanism of action is twofold. It stimulates colonic motility, augmenting propulsion and accelerating colonic transit, which reduces fluid absorption from the faecal mass. It also increases paracellular permeability across the colonic mucosa probably owing to an inhibition of Na^+, K^+ -adenosine triphosphatase or to an inhibition of chloride channels, which results in an increase in the water content in the large intestine. (WHO)

Clinical pharmacology
The laxative effects of Aloe are due primarily to the 1,8-dihydroxyanthracene glycosides, aloin A and B (formerly designated barbaloin). After oral administration aloin A and B, which are not absorbed in the upper intestine, are hydrolysed in the colon by intestinal bacteria and then reduced to the active metabolites (the main active metabolite is aloe-emodin-9-anthrone), which acts as a stimulant and irritant to the gastrointestinal tract. The laxative effect of Aloe is not generally observed before 6 hours after oral administration, and sometimes not until 24 or more hours after. (WHO)

Aloe Vera Gel

Pharmacology
Clinical investigations suggest that Aloe Vera Gel preparations accelerate **wound healing**. Furthmore, acemannan, a complex carbohydrate isolated from *Aloe* leaves, has been shown to accelerate wound healing and reduce radiation induced skin reactions. The therapeutic effects of Aloe Vera Gel also include prevention of progressive dermal ischaemia caused by burns, frostbite, electrical injury and intraarterial drug abuse.
The anti-inflammatory activity of Aloe Vera Gel has been revealed by a number of *in vitro* and *in vivo* studies.
Fresh Aloe Vera Gel significantly reduced acute inflammation in rats, although no effect on chronic inflammation was observed. Three plant sterols in Aloe Vera Gel reduced inflammation by up to 37% in croton oil-induced oedema in mice. Lupeol, one of the sterol compounds found in *Aloe Vera*, was the most active and reduced inflammation in a dose-dependent manner.
Aloe Vera Gel has been used for the treatment of radiation burns in humans.
In preliminary human clinical studies, the gel has shown significant results in the treatment of asthma, peptic ulcers, and diabetes mellitus (Shida, 1985; Blitz et al., 1963). The gel has been sold in the health food market as a tonic, as well as for “supporting the immune system” and “supporting healthy breathing”. But on May the 9th, 2002, the U.S. Food and Drug Administration issued a final rule banning the use of aloe (*Aloe* spp., Aloaceae) as a laxative ingredient in over-the-counter (OTC) drug products.

Externally, the gel has been used in many ways: cosmetics, dermabrasion, wound-healing, and psoriasis (Fulton, 1990; Heggers et al., 1993; McCauley et al., 1990; Syed et al., 1996). In cosmetics, the gel is added to moisturizers, cleansers, shampoos, suntan lotions, and sunburn treatments (Bruneton, 1994; Grindlay and Reynolds, 1986).
The drug derived from aloe latex is part of one of the strongest anthraquinone groups that function as stimulant laxatives (Wichtl and Bisset, 1994). The latex is officially approved as a laxative in the U.S., England, and Germany. The German Commission E recommends the use of aloe for occasional constipation and for conditions that require a soft stool, such as anal fissures, hemorrhoids, and after rectal or anal surgery (Blumenthal et al., 1998). Externally, the latex is used like the gel and as a soothing agent in treating burns and mild cuts (Bradley, 1992).

4.1 Animal Studies

Aloe gel has demonstrated wound healing (Davis et al., 1989; 1994a; 1994b; Heggers et al., 1993; 1995; 1996), anti-inflammatory (Vazquez et al., 1996), antiviral (Saoo et al., 1996), spermicidal (Fahim and Wang, 1996), and gastroprotective (Danhof, 1991) properties. It has also shown immune-stimulating (Zhang and Tizard, 1996) and cholesterol-lowering (Tizard et al., 1989) activity.
Aloe gel is thought to speed recovery from wounds by enhancing the activity of macrophages and fibroblasts (Davis et al., 1994a; Heggers et al., 1993). A recent study showed aloe to be more effective than conventional treatments for burns, frostbite, and intra-arterial damage from intravenous drug abuse, as well as tissue injuries from electrical shocks (Heggers et al., 1993). Aloe gel also helped speed the rate of wound closure and the resistance of the healed wound to tearing (Heggers et al., 1995 and 1996). Its wound-healing activity has been partially attributed to the polysaccharide, mannose-6-phosphate (Davis et al., 1994a).
It has been suggested that aloe works as an anti-inflammatory when used topically and when taken orally (Davis et al., 1989). An animal study examined the anti-inflammatory activity of extracts derived from aloe gel. The researchers concluded that the action may be due to inhibition of the arachidonic acid pathway through cyclooxygenase (Vasquez et al., 1996). Another study inhibited edema in mice by 37 percent and attributed the activity to the sterol compounds in aloe, especially lupeol (Davis et al., 1994b).
Antiviral activity was shown in an in vitro study using a purified extract from the gel of Aloe barbadensis. The main activity against cytomegalovirus was shown 12-36 hours after infection (Saoo et al., 1996). An in vitro study using zinc acetate and lyophilized Aloe barbadensis (7.5 and 10 percent) demonstrated an antiviral and spermicidal effect. The authors concluded that it may be useful as a contraceptive, especially significant in preventing the transmission of HIV. Spermicidal activity from Aloe was thought to be due to microelements (boron, barium, calcium, chromium, copper, iron, potassium, magnesium, manganese, phosphoru, and zinc) that immobilize the tails of sperm without causing vaginal irritation (Fahim and Wang, 1996).
Gastroprotective properties were shown in several animal studies. When aloe gel was administered to rats prior to ulcer-inducing stress, the number of ulcers decreased by 80 percent. After developing ulcers, the animals given aloe gel recovered three times faster than the control animals (Galal et al., 1975; Danhof, 1991). Another animal study demonstrated similar results (Kandil and Gobran, 1979). The triterpenoids contained in aloe were found to protect against the formation of gastric ulcers (Gupta et al., 1981).
Research on immune stimulation in mice has indicated that acemannan, a polysaccharide within aloe, has demonstrated dose-dependent macrophage activation (Zhang and Tizard, 1996; Ramamoorthy et al., 1996). When given orally to animals, mannans have also been shown to inhibit cholesterol absorption and lower cholesterol (Tizard et al., 1989).
The pharmacological difference between aloe gel and aloe latex is that the gel does not contain any anthraquinone compounds and does not, therefore, exert any laxative action (Newall et al., 1996). Anthraquinones induce secretion of water and electrolytes into the lumen of the gut and inhibit the absorption of electrolytes and water by the colon. This action in turn activates peristalsis (Blumenthal et al., 1998; Bradley, 1992). Aloe-emodin, a degradation product of B-glycosides in the colon, is the laxative metabolite (Blumenthal et al., 1998). Animal studies have found that aloe-emodin and an alcoholic extract of aloe possess antitumor and anticancer activity. In vitro research has reported that aloctin A could induce cells cytotoxic to syngenic and

allogenic tumor cells (Leung and Foster, 1996; Newall et al., 1996; Wren, 1988). However, the National Cancer Institute is no longer studying the latex as an anticancer treatment.

4.2 Recent Human Clinical Studies

Clinical studies have focused upon the external use of aloe gel for wound healing and psoriasis. In a study that compared aloe to polyethylene oxide gel dressing in the treatment of patients with dermabrasion (surgery to remove tattoos, acne, scars, and wrinkles), aloe accelerated healing by 72 hours (Fulton, 1990). The wounds of patients recovering from burns, frostbite, and intra-arterial drug abuse healed faster, had less tissue loss or morbidity, and fewer complications than those patients treated with conventional methods. Seven percent of the frostbite patients treated with aloe required amputation, compared with 32 percent in the nontreatment group (Heggers et al., 1993). In another study, aloe also was shown to assist patients recovering from wounds associated with frostbite (McCauley et al., 1990). One study contradicted these results by showing a delay of 30 days in the healing of wounds on patients treated with aloe gel (Schmidt and Greenspoon, 1991). A double-blind, placebo-controlled study examined the effect of aloe extract (0.5 percent in a hydrophilic cream) for 60 psoriasis vulgaris patients, aged 18 to 50 years. The aloe cream cured 25 out of 30 patients compared to a placebo rate of 2 out of 30. The authors concluded it was a safe alternative treatment for treating psoriasis (Syed et al., 1996).

The internal use of the gel has been studied for treating asthma, peptic ulcers, and diabetes mellitus. A clinical study found that a fraction of aloe extract enhanced phagocytosis (causing the elimination of bacteria and dust particles) in adult bronchial asthma (Shida et al., 1985). Another study examined the effect of aloe on the blood sugar levels of 50 men and 22 women with a diabetic curve on glucose tolerance tests. By week two, cholesterol levels were unchanged, but changes to blood sugar levels and triglyceride levels were statistically significant (Yongchaiyadha et al., 1996). A small study of 12 peptic ulcer patients reported positive results from treatment with aloe. The results were attributed to the inhibition of hydrochloric acid secretion, coacervation of pepsin and general detoxification (Blitz et al., 1963).

5. Microbiology

Aloe showed antiviral, spermicidal and anti- inflammatory effects and macrophage activation is reported. More see 4.

6. Efficacy

Aloe gel is reported to contain glycoproteins, polysaccharides, and other contituents (enzymes, etc.) and is essentially used for the treatment of various skin conditions, like burns, abrasions, bruises, cuts, psoriasis, herpes simplex, etc. On the other hand, there is a general agreement that aloe gel used in a fresh state, not on storage, is more able to show anti-inflammatory properties.

The internal use of Aloe drug has a laxative effect. It acts as a stimulant and irritant to the gastrointestinal tract.

7. Toxicology

Aloe drug

The major symptoms of overdose are griping and severe diarrhoea with consequent losses of fluid and electrolytes. Treatment should be supportive with generous amounts of fluid. Electrolytes, particulary potassium, should be monitored, especially in children and the elderly. [WHO]

Aloe Vera Gel
No information available.

7.1. SAFETY

Aloe drug
The decreased intestinal transit time may reduce absorption of orally administered drugs. Existing hypokalaemia resulting from long-term laxative abuse can potentiate the effects of cardiotonic glycosides (digitalis, strophanthus) and antiarrhythmic drugs such as quinidine. The induction of hypokalaemia by drugs such as thiazide diuretics, adrenocorticosteroids, and liquorice root may be enhanced, and electrolyte imbalance may be aggravated.
Laxatives containing anthraquinone glycosides should not be used continuously for longer than 1-2 weeks, owing to the danger of electrolyte imbalance. (WHO)

Pregnancy
No teratogenic or fetotoxic effects were seen in rats after oral treatment with aloe extract (up to 1000 mg/kg) or aloin A (up to 200mg/kg) [WHO]
Strong indirectly- induced uterus contractions can be induced, therefor could Aloe induce abort in pregnant animals Reichling et al., 2005).

Nursing animals
Anthranoid metabolites appear in the milk.The milk has a yellow-greenish to red colour and a bitter taste. The milk of animals, which are treated with *Aloe* has a laxative effect. These specialties of *Aloe* have to be considered by application and for the release of milk for consumption (Reichling et al., 2005).

Contraindication
Pregnant or lactating animals; Inflammation in the Gastric- and Intestinaltract (Reichling et al., 2005).

Dosages
The correct individual dose is the smallest amount required to produce a soft-formed stool. Short internal use is useful in case of obstipation. Aloe is a perfect laxative for horses. Therapeutic use in case of obstipation and chronical meteorism [Rabinovich 1981]
Horses are very sensitive on the laxative effects of *Aloe*. The laxative effect is only moderate for Cattle, small ruminants and pigs. [Rabinovich 1981]

Internal Use of Aloe (Aloe drug):
Dosages
recommended daily allowance:

Cattle	25,0 - 40,0 g
Horse	20,0 - 35,0 g
Sheep, Goat	5,0 - 15,0 g
Pig	3,0 - 10,0 g
Dog	0,5 - 3,0 g
Cat	0,2 - 1,0 g
Fowl	0,2 - 0,5 g

(changed by Rabinovich 1981; Gachnian and Assenov 1986, 1990) (Reichling et al., 2005).

7.2. PRECAUTIONS

Aloe drug
Drug aloe should not be taken for more than 10 days. It should not be used by pregnant or lactating women and is contraindicated in the following conditions: intestinalobstruction or inflammation (e.g., appendicitis, colitis, Crohn's disease, irritable bowel syndrome, etc.), abdominal pain of unknown origin, hemorrhoids, kidney dysfunction, menstruation, and in children less than 12 years old (McGuffin et al., 1996; Wichtl and Bisset, 1994).

Aloe vera Gel

It should not be used externally after laparotomy or caesarean delivery because it may delay wound healing (McGuffin et al., 1996).

Care should be taken for the preparation of fresh gel: harvest the leaves and wash them with water and a mild chlorine solution. Remove the outer layers of the leaf including the pericyclic cells, leaving a "fillet" of gel. Care should be taken not to tear the green rind which can contaminate the fillet with leaf exudate. [WHO]

Aloe Vera Gel is contraindicated in cases of known allergy to plants in the Liliaceae. [WHO]

No information is available concerning general precautions, or precautions dealing with carcinogenesis, mutagenesis, impairment of fertility; drug and laboratory test interactions; drug interactions; nursing mothers; paediatric use; or teratogenic or non-teratogenic effects in pregnancy. [WHO]

Legal status of other use than as feed additives:

It is allowed to use Aloen, Barbados and Cape, standardised dried extract and preparations out of the dried extract, as an active agent for foodstuff delievering animals in the EU. [VO (EWG) Nr. 2377/90] (Reichling et al., 2005).

KBB

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ASTRAGALUS GUMMIFER

Astragalus gummifer

1. INTRODUCTION

Astragalus gummifer (Labill.), *A. microcephalus* and some other *Astragalussp.*, family Fabaceae (formerly Leguminose), habitat Asia minor, Persia and Kurdistan, are the most important sources of tragacanth gum. From wounds in *Astragalus shrub*s exudes gum tragacanth and hardens as curled ribbons or flakes, which can be collected after a few weeks.
Gum tragacanth is defined by JECFA as: "a dried exudation obtained from the stems and branches of *Astragalus gummifer* Labillardiere and other Asiatic species of *Astragalus* (Fam. Leguminosae)" (FAO 1992b), to be not confused with gum karaya that is a dried gummy exudation from *Sterculia urens* Roxburgh and other species of *Sterculia*, or from *Cochlospermum gossypium* or other species of *Cochlospermum Kunth* (Weiping, 2000). Tragacanth occurs in two main forms, ribbons and flakes. Ribbons are considered to be of superior quality (Gecgil *et al*., 1975). The flakes break with a short fracture, are odourless and nearly tasteless. In EU countries, tragacanth gum is used as a food and feed additive (E413). In non-food application it is widely used as an effective suspending agent, as a stabilizer in dermatological creams, and as a binding agent in the production of tablets. Iran dominates the gum tragacanth market and supplies the highest quality gum. Turkey is the second largest producer, but Turkish gum is considered of inferior quality. Much smaller amounts of gum are exported from Afghanistan and Syria (Anderson *et al*., 1975).

2. DESCRIPTION OF THE PLANT

Astragalus sp. L. is the largest genus of the Fabaceae family consisting of more than 1.500 species mainly distributed over the northern hemisphere. In tropical regions on high mountains only, missing in Australia, *Astragalus* has its center of diversity in South-West Asia.

These small shrubs, varying in height from hardly 10 cm (*A. microcephalus*) up to 1 m (*A. gummifer*), grow in the highlands and deserts of Turkey, Iran, Iraq, Syria, Lebanon, Afghanistan, Pakistan, and Russia. Exudate gums are produced by many trees and shrubs as a natural defense mechanism, particularly in semiarid regions. When the plants bark is injured, an aqueous gum solution is exuded to seal the wound, preventing infection and dehydration of the plant. The solution dries in contact with air and sunlight, to form hard, glass-like lumps which can easily be collected. The primary source of the gum are the rather large tap roots of the bush,

inside of which gum is contained in a central gum cylinder. Incisions are also made in the bark of the branches, although this usually yields gum of an inferior quality. After collection, the gum is sorted manually into various grades of ribbon and flake. Iranian tragacanth ribbons are sorted into five grades, while flakes are sold in seven different grades (FAO, 1995). After arrival in the importing country, the gum is usually ground into powder, with particle size varying according to the desired viscosity.

Most commercial gum tragacanth is obtained from *Astragalus gummifer* Labill. and *A. microcephalus* Willd., but the contribution of other *Astragalus* species such as *A. adscencens* Boiss., *A. echidnaeformis* Sirjaev, *A. gossypinus* Fisch., and *A. kurdicus* Boiss. is also significant.

From other *Astragalus sp. a*s e.g. *A. membranaceus and A.mongholicus* indigenous to East Asia the roots are used due to their content on saponins and flavonoids. In traditional Chinese medicine (TCM) preparations are used at cardiovascular, gastrointestinal and immune disorders. This drug should not be mixed up with tragacanth (gum).

3. INGREDIENTS/CONSTITUENTS (CHEMICAL ANALYSIS, STANDARDIZATION)

Gum tragacanth is a complex, highly branched, heterogeneous polysaccharide, naturally occurring as a slightly acidic calcium, magnesium, and potassium salt. It has a molecular mass of about $8{,}4x10^5$ Da and an elongated shape of $4{,}500x10^9$ Å (Verbeken *et al*., 2003).
Gum tragacanth contain 20 – 30% of a water soluble fraction called tragacanthin (consisting of tragacanthic acid and an arabinogalactan) and 60-70% of a water-insoluble fraction called bassorin. Both fractions contain small amounts of proteinaceous material and methoxyl groups, the latter present in higher amounts in the watersoluble fraction. The ratio of the two fractions strongly varies between species. The water-swellable tragacanthic acid fraction has a high molecular weight and a rodlike molecular shape. The main chain is formed by 1,4-linked d-galactose residues with side chains of d-xylose units attached to the main chain by 1,3 linkages. The water-soluble tragacanthin is a neutral, highly branched arabinogalactan with a spherical molecular shape. Its structure probably consists of a core composed of 1,6- and 1,3-linked d-galactose with attached chains of 1,2-, 1,3- and 1,5-linked l-arabinose.
When solubilized in water, gum tragacanth forms viscous solutions, even at low concentrations. Depending on the grade, the viscosity of 1% solutions may range from 0.1 Pa s up to 3.5 Pa s. Higher viscosities are obtained with ribbon types than with flake types. When shear is increased, the viscosity of the solution decreases, indicating pseudoplastic behavior. This decrease is reversible and the viscosity regains its original level as shear is reduced.

3.1 CHEMICAL ANALYSIS

A gas chromatographic method is described for the determination of food grade gums in milk products, salad dressings and meat sauces. The extraction method essentially involves removal of fat followed by starch degradation and precipitation of protein. The isolated gums are hydrolysed with trifluoroacetic acid, and the resulting neutral monosaccharides are converted to their aldonitrile acetate derivatives for gas chromatographic analysis on a 1 m x 2 mm stainless steel column packed with Gas Chrom Q (100-200 mesh) coated with 3% neopentylglyclosuccinate. Samples components did not interfere in the determination of the gum. Recoveries of gum added to 13 different foods averaged 85%; where more than 1 gum is present in a food, estimation is best accomplished by using monosaccharide peaks which are unique for a particular gum (Lawrence *et al*., 1985).
In another analytical method the monosaccharide constituents of plant gums were separated by capillary electrophoresis at pH 12.1 and detected with indirect UV absorbance. The monosaccharides obtained after hydrolysis with 2 M trifluoroacetic acid and lyophilisation of the hydrolysate were arabinose, galactose, mannose, rhamnose, xylose, fucose, and glucose, and the two sugar acids galacturonic and glucuronic acid, in accordance with the literature. They were separated in a background electrolyte consisting of NaOH to adjust the pH, 20 mM 2,6-pyridinedicarboxylic acid as chromophore for detection and 0.5 mM cetyltrimethylammonium

bromide as additive to reverse the electroosmotic flow. Based on their electropherograms, the plant gums could be identified by their typical composition as follows: peaks of galacturonic acid and fucose point to gum tragacanth (Groessl *et al*., 2005).
Furthermore the complex structure of the arabinogalactan is now further elucidated using GC-MS, NMR, and ESI-MS techniques.

3.2 STANDARDIZATION, STABILITY

Gum tragacanth is one of the most acid-resistant gums. The pH of a 1% solution varies between 5 and 6, but the viscosity remains quite stable over a broad range (pH 2–10). Because of this high acid stability, which is more pronounced for flake types, the gum is widely used in acidic conditions. Traganth is also heat stable. The gels were unaffected by storage and the pH of the gels suggest that the gum substance was totally neutral in reaction (Leung *et al*., 2003).

4. PHARMACOLOGY

4.1 ANIMAL STUDIES

In a study, bassorin was administered for 2 weeks to piglets. No alterations of bile acids, lipidic composition, or lithogenicity of bile were observed in piglets.
In rats tragacanth increased fecal SCFA (Short Chain of Fatty Acid) and fecal water (Edwards et al., 1995).
In rats fed ground barley, gum tragacanth accelerated the passage of digesta trough the gastrointestinal tract, gave the faeces a lighter colour and increased their size and water content (Gohl *et al*., 1977).
Fe absorption was not inhibited by gum tragacanth (Gillooly *et al*., 1984).
In rats that were fed hyperlipidaemic diet containing gum tragacanth, a marked improvement in the plasma LDL cholesterol and total cholesterol concentration was observed. No significant difference in the plasma triglyceride concentration was detected. Plasma HDL concentration was significantly higher in the non-hyperlipidaemic group. Addition of gum tragacanth to the hyperlipidaemic diet significantly improved the plasma HDL concentration. These results suggest that fiber from gum tragacanth lowers plasma cholesterol and LDL in hyperlipidaemia (Amer *et al*., 1999).
In rabbit tragacanth mucilage exhibited a considerable potency for wound healing.

4.2 HUMAN STUDIES

In a study on volunteer males, with age ranging from 21-57 yr, gum tragacanth increased stool weight, with no effect on serum cholesterol or on hydrogen excretion but increased fecal bile-acid excretion Eastwood *et al*., 1986). In man the addition of TG significantly reduced blood glucose at one or more points, during the glucose tolerance test, and reduced serum insulin concentration. The reduction in the mean peak rise in blood glucose concentration and the delay in mouth-to-caecum transit time, were correlate positively with viscosity (Eastwood *et al*., 1984, Jenkins *et al*., 1978).
Tragacanth gum evidenced beneficial pharmaceutical and nutraceutical properties for its protective effect of LDL from oxidation (Nikolaos *et al*., 2003).

4.3 ADME

In a study with ten *Bacteroides* species from human colon, gum tragacanth was completely fermented during 21 h as its viscosity was abolished and SCFA and gases were produced. *Bacteroides* species were able to ferment plant polysaccharides including gum tragacanth. The ability to utilize plant polysaccharides varied considerably among the *Bacteroides* species (Salyers *et al*., 1977).
The mechanisms by which non-starch polysaccharides (NSP) increase faecal output, and in particular faecal water, are not fully understood. NSP which are resistant to fermentation by bacteria in the large intestine are the most effective faecal bulkers, probably because they retain their water-holding capacity (WHC). The extent to which a NSP is fermented in the large intestine of an animal is dependent not only on the ease of fermentation but also on the time it remains within the large intestine (Van Soest *et al*. 1982). The effect of fermentation products

on motility has not been investigated thoroughly and short-chain fatty acids (SCFA) have been shown to inhibit motility in the caecum of the sheep (Svendsen, 1972) and the isolated rat colon (Squires *et al*. 1992), but to stimulate contractions in rat mid- and distal colonic strips (Yajima, 1985).
The osmotic activity of the SCFA has been largely discounted as it has been shown that they are rapidly absorbed (McNeil *et al*. 1978). The major sites of fermentation are thought to be the caecum and proximal colon but it maybe possible, if propulsion is stimulated before fermentation is complete, for fermentation of a NSP to continue further along the large intestine. If this occurs, the SCFA produced at distal sites may have a greater effect on stool output as the water remaining in the gut. In addition, as SCFA have been shown to influence the metabolism and turnover of colonic enterocytes, higher concentrations of SCFA at more distal sites, where disease is more common, may have an important role in pathology and treatment.
Tragacanth had no significant effect on amount or concentration of SCFA. Tragacanth significantly reduced the molar proportions of acetic acid in the caecum but significantly increased the molar proportion of acetic acid in faeces and significantly decreased the proportion of butyric acid The predominance of acetic acid is generally higher in the faeces than in the caecum and reflects either a change in fermentation pattern in more distal colon or a difference in the utilization or absorption of the SCFA (Edwards et al., 1995).

5. MICROBIOLOGY

Tragacanthin polysaccharides were evaluated in mice infected with Punta Toro Virus (PTV), a phlebovirus member of the Bunyaviridae family. The polysaccharides administered as single intraperitoneal treatments protected the majority of mice from mortality not by activate lymphocytes or natural killer cell, nor interferon induced in treated mice but by activation of peritoneal macrophages (Smee *et al*., 1996).
The purified polysaccharides from the gum exudates of *Astragalus* species, Tragacantha, are immunomodulators and inhibit cancer cells and viruses *in vitro*.

6. EFFICACY

Tragacanth gum was studied in a 6-7 week feeding study on rats aiming to evaluate the effect on adaptive responses of nutritionally-controlled parameters in rats by feeding a fibre-free diet containing increasing additions of polysaccharides (0, 10, 20, and 40%). In general, the supplements reduced growth rates due to lower energy intakes, but did decrease energy utilization. All polysaccharides increased small intestine weights by up to about 30% without grossly altering mucosal protein or DNA per unit of length. On the large intestine, tragacanth gum had a pronounced effect on caecum weight, which increased by factors of 1.8, 2.0, and 4.2 for additions of 10, 20, and 40%, respectively.
Gum tragacanth was used to replace 2% starch in diets for chickens from 1 to 24 days old, Japanese quail from 4 to 35 or 37 days, Sprague-Dawley rats from weaning to 36 days and Tribolium castaneum larvae from 6 to 14 days. Gum tragacanth inhibited growth of chickens only. Growth of rats was not affected by any fibre (Vohra, 1979).

7. TOXICOLOGY

Following a 7-day control period, 5 male consumed 9.9 g gum tragacanth daily for 21 days. The results do not reflect any adverse toxicological effects arising from the ingestion of large daily doses of gum tragacanth. The intestinal transit time decreased and faecal fat concentration increased. These changes may be of nutritional and physiological interest but do not reflect any adverse toxicological effects arising from the ingestion of large daily doses of tragacanth.
No mutagenic activity of tragacanth gum was observed following *in vitro* microbial assays, with and without activation, on *Salmonella typhimurium* (strains TA1535, TA1537, TA1538, TA98, and TA100) and *Saccharomyces cerevisiae* strain D4. Tragacanth gum was not mutagenic in a number of tests using mammalian systems: Host Mediated Assay in vivo in rats and mice using *Salmonella typhimurium* strain TA1530 and G46 or mitotic recombination frequency in *S.*

cerevisiae D3, cytogenic study in vivo of rat bone-marrow cells, and in vitro study with human lung cells (wt. 38) in tissue culture (Litton Bionetics 1972; 1977).
Tragacanth gum showed no evidence of maternal toxicity or teratogenicity after oral administration (as a suspension in corn oil) at levels up to 1200 mg/kg b.w./day to pregnant mice (days 6-16 of gestation) or to pregnant hamsters at dose levels up to 900 mg/kg b.w./day (days 6-10 of gestation). Similar studies with pregnant rats at dose levels up to 1200 mg/kg b.w. (days 6-15 of gestation) and with pregnant rabbits at dose levels up to 700 mg/kg b.w. (days 6-18 of gestation) resulted in significant maternal mortality in rats at the 1200 mg/kg b.w. dose level and in rabbits at dose levels of 150 and 700 mg/kg b.w.
Although there are only a few reports on sensitization to tragacanth gum, the available information indicates that tragacanth gum is a powerful allergen capable of causing extremely severe reactions. Allergic reactions may occur as a result of inhalation or oral ingestion. The immunogenicity of tragacanth gum was demonstrated in an *in vivo* test using a foot pad swelling test in mice. Purification of the gum led to a marked reduction of the immune response (Gelfand, 1943; 1949).
Groups of 50 male and 50 female Osborne-Mendel rats (approximately 21 days of age) were maintained on diets containing 0, 0.006, 0.06, 0.6, or 6.0% ppm tragacanth gum. After 13 weeks on the test diets, the rats were bred to produce an F1 generation. Both males and females in the 6% group showed significantly lower body weights, as well as decreased food efficiency, than the controls. Lower body weights were also observed in the F1 generation, particularly in the males. Haematological measurements showed no compound-related effects. Only minor effects were noted in the various clinical chemistry parameters. Reproduction data were comparable for test and control animals. Histological studies did not show any compound-related effects. Enlarged livers were noted in the 6% group, but the enlargement was not associated with any significant change in liver composition or with histological changes. The ATP/ADP ratio in liver preparations for F0 animals was markedly decreased, but this effect was not observed in F1 animals (Graham et al., 1985).

7.1 SAFETY

Depending on the oil content. In low-calorie, oilfree dressings, gum tragacanth is used to imitate the creamy mouthfeel and body of dispersed oil droplets. In fish and citrus oil emulsions with a long shelf-life, gum tragacanth is used in combination with gum arabic. The interaction between the two gums reduces viscosity, allowing the production of thin, pourable, smooth emulsions. Gum tragacanth is used in ice cream to provide texture to the product. It also prevents texture changes during storage due to the formation of ice crystals. In water ices, sorbets, and ice pops, the gum prevents the migration of flavor and color components during storage and consumption. It is also used in milkshakes as a viscosity control agent, providing texture and stabilizing incorporated air.
Gum tragacanth is used in bakery fruit-based toppings and fillings to give a shiny, clear appearance and a creamy texture. In chewy sweets, such as lozenges, it acts as a thickener and provides texture. The gum is used in fruit tablets, gum drops, and pastilles as a binding agent during compression. It also provides body and mouthfeel and ensures good flavor-release during consumption. In icings, gum tragacanth is used as a waterbinder, maintaining pliability and preventing cracks and breaks. It also provides consistency, smooth texture, and creamy taste to the product. Gum tragacanth is also an excellent emulsifying agent for oil-in-water emulsions. It is very effective in lowering the interfacial tension, even at low concentrations, and simultaneously acts as a thickener of the continuous phase of the emulsion, preventing flocculation and coalescence of the dispersed oil droplets.

Recentely, there have been fatal accidents, mainly in children and elderly persons resulting from asphyxiation following the ingestion of jelly mini-cups due to their consistence, shape, size and manner of ingestion, giving rise to a risk that they remain blocked in the throat and provoke choking. In view of this situation, on 13 april 2004, a Commission Decision (adopted on 12 july 2004) suspended the placing on the market of jelly mini-cups containing one or more of the gel forming food additives, among others E413, in order to protect human health.

Non-food applications

Gum tragacanth is widely used in the pharmaceutical industry. It is an effective suspending agent and prevents insoluble materials, which are usually the active ingredients, from settling-out in aqueous mixtures. By stabilizing the emulsion and keeping the oil droplets uniformly distributed throughout the product, the gum facilitates the absorption of the water-insoluble components, thus improving the action of the pharmaceutical. Gum tragacanth is used as a stabilizer in dermatological creams and lotions, also contributing a protective coating and smooth handfeel. Combined with glycerin, it acts as a binding agent in the production of tablets.

Interactions with other drugs

In vitro digestibility of casein was substantially decreased by tragacanth. The loss appeared to be related to the structure of the gum (degree of branching and extent of ionization) (Acton *et al*., 1982).

Caution

Tragacanth gum from *Astragalus gummifer* or *A.microcephalus* may not be admixed with *Astragalus* roots
from East Asia (TCM).

DT/SG/LG/AT

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BORAGO OFFICINALIS

Borago officinalis

1. INTRODUCTION

Borage is also known by the names Bugloss, Burage and Burrage. Some authorities consider that the Latin name *Borago*, from which our popular name is taken, is a corruption of *corago*, from *cor,* the heart, and *ago*, I bring, because of its cordial effect. However, some assert that the name might be derived from the Latin "borra", which means "rough hair" due to the hairy leaves and stems of this plant. In all the countries bordering the Mediterranean, where it is plentiful, it is spelt with a double “r”, so the word may be derived from the Italian “borra” French “bourra”, signifying hair or wool, in reference to the thick covering of short hairs which clothes the whole plant. Some suggest that the name is a Celtic derivative from "borrach", meaning "courage". Borage flowers nourish the adrenal glands, acts as a restorative agent on the adrenal cortex, and may be why it has long been thought to bolster courage. The parts of this plant used medicinally are the leaves, flowers, and the oil from the seeds. The seeds contain essential fatty acids, linoleic acid, and gamma-linolenic acid (GLA), the same substance as in Evening Primrose oil. The oil from Borage seeds is used for inflammatory disorders, immunomodulatory effects, dermatitis, menstrual and menopausal problems, eczema, arthritis and rheumatism.
The herb can be confused with *Echium vulgare.*

2. DESCRIPTION OF THE PLANT

Borago officinalis is an annual plant native of southern Europe and Asia minor, but is now found all over Europe and the U.S.
The whole plant is rough with white, stiff, prickly hairs. The round stems, 15 – 100 cm high, are branched and hollow; the leaves are alternate, large, wrinkled, deep green, oval and pointed. The flowers, which terminate the cells, are bright blue and star-shaped, with prominent black anthers which form a cone in the centre. The fruit consist of four brownish-black nutlets.

2.1 SYSTEMATICS

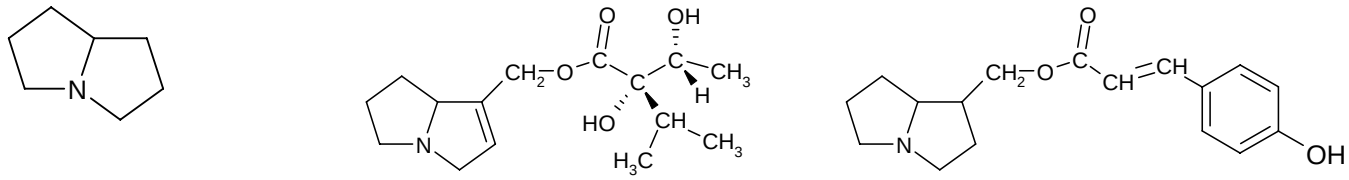
The genus *Borago* comprises of 3 species, *B.longifolia* Poiret in North-West Africa, *B.pymaea* (D.C.) Chater and Greuther, and *Borago officinalis* L., the only one used as herb and crude drug. It belongs to the Family Boraginaceae, closely related to Scrophulariaceae and Lamiaceae.

2.2 PLANT PARTS USED

Borago officinalis (the common Borage) is grown for its leaves, which are used in flavorings, and its flowers, which are used in potpourri. Borage has been used medicinally and in the preparation of various cordials and cups. The young leaves are used in salads to which they add a cucumber flavour. Seed oil (28% - 38% lipids) get recent interest as the richest source of γ-linolenic acid (GLA) 17 – 25%.

3. INGREDIENTS / CONSTITUENTS

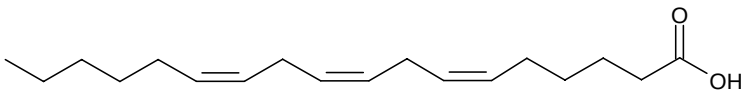
The leaves contain small amounts of pyrrolizidine alkaloids including lycopsamine, intermedine, their acetyl derivatives, supinine, supinidine, plus amabiline and choline; Amabiline (I) was found in seeds and leaves and thesinine (II) was found in flowers and seeds of *Borago officinalis*. (Fig. 1).



pyrrolizidine amabiline thesinine

Figure 1 Chemical structure of pyrrolizidine, amabiline, thesinine

Dry matter is mainly constituted by neutral detergent fiber, ash, protein and 9,1% fatty acid, including α-linolenic acid (55%) and γ-linolenic acid (>4%). Borage has also adequate levels of minerals, and K reaches a higher proportion than either elements. Whole leaves include: K 400, Na 63, Ca 130, Mg 30, Fe 1.8, Mn 0.44, Zn 0.23, and Cu 0.13 mg/100 g. Also present are small amounts of silicic (1,5– 2,2%), acetic, lactic and malic acid.
Aerial blooming parts contain larger quantities of heterogeneous polysaccharides (19,90 - 20,30 g%), polyphenolcarboxilic acids (0,545 - 0,546 g%) and smaller quantities of flavonoids (0,028 - 0,029 g%) than seeds (11,9 - 12,51 g%; 0,402 - 0,403 g%; 0,032 - 0,033 g% respectively) (Dinu et al., 2002).
Fresh leaves contain up to 30% mucilage hydrolyzing to glucose, galactose, arabinose and allantoin.
Seed oil is obtained from seeds of *Borago officinalis* L. by extraction and/or pression and it is then refined. A suitable antioxidant may be added.
Solubility: practically insoluble in water and in ethanol (96 %), miscible with light petroleum.
Relative density: about 0.921.
Refractive index: about 1.476.
Seed oil (28% - 38% lipids) get recent interest as the richest source of γ-linolenic acid (GLA) 17 – 25% (Fig. 2).



γ-linolenic acid

Figure 2 Chemical structure of gamma linolenic acid

Other seed fatty acids include linoleic (30% - 41%), oleic (14,5% – 23%), α-linolenic acid (max 0,5%), palmitic acid (9% - 12%), palmitoleic acid (max 0,6%) and stearic (2% - 6%) (Leung and Foster, 2003; PDR for Herbal Medicines, 2004; Ph.Eur. 5, 2005)

Ferulic acid represents a high proportion of the free phenolic acids, but hydroxycaffeic acid is the major constituent of phenolic acids liberated from esters of borage seeds. New compounds were found: rutoside in aerial blooming parts, quercitol in aerial blooming parts and seed .
The evolution of the quality of borage is closely related to the ageing of the plant, with a progressive increase in the fibrous fraction and N-free extracts and a slight decrease in crude protein content and organic matter digestibility. The fatty acid components of borage are myristic, palmitic, stearic and oleic acids, and four polyunsatd. fatty acids (PUFA): linoleic (LA), α-linolenic (ALA), γ-linolenic (GLA), and stearidonic (SDA). The n-6/n-3 PUFA ratio increases from 0.43, at late vegetative stage, to 1.59 at early seed stage, as the relative GLA and LA content increase during the growth cycle, while the SDA and ALA content decrease (Peiretti et al., 2004).
The content of γ-linoleic acid and other fatty acids of the seed oil of germoplasm is different in wild and cultivated borage. GLA showed an important range of variation, from 8.7% to 28.6% of the seed oil. Oleic, linoleic and erucic acid also showed wide ranges of variation. White flowered cultivated genotypes had higher contents of GLA than blue flowered wild material collected in Spain. Blue flowered germplasm from Northern Europe showed higher values of erucic acid (De Haro et al., 2002).

3.1 CHEMICAL ANALYSIS: METHODS TO DETERMINATE THE COMPOUND

Analytical methods specifications for oil evaluation
from borage have been published (European Pharmacopea 5.1; 04/2005:2105). The oil is clear, light yellow or yellow liquid and the official method for the chemical identification of fatty acid is the Thin-Layer Chromatography.
The total chemical composition of lipophilic extracts from *Borago officinalis* seeds was determinated by capillary Gas-Chromatography/Mass-Spectrometry, revealing the presence of tocopherols and sterols along with important quantities of fatty acids. The composition of phenolic acids, both free and liberated from esters and glycosides, was determinate in borage seeds by GC and MS.
The pyrrolizidine alkaloid content of crude borage oil and borage oil from different processing stages was deteterminated by GC-MS. The results showed that no pyrrolizidine alkaloids were present above a detection limit of 20 ppb. The reduction factors for pyrrolizidine alkaloids at various stages in the oil refining process were determined by means of spiking experiments using the available pyrrolizidine alkaloid crotaline. The pyrrolizidine content in crude borage oil was reduced overall by a factor of approximately 30,000 in the refining process (Wretensjoe and Karlberg, 2003).

3.2 STANDARDIZATION, STABILITY

The oil is kept under an inert gas, in a well-filled, airtight container, protected from light.
It was stated that plant oils rich γ-linolenic acid (borago oil) become subject to oxidation processes very easily. One of the methods to increase their oxidative stability is to introduce antioxidants. The addition of alpha tocopherol, ascorbyl palmitate and lecithin was the most effective for borago oil.
Edible flowers were stored in polyethylene bags at -2.5 to 20 °C. *Borago officinalis L.* stored at 0-5 °C were marketable after 1 week, and those stored at -2.5°C were still marketable after 2 weeks.

4. PHARMACOLOGY

Borage is a plant of renewed interest because the seeds are rich in γ-linolenic acid, an important polyunsaturated fatty acid of medicinal value in human and animal diets, helpful in the treatment of a wide range of disorders.
GLA play an important role in hormone regulation and fatty acid metabolization. Furthermore it is also the biological precursor of a group of molecules, including prostaglandins, leukotrienes and thromboxanes. Small number of oilseed plants produce γ-linolenic acid but the majority produce linoleic acid (LA, C18:2delta9.12) as its substrate. There are reports that vegetable oils

containing gammalinolenic acid (GLA) may exert beneficial effects on inflammatory skin disorders. Different in vitro and in vivo studies have been carried out.

4.1 IN VITRO STUDIES

Antioxidative activity

Crude extract of meal of borage was prepared under optimum extraction conditions and were subjected to Sephadex LH-20 column chromatography. Six fractions from each of the crude extracts were obtained and their content of total, hydrophilic and hydrophobic phenolics determined.

The objective of this study was to investigate the ROS-scavenging properties of extracts of borage (defatted ground seeds). The crude extracts and their fractions (at 100 and 200 ppm as sinapic acid) were investigated for their reactive-oxygen species- (ROS; H2O2, O2-, OH) and 2,2-diphenyl-1-picrylhydrazyl- (DPPH) scavenging efficacies. At 200 ppm, borage crude extract and some its fractions, exhibited a 100% scavenging of H2O2. Borage crude extracts and their fractions, separated via a column chromatographic procedure, possess strong ROS- and DPPH scavenging properties. These effects are attributable to varying hydrogen-donating and metal-chelating capacities of the phenolic compounds present in the crude extracts/fractions ((Wettasinghe and Shahidi, 2000).

The rapid evaluation of antioxidant activity and a screening of several radical scavenging components of crude borage (*Borago officinalis* L.) extract was determinate by HPLC-DPPH free radical method. The extract of borage showed very high hydrogen-donating capacity towards the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical. The dominant antioxidative compound in the crude extract of borage leaves was identified by HPLC as rosmarinic acid (Bandoniene and Murkovic, 2002). Rosmarinic acid is separated and identified on the basis of high-performance liquid chromatographic (HPLC)-UV-mass spectrometry data in 80% methanol in water extracts from the leaves of *Borago officinalis*. The amount of rosmarinic acid in borage leaves is similar compared with Salvia officinalis (15 mg/g) (Bandoniene et al., 2005).

4.2 ANIMAL STUDIES

Immunological modulation

The effect of diets enriched in gamma linolenic acid (GLA) enrichment were considered in the rat subcutaneous air pouch and in rats with adjuvant induced arthritis. It was observed that the diet with plant seed oils rich in GLA may provide a way to alter generation of prostaglandins and leukotrienes and to influence acute and chronic inflammatory responses. Gamma linolenic acid can also have an influence on immune system. It has been shown that dietary supplementation with borage oil can affect the activation response of polimorphonuclear neutrophils. Studies on mice have shown that oral feeding of gamma-linolenic acid-rich plant lipid markedly affects the disease course of acute experimental autoimmune encephalomyelitis (EAE).

Feeding γ-linolenic acid-rich plant (*Borago officinalis*) to mice, had protective effects on EAE associated with production of PGE2 and gene transcription and secretion of TGF-β1. EAE was induced by an encephalitogenic peptide (92-106) of myelin oligodendrocyte glycoprotein (MOG), and mice were fed the plant lipid daily from 7 days after EAE induction. The clinical incidence and histological manifestations of acute EAE, and the clinical relapse phase of chronic relapsing EAE (CREAE) were markedly inhibited by ω-6 fatty acid feeding. A significant increase in the production of TGF-β1 and PGE2 were detected in spleen mononuclear cells from fatty acid-fed mice. There was no difference in interferon-γ, IL-4, and IL-2 production between the fatty acid-fed and control groups. Significantly higher TGF-β mRNA expression was found in the spleens of ω-6 fatty acid-fed mice at day 21. γ-linolenic acid-rich plant lipid markedly affects the disease course of acute EAE and CREAE and is associated with an increase in cell membrane long chain ω-6 fatty acids, production of PGE2 and gene transcription and, on activation, secretion of TGF-β1 (Harbige et al., 2000). In an other study using a classical oral ovalbumin gut-induced tolerance protocol in BALB/c mice, the effects of dietary n-6 polyunsaturated fatty acids (PUFA) on high-and low-dose oral tolerance (and in non-tolerised animals, i.e. effects of antigen challenge alone) in relation to lymphoproliferative, cytokine and antibody responses was investigated. Borage (*Borago officinalis*) oil rich in n-6 PUFA, of which gamma-linolenic acid is rapidly metabolised to longer-chain n-6 PUFA, increased Th1-like responses and decreased Th2-like responses, and possibly enhanced suppressor cell or Th3-like activity. These findings

explain more precisely and broadly the immunological regulatory mechanisms involved (Harbige et al., 2001).
The effects of dietary supplements of oils rich in EPA or GLA on neutrophil (PMN) membrane potential (delta gamma), secretion, and superoxide (O2-) responses were evaluated in guinea pig. At 4-week intervals, 12% sterile casein-elicited peritoneal neutrophils (PMN) were assessed for membrane polyunsaturated fatty acid (PUFA) profiles. The peritoneal PMN of all groups demonstrated in vivo activation and this was significant only with borage oil (Fletcher and Ziboh, 1990).

Lipid metabolism
The polyunsaturated fatty acid (PUFA) composition of murine peritoneal macrophage phospholipids was altered in vivo following the four-week feeding of specific dietary oils. So fish oil (containing 20:5n-3 and 22:6n-3) feeding significantly increased macrophage 20:5n-3, 22:5n-3, and 22:6n-3 ($P<0.05$), while borage oil (containing 18:2n-6 and 18:3n-6) increased ($P<0.05$) the macrophage 20:3n-6/20:4n-6 ratio, relative to safflower oil (containing 18:2n-6) and hydrogenated coconut oil (containing 12:0)-fed animals.
Male rats were given liquid diets by pair-feeding for 24-30 days, and phosphatidylinositols in pancreas were analyzed as derivatives of diacylglycerols and fatty acids. Addition of arachidonic acid or changing the fat component (35 energy%) in the liquid diet from olive oil/corn oil to oil from *Borago officinalis*, which contains 22% γ-linolenic acid, increased the fraction of arachidonoyl species.
Hypocholesterolemic effects in older animals after long-term feeding are unknown. Therefore, aged rats (24 wk of age) fed a conventional diet were shifted to diets containing 10% perilla oil, borage oil, evening primrose oil mixed oil, or palm oil with 0.5% cholesterol for 15 week in this experiment. The results of this study demonstrate that both n-6 fatty acid and n-3 fatty acids such as gamma-linolenic acid and alpha-linolenic acid inhibit the increase of serum total cholesterol and VLDL + IDL + LDL-cholesterol concentrations of aged rats in the presence of excess cholesterol in the diet compared with dietary saturated fatty acid.
In an other this study was evaluate the effect of different doses and sources of dietary gamma-linolenic acid (GLA) on the tissue phospholipid fatty acid composition. Rats fed four different levels of GLA (2.3, 4.6, 6.4 and 16.2 g of GLA/kg diet) in the form of either borage oil or evening primrose oil during 6 week were compared with animals fed corn oil. The levels of dihomo-gamma-linolenic acid (DHLA) and GLA showed a significant dose-related increase in liver, erythrocyte and aorta phospholipids. Moreover, the arachidonic acid/DHLA ratios in tissues decreased with increasing intake of dietary GLA.

Blood pressure activity
Blood pressure in hypertensive mammals is reduced by administering a compound comprising a combination of eicosapentaenoicacid (EPA) and γ-linolenic (GLA) in a ratio of (2-8):1.
The combination increases the production of PG1 prostaglandins from GLA which cause vasodilation and prevents the production of PG2 prostaglandins from arachidonic acid which cause vasoconstriction. EPA/GLA ratio of 8:1 were most effective in reducing blood pressure in hypertensive subjects.
The effects of dietary gamma-linolenic acid upon blood pressure, aortic reactivity and cholesterol metabolism were evaluated in spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. SHR and WKY rats were fed a purified diet containing either sesame or borage oil rich in gamma-linolenic acid for 7 weeks. Dietary borage oil has a blood pressure lowering effect in hypertensive and normotensive rats. However, the effect cannot be explained by altered sensitivity to humoral and neural vasoconstrictors or changes in cholesterol metabolism. This effect may be attributed to changes in the metabolism of GLA to dihomo-γ-linolenic (DGLA) and arachidonic acids (AA). GLA and DGLA levels in the plasma, liver, aorta, and renal artery tissues increased both in SHR and WKY. AA levels were also increased in both plasma and liver of SHR and WKY rats fed the borage oil-enriched diet. Thus, dietary borage oil produces marked changes in the metabolism of GLA which may contribute to its blood pressure-lowering effect in WKY and SHR.
The effects of dietary GLA on established hypertension in adult rats, as well as its effects on components of the renin-angiotensin-aldosterone axis were evaluated. The borage-enriched diet

reduced adrenal ANG II receptor density and affinity compared to the control group. Results suggest that borage inhibits adrenal responsiveness to ANG II by an action on adrenal receptors. This effect may be mediated, at least in part, by interference with the renin-angiotensin-aldosterone system at the level of adrenal ANG II receptors.

Platelet interaction

Effects of a dietary intake of the polyunsaturated omega-6 essential fatty acids (EFAs) linoleic and gamma-linolenic acids (GLA) on blood lipids, platelet function, and vascular prostacyclin production were studied 12 hyperlipidemic patients (doses of 3 g/day) and 12 male Wistar rats (doses of 3 mg/kg/day) for 4 months. In humans, GLA supplementation decreased plasma triglyceride (TG) levels by 48% and increased HDL-cholesterol concentration by 22%. Total cholesterol and LDL-cholesterol levels were significantly decreased by omega-6 EFAs. Platelet aggregation induced by low concentrations of adenosine diphosphate (ADP) and epinephrine, and serum thromboxane B2 decreased by 45% both in humans and animals after GLA supplementation. Bleeding time increased 40%. In rats, vascular prostacyclin production measured by radioimmunoassay of 6-keto-PGF1 alpha was enhanced by GLA intake.

Skin disease

The fatty acid constituents of dietary oils exert their effects by altering the levels of cutaneous eicosanoids. It has been shown that guinea pigs supplemented with dietary borage oil had elevated levels of 20:3(n-6) in epidermal phospholipids and elevated epidermal levels of 15-hydroxyeicosatrienoic acid compared with animals fed olive oil or fish body oil. There were no significant changes in epidermal levels of prostaglandins. Both 15- hydroxyeicosapentaenoic (HEPE) and 15- hydroxyeicosatrienoic (HETrE) have been identified as possible anti-inflammatory metabolites, and their elevated presence in the epidermis of animals fed oils rich in 20:5(n-3) or 18:3(n-6) may provide a mechanism for the beneficial effects of these oils on inflammatory conditions.

The effects of conjugated linoleic acid (CLA), γ-linolenic acid (GLA), linoleic acid (LA), and their combinations, on skin composition in mice were investigated. Mice (8 weeks old) were orally administered with either LA, GLA, CLA, LA + GLA, LA + CLA, or CLA + GLA for 4 weeks. Then, the skin was analysed for triacylglycerol content, fatty acid composition and collagen content. Additionally, thicknesses of the dermis layer and subcutaneous tissue layer, and the size and number of adipocytes were measured histologically. The skin fatty acid composition was modified depending upon the fatty acid composition of supplemented oils. In each oil-alone group, skin triacylglycerol content was the highest in LA, followed by GLA and CLA treatments. No significant changes in collagen content were observed among any treatments. The effects on subcutaneous thickness were similar to the results obtained in the triacylglycerol contents. However, no significant difference was detected in the thickness of the dermis layers.

To determine whether or not dietary GLA exerts any modulatory role on cutaneous eicosanoids, guinea pigs were fed either a control diet containing safflower oil (less than 0.5% GLA) or borage oil, a GLA-rich diet containing 25% GLA. After an 8-week feeding period, epidermal samples from both animal groups were analyzed for fatty acid composition and tissue eicosanoids. Analysis of epidermal neutral lipids and phospholipids in borage oil-fed animals showed a marked increase in GLA and its elongase product, dihomogammalinolenic acid (DGLA). Similarly, analysis of epidermal eicosanoids in the borage oil-fed animals revealed significant increases in the amounts of the 15-hydroxy fatty acid (15-OH-20:3n-6) and prostaglandin PGE1, both metabolites of DGLA. Since these metabolites have anti-inflammatory potential, the results suggest that increased dietary GLA result in the generation of local anti-inflammatory metabolites.

Diabetes and pregnancy

Unsaturated fatty acids are a major target for ROS and essential fatty acid metabolism is impaired by diabetes. γ-Linolenic acid stimulates vasodilator prostanoid production and there are marked synergistic interactions between γ-linolenic acid and antioxidants.

Experimental diabetes in rats is associated with excessive electrolyte loss in the urine, which is further accentuated by pregnancy, particularly of Ca. Supplementation with essential fatty acids has proven beneficial in treating several types of complications, including nephropathy. The

effect of γ-linoleic acid (GLA; 500 mg/kg per day) was investigated on urinary electrolyte output and skeletal compound in pregnant streptozotocin-diabetic rats. The treatments reduced urine volume with no effects on electrolyte concentration. A reduced bone size and bone Ca content was partially ameliorated by the diet supplementation. The authors have concluded that GLA , alone or in combination, prevent urinary electrolyte loss in pregnant rats and do so by reducing urine production.

4.3 HUMAN STUDIES

Immunological modulation

In a study borage seed oil (9 capsules/day) was administered for 12 weeks to 7 normal controls and to 7 patients with active rheumatoid arthritis. Significant reductions in prostaglandin E2, leukotriene B4, and leukotriene C4 produced by stimulated monocytes were seen after 12 weeks of GLA supplementation. The anti-inflammatory effects of GLA administration observed in animal models, and the apparent clinical improvement experienced by 6 or 7 rheumatoid arthritis patients given borage seed oil may be due in part to reduced generation of arachidonic acid oxygenation products.

Platelet interaction

The authors studied the effects of 3 g/day borage oil supplementation on platelet aggregation and biological and clinical parameters over a 6-week treatment period in 30 healthy male volunteers aged 18 to 45 year. The results showed that borage oil supplementation could be used as a source of polyunsaturated fatty acid, in particular γ-linolenic acid, without any adverse effects on platelet aggregation or any increased risk of platelet mediated thrombosis.

Anti-Hepatoma Activity

Borage was evaluated for anti-hepatoma activity on five human liver-cancer cell lines. The samples were examined by in vitro evaluation for their cytotoxicity. The results showed that the effects of crude drugs on hepatitis B virus genome-containing cell lines were different from those against non hepatitis B virus genome-containing cell lines. The results indicated that B. officinalis exhibited an inhibition percentage of 38.1.

4.4 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

In tissues, dietary essential fatty acids (EFA), EFA, should be converted into fatty acids of longer and more unsaturated chain by alternate desaturation (delta 6, delta 5, delta 4)-elongation reactions. Animal tissues are more active in this biosynthesis than human tissues. Liver is one of the most active organs and its role is critical in providing less active tissues, particularly the brain, with long-chain PUFA secreted in VLDL (very low density lipoprotein). In liver, many nutritional, hormonal and physiological factors act on the PUFA biosynthesis. Dietary fatty acids exert a great influence and are often inhibitory. The desaturation products AA, EPA, and DHA inhibit delta 6 desaturation of LA and delta 5 desaturation of DGLA (dihomo-gamma-linolenic acid).

With regard to hormones, insulin and thyroxin are necessary to delta 6 and delta 5 desaturation activities, whereas other hormones (glucagon, epinephrine, ACTH, glucocorticoids) inhibit desaturation. Concerning the physiological factors, the age of individuals is critical. Just after birth, in animals, the delta 6 desaturation activity increases in the liver and decreases in the brain. In aging, the capacity of the whole liver to desaturate LA and DGLA is equal at 1.5 and 25 months of age in rats.

In rats, parenteral nutrition with 2.7%, 4.4%, and 6.1% gamma-linolenic acid from borage oil increased the plasma percentages (mol%) of gamma-linolenic acid and dihomo-gamma-linolenic acid in a dose-dependent fashion. Supplementation with gamma-linolenic acid did not increase the plasma percentage of arachidonic acid as compared with the 0% gamma-linolenic acid lipid emulsion. The ratio of dihomo-gamma-linolenic acid to arachidonic acid was significantly increased in response to 4.4% and 6.1% gamma-linolenic. In conclusion: supplementation of parenteral nutrition with gamma-linolenic acid had the following effects: a) increased plasma gamma-linolenic acid, dihomo-gamma-linolenic acid, and bicyclo-prostaglandin E; b) increased the plasma ratio of dihomo-gamma-linolenic acid to arachidonic acid (Karlstad et al., 1993).

Hoey et al. (1995) examined borage oil, and other seed oils, with respect to fatty acid profile and fatty acid composition. Only borage oil was specifically structured with respect to 18:3n-6, this fatty acid being preferentially esterified in the sn2 position. During absorption, the composition of the lymph largely reflected the fatty acid profiles of the oils administered. The total absorptions as indicated by the transport of fatty acids in the lymph indicated that borage oil was well absorbed. In the chylomicrons, a redistribution of 18:3n-6 from the sn2 position to the sn1/3 positions in the borage oil-fed rats was found.
Dietary oils influence the distribution of PUFA in epidermal phospholipids and the epidermal levels of PUFA-derived hydroxy fatty acids. Animals supplemented with esters of borage oil preferentially incorporated dihomogammalinolenic acid (DGLA), and epidermal elongase product of GLA, into the epidermal accumulation of 15-hydroxyeicosatrienoic acid (15-HETrE) (Craig et al.,1991).

5. MICROBIOLOGY

Antibacterial activity
Borage oil is generally regarded as lacking in antimicrobial activity.

6. EFFICACY

Borage is occasionally used in salads and soups and is stated to possess diaphoretic, expectorant, tonic and galactogogue properties. Traditionally, borage has been used to treat many ailments including fevers, coughs and depression. Thus, no documented pharmacological data were located to support the traditional uses. Interest has focused on the Borage oil as a source of γ-linolenic acid.

6.1 QUALITY OF THE ANIMAL PRODUCT

The use of Borage oil in animal diets is indicated when an important source of essential fatty acid (especial γ-linolenic acid) is necessary.
Borage meal is derived from the processing of borage seeds extraction. The crude protein content of borage meal is 334 g/kg. A series of experiments was conducted to determinate the nutritive value of Borage meal as a protein supplement for ruminants and pigs.
In situ ruminal effective crude protein degradability (ECPD) was determinate relative to four other protein supplements using two ruminally fistulated cows in a randomized complete block design. The protein supplements used for comparison purposes included soybean (SBM) and canola (CM) meals (high rumen degradable protein) and corn gluten (CGM) and heated canola (HCM) meals (high rumen undegradable protein). ECPD of borage meal was intermediate with the following order observed: SBM > CM >BM > HCM > CGM.
In ruminants no effect of borage meal inclusion rate was observed on voluntary intake, nutrient digestibility coefficients and digestible energy (DE) values. Relative to four other protein source selected for their extremes in rumen crude protein degradability, the effective CPD of borage meal was high. Inclusion of borage meal in rations of growing lambs up to 18% of the diet DM, had no adverse effects on intake or nutrient digestibility.
In two experiments with swine, borage meal was included at 0, 10, 20, 30, and 40% in grower diets and 0, 6.75, 13.5, 20.25 and 27% in finisher diets. Both rate and efficiency of gain were depressed in a linear fashion ($P<0.05$) as the level of BM in the diet increased. Digestibility coefficients for dry matter, crude protein and gross energy also declined linearly as dietary BM increased. However, results of the pig experiments showed poor performance when BM was included in grower and finisher diets. Borage meal inclusion resulted in reduced intake and poor nutrient utilization especially for growing pigs. The adverse effects of borage meal on pig performance were attributed to bitter taste and to its poor digestibility.

The reduction in feed intake with borage can likely be attributed to the presence of pyrrolizine alkaloids in borage. Pyrrolizine alkaloids have been reported to have a bitter taste which may cause limit intake. In addition, these compounds are toxic and have been shown to cause liver damage at high concentration. Some authors suggested that the concentration of pyrrolizine alkaloids in borage is likely too low to cause a significant health hazard, caution should be exercised before including high levels of borage meal in swine diets.

7. TOXICOLOGY

Pyrrolizidine alkaloids (Pas) are very common in plants used in livestock feeds and in rangeland weeds such as tansy ragwort, Senecio jacobea, Heliotropium spp. and Crotolaria spp.. It appears that the pyrrolizidine alkaloids themselves are not toxic, rather, some of their metabolites, primarily their "pyrrolic" derivatives are highly toxic. Toxic alcohols can also be produced as secondary metabolites. The common PAs, heliotrine and lasciocarpine, are partially reduced to the non-toxic 1-methylene and 7-hydroxy-1 methyl derivatives in the rumen.
However, these can be activated by oxidases in the animal's liver to pyrrolic derivatives and thus have pathological effects in the heart, liver, kidney and respiratory system. Especially unsaturated PAs have been shown to be hepatotoxic and carcinogenic. The basis for alkaloid regulation and its effects in the ruminal ecosystem are not yet fully understood, but is a well-known fact that its degradation is directly proportional to its concentration in the rumen. Heliotrine degrading bacteria have been isolated and identified in the rumen. Although these bacteria appear to get very little useful energy from the clevage of heliotrine, this characteristic may well improve their ability to successfully compete in the rumen of an animal exposed to this type of alkaloid.
The glycosylated pyrrolizidine alkaloid, thesinine-4'-O-β-D-glucoside, has been isolated from the aqueous methanol extract of dried, defatted seeds of *Borago officinalis* (Boraginaceae). So far seven Pas were identified in leaves, flowers and seeds of borage with thesinine, a saturated and therefore probably non toxic necine base described as a major alkaloid. Besides thesinine six unsaturated PAs, amabiline, supinine, lycopsamine, intermedine, acetyllycopsamine and acetylintermedine, were identified as minor constituents.
The total alkaloid amount of the plant was determined as less than 0.001% whereas mature seeds yielded about 0.03% crude alkaloids.
The pyrrolizidine alkaloid content of crude borage oil and borage oil from different processing stages was deteterminated by GC-MS. The results showed that no pyrrolizidine alkaloids were present above a detection limit of 20 ppb. The reduction factors for pyrrolizidine alkaloids at various stages in the oil refining process were determined by means of spiking experiments using the available pyrrolizidine alkaloid crotaline. The pyrrolizidine content in crude borage oil was reduced overall by a factor of approximately 30,000 in the refining process (Wretensjoe and Karlberg, 2003).

7.1 SAFETY (INFORMATION ON THE DOSAGE)

Administration – Dosage

Borago officinalis is marketed as oil or as food supplement containing natural gamma linolenic acid (GLA).
The parts used are the nutlets (seeds), leaves and flowers. The leaves are used in flavoring and fresh flowers with saline, cucumber like flavor (free of toxic pyrrolizidine alkaloids) are used in salads.
The edible part of the plant corresponds to the basal leaf petioles. In this part, water was the major constituent with a value of 94%. Borage (*Borago officinalis* communis) is a plant commonly cultivated for consumption in Spain and other countries.
Borage is included in the food tables within the leafy vegetable group.
Borage contains toxic pyrrolizidine alkaloids although at concentrations considerably lower than comfrey for which human toxicity has been documented . However, it would seem wise to avoid excessive or prolonged ingestion of borage. Borage is included in the food tables within the leafy vegetable group.

Borage seed oil, clear, light yellow or yellow liquid, is a rich source of the essential fatty acid g-linolenic acid and is, therefore, used in nutritional, cosmetical and medical applications. Dried tops sometimes are used in teas.
Capsulated seed oil are products as dietary supplement, used as an alternative source of γ-linolenic acid. As additive γ-linolenic acid, or equivalent ester or salt is used in infant foods, geriatric preparations or externally in skin care products (cosmetics, bath preparations and detergents). The content of these compounds in the formula may vary from 0.005-1.0%.
The food additive may contain from 1-90% of germ oil obtained from Oenothera lamarkiana biennis or from *Borago officinalis* instead of the essential fatty acid compounds. The germ oils of both plants contained 2-26% γ-linolenic acid.

Side effects/ Adverse reaction /Contraindications/ Precautions
No data in literature suggest possible adverse effects in animals.
The unsaturated pyrrolizidines, lycopsamine and supinidine viridiflorate, are suspected poisons, but are found in borage at very low concentrations. The low alkaloid level may account for the lack of acute toxicity reports during borage use. A mixture of lycopsamine and intermedine has, however, been reported to carcinogenic.
Borago officinalis preparations should not be to used during pregnancy in view of the documented pyrrolizidine constituents. Borage oil is recommended to be used with caution in epileptic patients.
In oil products from *Borago officinalis* no toxic pyrrolizidine alkaloids were detected.

Interactions with other drugs

No negative interactions are known.

DT/SG/LG/AT

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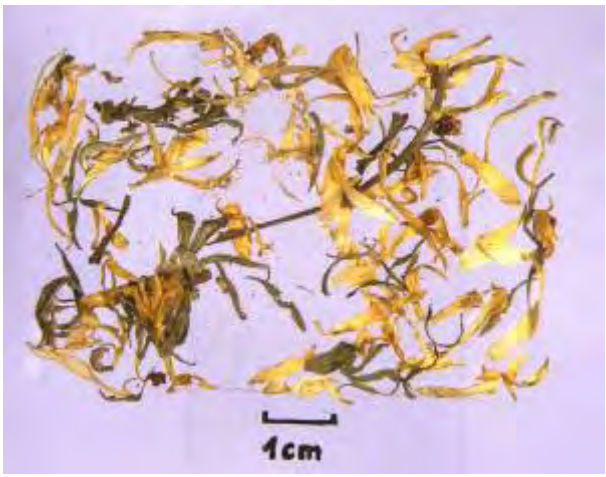
CALENDULA OFFICINALIS



Calendula officinalis L

1. INTRODUCTION

Marigold owes its botanical name *Calendula officinalis L*. to the Latin word "*Calendae*", the first day of the month, reflecting the fact, that the blossom opens and closes daily [Genaust, 1996]. In folk medicine the drug is used as a diaphoretic, diuretic, antispasmodic, anthelmintic, emmenagogue, and for liver complaints. However, a scientific basis of these applications is lacking. Therapeutic indications described in the ESCOP monograph are the treatment of minor inflammations of the skin and mucosa as well as an aid to the healing of minor wounds.
Synonyms are Calendula, Mary bud, Gold Bloom, Garden Marygold, Common Marygold, and Pot Marigold, however it should be noted that the trivial name marigold is also used for members of the genus *Tagetes*.

2. DESCRIPTION OF THE PLANT

The annual or biennial plant grows to a height of 30-60 cm. The stem is angular, hairy and solid. Lower leaves are spatulate, 10-20 cm long and 1-4 cm wide; higher leaves oblong and mucronate, 4-7 cm long. The flower heads range from bright yellow to orange. Marginal flowers in cultivated plants are often multi-seriate, corolla oblong-spatulate, 15-25 mm long and 3 mm wide. The corolla of disc flowers is rounded, at the top tridentate, 1.5-2.5 cm long and 4-7 mm in diameter with 5 mm long tubular florets [Auster and Schäfer, 1958; Bisset and Wichtl, 2001].

2.1 SYSTEMATICS

The genus *Calendula* L. includes 12 predominantly Mediterranean species: *C. arvensis* L., *C. eckerleinii* OHLE, *C. incana* WILLD., *C. lanzae* MAIRE, *C. maroccana* BALL, *C. meuselii* OHLE, *C. officinalis* L., *C. pachysperma* ZOH., *C. suffructicosa* VAHL and *C. tripterocarpa* RUPR. For pharmaceutical purposes only the annual *C. arvensis* and the annual or perennial *C. officinalis* are used [Hänsel et al., 1992].
Due to the commercial cultivation of *Calendula officinalis* L., confusions with other species of the genus *Calendula* are very scarce.

2.2 OCCURRENCE

Indigenous to central, eastern and southern Europe *Calendula officinalis* is nowadays cultivated in Mediterranean countries, in North America, in the Balkans, in eastern Europe and to a smaller

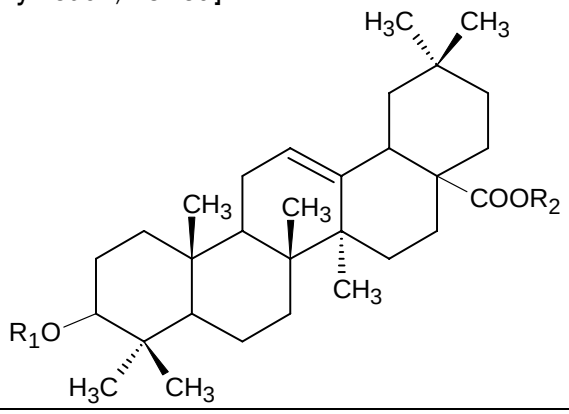
extent in Germany. Imported drug also originates from Egypt, Poland, and Hungary [Bisset and Wichtl, 2001; Leung and Foster, 1996].

2.3 Plant Parts and Products

The plant parts used are the dried ligulate florets (Calendulae flos, Calendulae flos sine calyce), the composite flowers (Calendulae flos cum calyce), and the dried or fresh aerial parts of the herb (Calendulae herba), collected in flower [Hänsel et al., 1992]. The flowers are harvested manually or automatically from May to August. Continuous and frequent harvest stimulated flower formation, and therefore increased the production [Helemiková, 1991]. For therapeutical use, the flowers should be dried expeditiously in the shade or at of 35-40°C to avoid carotenoid and flavonoid losses. Storage of freshly collected plant material for 3.5 h decreased carotenoid and flavonoid content by 28-30% and 24-26%, respectively, whereas the loss was independent of temperature. Due to the hygroscopicity of the dried flowers, the drug should be stored in well-closed container protected from light [Isaac, 1992; Omel'chuk et al., 1984].

3. Ingredients/Constituents

The major constituents of *Calendula* flowers are triterpene saponins (2-10% on a dry weight basis) based on oleanolic acid, i. e. calendulosides [Kasprzyk et al., 1965; Vidal-Ollivier et al., 1990]. The structures of saponosid A-F are given in Figure 3.1. [Vidal-Ollivier et al., 1989]. The triterpenoid content increased until the stage of full development of flowers is reached [Kasprzyk et al., 1970a].



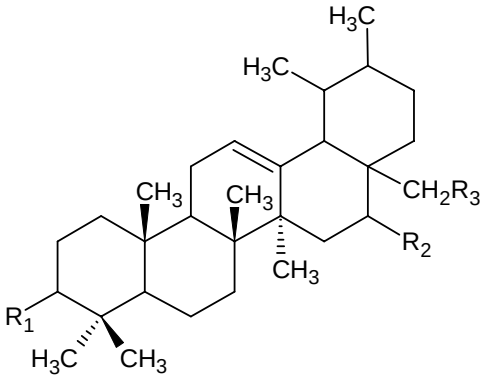
	R_1	R_2
Oleanolic acid	- H	- H
Saponoside A	- Glu - (2→1) - Glc (3→1) - Gal	- Glc
Saponoside B	- Glu - (2→1) - Glc (3→1) - Gal	- H
Saponoside C	- Glu - (3→1) - Gal	- Glc
Saponoside D	- Glu - (3→1) - Gal	- H
Saponoside D_2	- Glu	- Glc
Saponoside F	- Glu	- H

Glu = Glucuronic acid; Gal = Galactose; Glc = Glucose

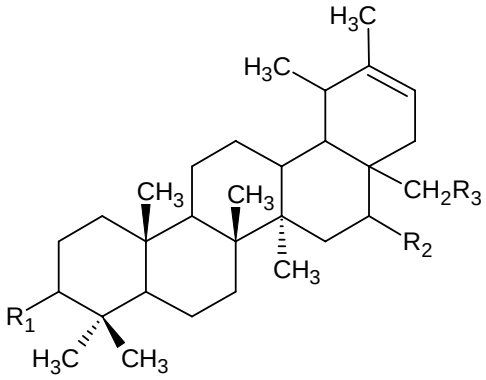
Figure 3.1. Structures of triterpene saponins from *Calendulae*

Besides the triterpeneglycosides, a number of pentacyclic triterpenic alcohols (monohydroxyalcohols, dihydroxyalcohols and trihydroxyalcohols), predominantly acylated, were identified [Kasprzyk and Pyrek, 1968; Kasprzyk et al., 1969]. They are derived from Ψ-taraxen, taraxen, lupane, oleanane and ursane (Figure 3.2). Triterpene monols are esterified with acetic

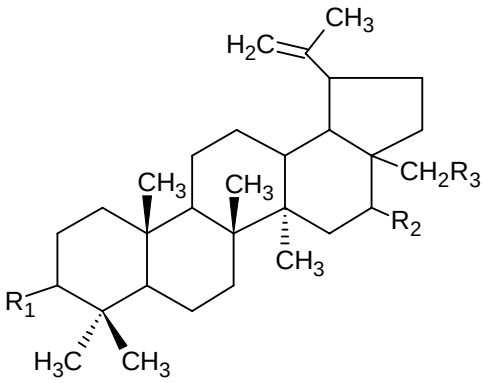
acid, whereas triterpene diols are mainly esterified with lauric, myristic and palmitic acids [Wojciechowski et al., 1972]. The main diol esters are monoesters (98%) and only 2% are diesters [Adler and Kasprzyk, 1976]. Dried flowers contain 0.6% triterpene monols, with 14% α-amyrin, 26.1% β-amyrin, 6.0% lupeol, 2.8% taraxasterol and 51.1% Ψ-taraxasterol. About 11% of the triterpene monols are esterified [Stevenson, 1961; Kasprzyk and Pyrek, 1968; Kasprzyk



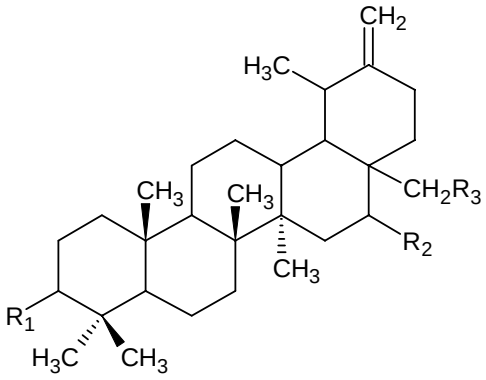
$R_1 = R_2 = R_3 = H$: Ursane
$R_1 = OH; R_2 = R_3 = H$: α-Amyrin
$R_1 = R_2 = OH; R_3 = H$: Brein
$R_1 = R_2 = R_3 = OH$: Ursatriol



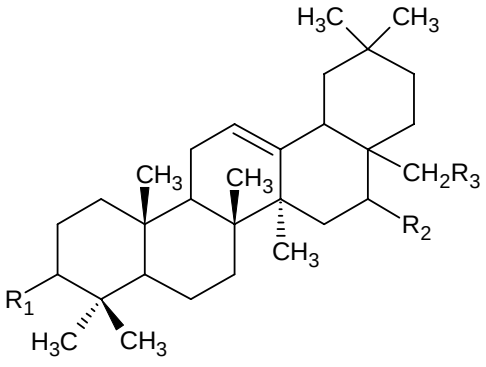
$R_1 = R_2 = R_3 = H$: Ψ-Taraxen
$R_1 = OH; R_2 = R_3 = H$ Ψ-Taraxasterol
$R_1 = R_2 = OH; R_3 = H$: Faradiol
$R_1 = R_2 = R_3 = OH$: Heliantriol B_0



$R_1 = R_2 = R_3 = H$: Lupane
$R_1 = OH; R_2 = R_3 = H$: Lupeol
$R_1 = R_2 = OH; R_3 = H$: Calenduladiol
$R_1 = R_2 = R_3 = OH$: Heliantriol B_2



$R_1 = R_2 = R_3 = H$: Taraxen
$R_1 = OH; R_2 = R_3 = H$ Taraxasterol
$R_1 = R_2 = OH; R_3 = H$: Arnidiol
$R_1 = R_2 = R_3 = OH$: Heliantriol B_1



$R_1 = R_2 = R_3 = H$: Oleanane

R_1 = OH; R_2 = R_3 = H: β-Amyrin
R_1 = R_2 = OH; R_3 = H: Maniladiol
R_1 = R_2 = R_3 = OH: Longispinogenin

Figure 3.2: Structures of triterpenic alcohols from *Calendula officinalis*

et al., 1969; Wilkomirski and Kasprzyk, 1979]. The content of triterpendiol-3-monoester averages 2.0 to 4.0% with 85.4% faradiol esters, 6.0% calenduladiol esters, 4.9% brein esters, and 3.3% ursadiol esters. The remaining 0.4% are esters of erythrodiol, maniladiol and arnidiol [Zimmermann, 1946; Kasprzyk and Pyrek, 1968; Wilkomirski and Kasprzyk, 1979; Wojciechowski et al., 1972; Pyrek, 1997a, b; Kasprzyk et al., 1970b]. Moreover, 0.2% triterpentriols, mainly in the free form, were found in the flowers [Kasprzyk and Wilkomirski, 1973; Pyrek, 1979; Wilkomirski, 1985; Wilkomirski, 1986].

Throughout the whole plant free sterols, their esters and glycosides were found. The period of flowering is characterized by sterol accumulation in all plant organs, particularly in the flowers. The sterol content of the dried flowers ranged from 0.06-0.08%, whereof 15-20% were esterified with acetic, lauric, myristic or palmitic acid [Kasprzyk et al., 1968; Wojciechowski et al., 1972; Wilkomirski and Kasprzyk, 1979].

Furthermore, carotenoids with lutein and zeaxanthin being the major compounds (88-92%), were identified in flowers. In orange flowering species lycopene is responsible for the colour, whereas in yellow flowering species xanthophylls are dominant. In deep orange coloured ligulate flowers carotenoid contents of 1.5g/100g were determined [Andreeva, 1961; Quackenbush and Miller, 1972; Isaac, 1992].

Flavonoid derivatives identified in flowers of *Calendula officinalis* are glycosides composed of isorhamnetin and quercetin as well as free isorhamnetin. Other methoxylated flavonoids characteristic of Compositae were not detected. Flavonoid contents in the flowers ranged from 0.3 to 0.8% [Steinegger and Hänsel, 1988; Hegnauer, 1989; Vidal-Ollivier et al., 1989b; Isaac, 1992].

Other constituents include essential oil (0.03% and 0.2% in fresh and dried flowers, respectively), phenolic acids (salicyl-, ferulic-, *p*-hydroxybenzoic-, caffeic-, chlorogenic acid), and polysaccharides [Isaac, 1992].

3.1 Chemical Analysis

The carotenoid composition in petals of three orange-flowered and yellow-flowered cultivars of *Calendula* was analysed by HPLC [Kishimoto et al., 2005].
Mrugasiewicz et al. (1979) proposed a photometric determination of saponosides after hydrolysation to oleanolic acid and reaction with vanillin in acidic medium. HPLC quantification of the saponosides A, B, C, and D was performed using a Water µ-Bondapak C18 column, aqueous methanol phosphoric acid eluent and UV detection at 210 nm [Vidal-Ollivier et al., 1989c].
Triterpenoid monoesters were quantified in a dichloromethane extract of dried flowers after semi-purification through TLC on silica gel using reversed-phase HPLC with isocratic elution [Neukirch er al., 2004]. Reversed-phase HPLC and micellar electrokinetic capillary chromatography have been used to separate flavonol-2-*O*-glucosides from *Calendula officinalis* [Pietta et al., 1992]. Zitterl-Eglseer et al. (2001) quantified triterpendiol esters (faradiol laurate, faradiol myristate, and faradiol palmitate) in ligulate and tubular florets, involucral bracts, receptacles, and leaves by means of reversed-phase HPLC.
The constituents of the essential oil were analysed by GC-MS after steam distillation of flowers and whole plants [Chalchat et al., 1991].

3.2 STANDARDISATION, STABILITY

According to the European Pharmacopoeia, Calendulae flos contains not less than 0.4% of flavonoids, calculated as hyperoside. The storage of the drug protected against air and light should not exceed 3 years (DAC 79).
A combined method of 2-dimensional thin-layer chromatography and HPLC was used for the determination of flavonoid glycosides and saponins in *Calendula officinalis* flowers [Heisig and Wichtl, 1990].

4. PHARMACOLOGY

Preparations of *Calendula officinalis* are mainly externally applied in form of infusions, tinctures, and ointments as a wound-healing remedy for inflammation of the skin and mucous membranes, for poorly healing wounds, bruises, boils, and rashes, e.g. pharyngitis, and leg ulcers [Bisset and Wichtl, 2001]. In contrast, the internal use as an antiphlogistic and spasmolytic for cholecystitis, cholangitis, gastritis, cystitis, and gastrointestinal spasms is scarce [Luckner et al., 1969; Madaus, 1976; Braun and Frohne, 1987]. The data given below are based on the ESCOP monograph (2003).

***In vitro* experiments**

Immunomodulatory effects

A dry 70% ethanolic extract of *Calendula* flower exhibited an inhibitory effect in the mitogen-induced lymphocyte proliferation assay. In the mixed lymphocyte reaction the extract showed stimulatory effects at 0.1-10 µg/mL, followed by inhibition at higher concentrations [Amirghofran et al., 2000]. Phagocytosis of human granulocytes was stimulated by polysaccharides isolated from *Calendula* flowers [Varljen et al., 1989].

Antioxidant effects

Extracts of *Calendula* flowers of differing polarities exhibited antioxidative effects on liposomal lipid peroxidation induced by Fe^{2+} and ascorbic acid. Marked activity was observed with ether, butanol and aqueous extracts. In combination with pro-oxidants different antioxidative effects were obtained: with pyralene the butanol and aqueous extracts were most effective; with carbon tetrachloride the chloroform and ethyl acetate extracts showed the strongest activity. Combination of the antioxidant fullerenol with each extract resulted in a further decrease in lipid peroxidation [Popovic et al., 1999; 2000].

Angiogenic effects

The angiogenic activity of a lyophilised aqueous infusion (1:10) of *Calendula* flowers was tested in the chick chorioallantoic membrane (CAM) test. The numbers of micro-vessels in tissue sections of treated CAMs were significantly higher ($p<0.0001$) than in control CAMs. All treated CAMs were positive for hyaluronan, while no hyaluronan was detected in control CAMs [Patrick et al., 1996].

Anti-inflammatory effects

Three isorhamnetin glycosides from *Calendula* flower at concentrations of 1.5×10^{-5} M showed inhibitory effects on arachidonate 12-lipoxygenase from rat lung cytosol [Bezákova et al., 1996].

Effects on buccal membranes

In a test system based on porcine buccal membranes, strong concentration-dependent adhesive processes were observed with a low-viscosity polysaccharide-enriched extract (98% carbohydrates) of *Calendula* flowers. These findings suggested that the polysaccharides may contribute to therapeutic effects in the treatment of irritated mucosa [Schmidgall et al., 2000].

4.1 ANIMAL STUDIES

Effects on wound healing

Dried alcoholic and aqueous extracts of *Calendula* flower in combination with allantoin, applied topically as a 5% ointment, stimulated epithelisation in surgically-inflicted standard wounds in rats [Klouchek-Popova et al. 1981; 1982]. Experimental incisions in rats were infected with *Staphylococcus epidermidis*. Topical treatment with a *Calendula* cream (not further specified) resulted in accelerated cicatrisation compared to controls. The results were confirmed by histological examination [Perri de Carvalho et al., 1991].

In a study in rabbits, the time taken for complete cicatrisation of experimental wounds was shortened by approximately 25 % after topical treatment with hydrogel or powder preparations containing 10% of an ethanolic dry extract of *Calendula* flower in comparison with vehicle-only controls [Oana et al., 1995].

Enhanced healing of experimental wounds in buffalo calves was observed after topical treatment with an ointment containing 5% of a dry extract of *Calendula* flower. Epithelization accelerated and histopathological parameters improved in comparision with wounds treated with normal saline only [Ansari at al., 1997].

Immunomodulatory effects

Polysaccharide fractions from aqueous extracts of *Calendula* flower appeared to enhance phagocytosis in the carbon clearance test in mice [Wagner et al., 1985]. The unsaponifiable fraction of a hydroalcoholic extract, administered intraperitoneally, protected mice from lethal sepsis due to *Escherichia coli* [Delaveau et al., 1980].

Anti-inflammatory effects

A lyophilised extract suppressed inflammatory effects and leukocyte infiltration induced by simultaneous injection of carrageenan and prostaglandin E_1 into rats [Shipochliev et al., 1981].

A dry 80% ethanolic extract of *Calendula* flower, given orally 1 hour before oedema elicitation, inhibited carrageenan-induced rat paw oedema by 11% at a dose of 100 mg/kg body weight. In the same experiment indometacin at 5 mg/kg resulted in 45% inhibition of oedema [Mascolo et al., 1987].

A 70% alcoholic extract and a supercritical CO_2 extract were applied topically in the croton oil ear oedema test in mice. The hydroalcoholic extract had a mild dose-dependent effect, inhibiting oedema by 20% at a dose of 1200 µg/ear, corresponding to 4.16 mg of crude drug. The CO_2 extract produced 30% inhibition at 150 µg/ear, corresponding to 3.6 mg of the crude drug, and 71% inhibition at 1200 µg/ear, corresponding to 28.6 mg crude drug. The activity at the higher concentration was comparable to that of indometacin at 120 µg/ear [Della Loggia et al., 1990]. In the same model, triterpenoids were shown to be the most important anti-inflammatory principles in the CO_2 extract [Della Loggia et al., 1994]. A faradiol monoester mixture (faradiol-3-myristic acid ester, faradiol-3-palmitic acid ester and Ψ-taraxasterol) isolated from Calendula flower showed significant ($p<0.05$) anti-oedematous activity in male albino Swiss mice at doses of 240 and 480 µg/cm² compared to controls [Zitterl-Eglseer et al., 1997]. Faradiol, which is not present in the free form in *Calendula* flowers, proved to be even more active than the esters with an anti-edematous effect comparable to that of an equimolar dose of indometacin, but 3-esterification reduces the activity by more than 50% [Della Loggia et al., 1994; Zitterl-Eglseer et al., 1997].

Helianol and Ψ-taraxasterol from *Calendula* flowers inhibited inflammation induced by 12-*O*-tetra-decanoylphorbol-13-acetate (1 µg per ear topically) in female ICR mice. The 50% inhibitory doses were 0.1 mg/ear for helianol and 0.4 mg/ear for Ψ-taraxasterol, compared to 0.3 mg/ear for indometacin [Akihisa et al., 1996].

Antitumour effects

A triterpene-enriched fraction given orally to mice inoculated with Ehrlich mouse carcinoma prevented the development of ascites and increased survival time compared to control [Boucaud-Maitre et al., 1998].

Other effects

An isolated saponin fraction administered orally at 50 mg/kg body weight to hyperlipaemic rats reduced the serum lipid level [Samochowiec, 1983; Wojcicki and Samochowiec, 1980].

4.2 HUMAN STUDIES

In a randomized, open, controlled study, the effects of three ointments were compared after topical treatment of patients with 2nd or 3rd degree burns for 17 days: a *Calendula* flower ointment (prepared by digestion in vaseline) (n = 53) or vaseline only (n = 50) or a proteolytic ointment (n = 53). The success rates were considered to be 37/53 for *Calendula* flower ointment, 27/50 for vaseline and 35/53 for the proteolytic ointment. *Calendula* flower ointment was marginally superior to its base, vaseline (p = 0,05) [Lievre et al., 1992].

In an open, uncontrolled pilot study 30 patients with burns or scalds (Degrees 1 and 2a) were treated 3 times per day for up to 14 days with a hydrogel containing 10% of a hydroethanolic extract. The symptoms reddening, swelling, blistering, pain, soreness and heat sensitivity were scored before, during and at the end of treatment. Total score and individual scores for each symptom improved [Baranov, 1999].

4.3 ADME (ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION)

No data available.

5. MICROBIOLOGY

Antimicrobial, antifungal and antiviral effects

Various hydroalcoholic extracts exhibited antibacterial activity, especially against *Staphylococcus aureus* and *Streptococcus fecalis* [Dumenil et al., 1980]. The essential oil of *Calendula* was active against *Campylobacter jejuni* in a microplate assay [Mendel et al., 2002]. Antifungal activity was observed with a 10% methanol extract [Wolters, 1966]. In a study to evaluate the antibacterial activity of different herbs against anaerobic and facultative anaerobic periodontal bacteria, extracts of *C. officinalis* showed an inhibiting activity against *Porphyromonas gingivalis, Fusobacterium nucleatum, Capnocytophaga gingivalis, Veilonella parvula, Eikenella corrodens, Peptostreptococcus micros* and *Actinomyces odontolyticus* [Iauk et al., 2003]. A flavonoid fraction isolated from the flowers inhibited exhibited *in vitro* antibacterial activity against *Staphylococcus aureus, Sarcina lutea, Escherichia coli, Klebsiella pneumoniae* and *Candida monosa* [Tarle and Dvorzak, 1989].

The essential oil of the flowers inhibited the *in vitro* growth of *Bacillus subtilis, Echerichia coli, Staphylococcus aureus, Pseudmonas aeruginosa* and *Candida albicans* [Janssen et al., 1986].

A 70% hydroalcoholic tincture of *Calendula* flowers had high virucidal activity against influenza viruses and a marked ability to suppress the growth of herpes simplex virus [Bogdanova et al., 1970].

A dry extract (dichloromethane-methanol 1:1) exhibited anti-HIV activity in an in vitro MTT (dimethylthiazole-diphenyltetrazolium)-based assay. In the presence of 10-30 µg/mL of the extract, 90% inhibition of HIV-1 replication in acutely infected lymphocytic Molt-4 cells was observed. In the concentration of 500 µg/mL the extract suppressed cell fusion and protected uninfected Molt-4 cells against subsequent HIV-induced cytolysis caused by cocultivation with persistently infected U-937/HIV-1 cells for up to 24 hours. The extract (50, 100 and 200 µg/mL) also caused marked dose- and time-dependent inhibition of HIV-1 reverse transcriptase activity by up to 85% in a cell-free system [Kalvatchev et al., 1997].

Extracts from the flowers have trichomonacidal activity [Samochowiec et al., 1979; Fazakas and Racz, 1965], which has been attributed to oxygenated terpenes [Gracza, 1987].

6. EFFICACY

An extract of freshly cut flowers and leaves of *Calendula officinalis* given orally to broiler chickens had an immunomodulatory effect against three different live viruses, resulting in reduction in the immune response to viruses, which were associated with improvements in body weights [Barbour et al., 2004].
Dried petals of *Calendula officinalis* supplemented in a rate of 2% in the diet of hens intensified the egg yolk pigmentation [Neamtu et al., 1981].
External applications in form of ointments and extracts are used to heal bad curatively wounds (lacerations, contused wound, burns) [Sviridonov, 1986; Lipnizkiji et al., 1987; Isaac, 2003].

7. TOXICOLOGY

The intravenous LD_{50} and the intraperitoneal LD_{50} of an aqueous extract from *Calendula* flowers was determined as 375 mg/kg and 580 mg/kg body weight, respectively [Manolov et al., 1964]. For a hydroalcoholic extract (drug to extract ratio 1:1, 30% ethanol) the subcutaneous LD_{50} was 45 mg in mice and the intravenous LD_{50} was 526 mg/100 g in rats [Boyadzhiev, 1965]. An ethylene glycol extract (drug to extract ratio 2:1) was non-toxic in albino mice after subcutaneous administration of 10 mL/kg [Russo, 1972].

7.1 SAFETY

Dosage/Administration

According to the German Commission E monograph: Unless otherwise prescribed, 1-2 g of drug to a glass of water (150 mL) or 1-2 teaspoonfuls (2-4 mL) of tincture in 0.25-0.5 mL water or as an ointment with the equivalent of 2-5 g of drug in 100 g of ointment.

Side effects/Adverse Reactions/Contraindications/Precautions

Weak skin sensitisation has been shown experimentally, but there are no clearly recorded cases of contact dermatitis [Hausen and Vieluf, 1997].

Interactions with other drugs

None known.

KS

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ECHINACEA SP

Echinacea pallida

1. INTRODUCTION

American Indians were the first to use Echinacea species for many different ailments, including cough, sore throat and tonsillitis (Caruso and Gwaltney, 2005).

At present, mainly *E. purpurea*, *E. angustifolia* and *E. pallida* are used as medicinal plants.

The plant parts used include the rhizomes of *E. purpurea*, *E. angustifolia* and *E. pallida*; the fresh or dried aerial parts of *E. purpurea*; and (in homoeopathy) the whole plant of *E. angustifolia* and *E. pallida* (Harborne and Williams, 2004). Tinctures or extracts in alcohol are the form most herbal authorities recommend. In Germany, the freshly pressed *E. purpurea* juice stabilized with alcohol is very popular. The preparations are for internal and (less often) for external use (Yu and Kaarlas, 2004).

The main indications nowadays are internally for the adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common colds) and the derivative urinary tract, and externally as an adjuvant for the treatment of poorly healing superficial wounds (Blumenthal, 1998; ESCOP, 2003a-c, WHO, 1999).

Although the immunomodulatory activity of *Echinacea* preparations has been demonstrated in many *in vitro* and *in vivo* studies, the nature of active constituents, bioavailability, pharmacokinetics, physiologic actions and their relevance for human health are not yet fully understood. Also the outcome of the clinical studies performed up to now is not fully conclusive. The number of studies dealing with potential immunostimulatory and growth promoting effects on productive livestock is limited, and also in this case results are not conclusive. In most of the studies no real growth promoting effect was demonstrated, but in some cases an enhanced feed conversion and also a positive influence on the immune system were found. Generally it is difficult to interprete and compare study results concerning *Echinacea*, because the tested preparations widely differ in used species, extraction procedure and content of marker compounds.

Key words: Echinacea purpurea, E. pallida, E. angustifolia, immune system

2. DESCRIPTION OF THE PLANT

The genus *Echinacea* belongs to the Asteraceae family, subfamily Asteroideae, tribus Heliantheae, subtribus Ecliptinae (Bauer and Liersch, 1992). The genus *Echinacea* has recently

been reclassified to comprise four species and eight varieties, together with a group of introgressant hybrids. *E. purpurea, E. angustifolia* and *E. pallida* are revised as *E. purpurea* (L.) MOENCH, *E. pallida* var. *angustifolia* (DC.) Cronq and *E. pallida* var. *pallida* (Nutt.) Cronq (Yu and Karlaas, 2004).
The genus naturally is endemic to North America, but nowadays cultivation has spread to Europe, South America, Australia and other areas of the world. In 2001, the global cultivation area of Echinacea was roughly estimated as several thousand hectares (Yu and Kaarlas, 2004).
E. purpurea is a herbaceous, perennial plant becoming 60-150 (-180) cm high. The stem is green to red, upright and slighty branched. The leaves are alternate, ovate to ovate-lanceolate, irregularly serrate, rugose on both surfaces, dark green with prominent light green veins. The involucral bracts of the large capitulum are arranged in 2 or 3 rows. Each of the outer violet ligulate florets (4-6 cm) and of the inner violet-pink tubular florets is attached to a reddish acute and coriaceous bract. The fruits are green to light brown achenes bearing a pappus.
E. angustifolia is becoming less high (10-50 cm), is coarsely hirsute, the leaves are more narrow and the ligulate florets rather short (2-3.8 cm). A typal anatomic characteristic are sclerenchymatic fibers in the pith tissue of the stem. It is very often mistaken for *E. pallida*, which becomes higher (40-120 cm), has white pollen, longer ligulate florets and oil tubes in pith and stem bark. Discrimination between the two species is also possible by the number of chromosomes (Bauer and Liersch, 1992).

2.1 Systematics

The genus *Echinacea* belongs to the Asteraceae family, subfamily Asteroideae, tribus Heliantheae, subtribus Ecliptinae (Bauer and Liersch, 1992). The genus *Echinacea* has recently been reclassified to comprise four species and eight varieties, together with a group of introgressant hybrids. *E. purpurea, E. angustifolia* and *E. pallida* are revised as *E. purpurea* (L.) MOENCH, *E. pallida* var. *angustifolia* (DC.) Cronq and *E. pallida* var. *pallida* (Nutt.) Cronq (Yu and Karlaas, 2004).

2.2 Occurence

The genus naturally is endemic to North America, but nowadays cultivation has spread to Europe, South America, Australia and other areas of the world. In 2001, the global cultivation area of Echinacea was roughly estimated as several thousand hectares (Yu and Kaarlas, 2004).

2.3 Plant Parts and Products

Purple coneflower herb consists of the fresh or dried, flowering aerial parts of *E. purpurea* (L.) MOENCH (ESCOP, 2003b). A monograph for the European pharmacopea is in preparation. As the morphologic discrimination of the aerial parts is difficult, the herbs of the three species are often mixed up with each other. A chemical discrimination is more adequate.
Pale coneflower root consists of the dried, whole or cut underground parts of *E. pallida* Nutt., and narrow leaved coneflower root consists of the dried, whole or cut underground parts of *E. angustifolia* (DC). The materials comply with the respective monographs in the European Pharmacopoea (Ph. Eur. 5). For purple coneflower root, a monograph is in preparation.
Also the roots of the three species are often mistaken for each other. Additionally, the adulteration with the roots of *Parthenium integrifolium* L. occurs (Bauer and Liersch, 1992). The roots can be discerned by macroscopic and microscopic characteristics, but the better method is chemical discrimination by TLC as described in the European Pharmacopoea (Ph. Eur. 5 a,b) or by HPLC.

3. CHEMICAL CONSTITUENTS

The lipophilic alkamides, the hydrophilic caffeic acid derivatives (CADs) such as echinacoside and cynarin, polysaccharides and glycoproteines are considered to be the most relevant constituents for activity. Additionally polyacetylenes, flavonoids, essential oil compounds and pyrrolizidine alkaloids have been isolated from the plants (ESCOP, 2003 a-c, Harborne and Williams, 2004). Variation in type and amount of constituents depending on species, varieties, cultivating stage and region, plant part and processing of plant products have to be considered in phytochemical standardization and batch to batch consistency of Echinacea preparations (Yu and Kaarlas, 2004). At present, mainly TLC and HPLC fingerprints of CADs and alkamides are used for standardization, quality control, and for the phytochemical discrimination of the Echinacea species (Bauer, 1999; Yu and Kaarlas, 2004). The European Pharmacopoea specifies the minimum echinacoside content to be 0.2% of dried drug for E. pallidae radix and 0.5% for E. angustifoliae radix (EAB, 2005a,b).
The use of CADs for product standardization has been shown to be problematic due to the polyphenol oxidases (POs) present in the plant material. POs can lead to the enzymatic degradation and oxidation of CADs in hydroalcoholic solutions during the extraction process (Nüsslein *et al.*, 2000, Wölkart *et al.*, 2004).
There are also efforts to use glycoproteins as marker compounds in Echinacea products. Classen and co-workers (2004) have developed a monoclonal antibody against an arabinogalactan protein from *E. purpurea* which might be a tool for quality control of *E. purpurea* preparations.

4. PHARMACOLOGY

4.1 IN VITRO AND ANIMAL TESTING

In vitro and animal studies have demonstrated the ability of various Echinacea preparations to enhance the activities of immune cells. Activity was found for hydrophilic and lipophilic extracts and fractions. The effect most widely demonstrated is the activation of macrophages and the increased activity of polymorphonuclear granulocytes resulting in an increased phagocytic activity. Stimulation of natural killer cells has also been reported (ESCOP, 2003a-c, Barrett, 2003). Other activities demonstrated *in vitro* or in animal studies are antioxidant and anti-inflammatory effects, wound healing and hyaluronidase inhibitory activity (ESCOP 2003 a-c).

4.2 VETERINARY STUDIES

Echinacea has been widely researched in laboratory animals for its potential clinical uses. Research on livestock is at best limited, however, some data exist which provide species-specific information on efficacy of Echinacea to various livestock species, including poultry, cattle, horses and swine. Practical applications are predominately, although not exclusively, associated with immune system stimulation (Pearson, 2004).

4.2.1 POULTRY

Schranner *et al.* (1989) studied the effect on the humoral immune response of a complex drug containing *E. angustifolia* homoeopathic mother tincture and *E. angustifolia* mother tincture alone on normal and immunodeficient chickens. The administration of the complex drug enhanced humoral immune parameters both types of chickens, but *E. angustifolia* mother tincture alone had no significant effect. Roth-Maier *et al.* (2005), who fed cobs prepared from *E. purpurea* aerial parts to healthy broiler and layer chickens, found no beneficial effect on feed intake and growth performance of the animals and concluded that *E. purpurea* cobs are not useful as a replacement of feed antibiotics.
There are two studies dealing with coccidia infections: Allen (2003) showed that feed supplementation with powdered *E. purpurea* root during the first two weeks of life of processed broiler chickens that were given a live oocyst vaccine, enhanced growth before coccidia

challenge and protected challenged chickens up to a certain extent against weight gain suppression and development of intestinal lesions. This indicates that *E. purpurea* may potentiate the immune response to live vaccination and may provide protective immunostimulation in the presence of natural coccidia populations in the litter.
Christaki *et al.* (2004) compared the effect of a mixture of herbal extracts containing *E. angustifolia* (plant part not given) to the anticoccidial drug lasolacid in broiler chickens experimentally infected with *Eimeria tenella*. The herbal feed supplement led to a certain coccidiostatic effect, which was however significantly lower than that of lasolacid.

4.2.2 SWINE

The outcomes of studies recently performed in pigs are rather controverse:
Holden and co-workers performed two series of experiments dealing with the potential of Echinacea as a growth promoter: In their first study (Holden *et al*., 1998), they fed 0-2% Echinacea (plant part not specified) to weaned pigs for a period of 5 weeks and compared their performance to pigs receiving a subtherapeutic level of the common antibiotic Mecadox and a negative control not receiving any feed supplement. In weeks 0-3 and 0-4 the the groups receiving 0.5 and 2% Echinacea were significantly better in feed efficiency but daily gain and feed intake and also the total performance for the entire experiment (weeks 0-5) were not statistically different. These data suggested that higher levels of Echinacea enhanced the feed efficiency compared to 0% Echinacea during the first two weeks and were greater than with the Mecadox diet during the weeks 0-3 and 0-4 (Holden *et al*., 1998). In a second study, the authors performed two experiments using lower (0.1-0.5%) and higher (1.5-3%) doses of Echinacea under the same conditions as described above. Compared with low dose Echinacea supplementation, the Mecadox diet performed significantly better whereas 3% Echinacea enhanced overall gain in the week 0-5 period compared to 0 and 1.5% Echinacea and supported gains equal to the Mecadox treatment (Holden and McKean, 2000). As the authors gave no information on the used Echinacea species (only a echinacoside content of 0.08% was given) and plant part, it would be of great value to repeat the experiment with a characterized and standardized Echinacea product (Pearson, 2004).
Hermann and coworkers challenged weaned pigs by inoculation with porcine reproductive and respiratory syndrome virus in order to study the potential antiviral and immunostimulatory effect of *E. purpurea* root powder containing 1.35% cichoric acid. The authors did not find any growth enhancing, antiviral or immune enhancing effects of Echinacea supplementation (Hermann *et al*., 2003).
Maass *et al.* (2005) studied the effect of supplementation with cobs and juice from the aerial parts of *E. purpurea* on general performance and immune parameters of sows during gestation and lactation, piglets and grower/finisher pigs. In all three groups no effect on growth performance was found, but supplemented grower/finisher pigs showed a significantly better feed conversion. No significant effects were found concerning blood and immune parameters of sows and piglets but since there was no response to flavomycin too, the authors concluded that the experimental conditions were not appropriate to effectively address the capacity of the Echinacea preparations due to the high hygienic conditions of the experiments. They suggest that there may be more efficiency under sub optimal ambient conditions. As an indicator of the specific reaction of the immune system, the antibody production after the vaccination of grower/finisher pigs with Swine erysipelas was tested, and the immune response turned out to be significantly higher in the Echinacea supplemented groups. The efficiency of the cobs was comparable to that of the commercial juice product, even though there was a great difference in the content of marker compounds present in the preparations.
In an experiment with 13 sows (6 Echinacea, 7 control), Kuhn *et al.* (2005) fed an *E. purpurea* pressed juice from the aerial parts, stabilized with alcohol (0.125 ml/kg b.w.) during the whole pregnant and suckling period of sows in 6 intervals. The influence on immune system, health status, growth performance and carcass quality of the offspring was investigated. The treatment resulted in immunostimulatory effects of Echinacea both in sows and piglets with highest changes in the peripartal period: In 1 day old piglets IgG, IgA and CRP concentrations were significantly increased in the Echinacea group. Up to day 70 of age the rate of therapeutical treatments of piglets form Echinacea-treated sows was decreased in tendency, but not

significantly. As in the studies mentioned before, growth performance was not influenced by Echinacea supplementation.

4.2.3 HORSES AND CATTLE

In a double blind, placebo controlled cross over trial O´Neill *et al*. (2002) investigated the effect of aqueous *E. angustifolia* root extract standardized to 4% echinacoside in 8 healthy horses. Animals were supplemented for 42 days. Echinacea treatment increased phagocytic activity of isolated neutrophils, boosted peripheral lymphocyte counts and appeared to stimulate neutrophil migration from peripheral circulation into the tissues. Moreover, the size and concentration of peripheral red blood cells and haemoglobin concentration increased significantly, an effect that had not been reported in previous studies with other animal species and may potentially improve the parameters of exercise physiology and performance.
The same extract was tested in a 14 day pilot study with 8 dairy heifers aged between 2 and 52 days. A trend to an increase in neutrophils and total plasma protein was observed. There were no significant effects on the incidence of scour or pneumonia. However this study is limited by its short time frame (Pearson, 2004).

4.3 HUMAN STUDIES

Clinical studies of Echinacea species have ended with mixed results. The majority of studies investigated whether Echinacea treatment shortens duration or decreases severity of symptoms of common cold over placebo, some studies also investigated the preventive effect of Echinacea preparations. The results of these studies are very different, and the studies are also difficult to compare due to different study design and Echinacea species and preparation used.
For example Brinkeborn *et al.* (1999) investigated the efficacy and safety of different doses and preparations of *E. purpurea* in the treatment of common cold in a randomized, double-blind placebo controlled study. The commercial *E.purpurea* product and its concentrated preparation showed to be significantly more active than a different *E. purpurea* preparation and placebo. The latest clinical study concerning Echinacea was performed by Turner and coworkers (2005). In this trial, the authors studied the efficacy of 3 *E. angustifolia* extracts of different polarity in the treatment of persons experimentally challenged with rhinovirus type 39. None of the 3 extracts led to statistically significant effects in this study.
Linde *et al.* (2006) reviewed 16 randomized trials investigating the effectiveness of a variety of Echinacea preparations for preventing and treating common colds. 2 of these trials investigated whether taking Echinacea preparations for 8 to 12 weeks prevents colds but found no clear effect. The majority of studies investigated whether taking Echinacea preparations after onset of cold symptoms shortens duration or decreases severity of symptoms over placebo. It seems that some preparations based on the herb of *E. purpurea* are effective for this purpose in adults, while it is unclear whether other preparations are effective and whether children benefit.

4.3.1 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

In *in vitro* studies using Caco-2 monolayers, a model of the intestinal epithelial barrier, alkamides were shown to be transported through the monolayer, presumably by passive diffusion, as the authors suggest. The diffusion time was dependent on structural variations in the alkamides. Caffeic acid derivatives only showed poor diffusion in this model. From these findings the authors conclude that alkamides, but not caffeic acid derivatives are likely to cross the intestinal barrier (Jager *et al.*, 2002; Matthias *et al.*, 2004).
Woelkart *et al.* (2005) investigated the pharmacokinetics of alkamides in humans after oral administration of a 60% ethanolic *E. angustifolia* root extract. Blood samples were taken from 11 healthy volunteers before and in short intervals after ingestion of a single dose of the extract or placebo using a crossover design. The 6 major alkamides from the extract were quantified in the plasma samples using LC-ESI-MS/MS. The maximum alkamide concentration was already reached after 30 minutes, thus the authors conclude that the mucous membranes of the mouth and the esophagus might be an important site of absorption. The absorption rate differed depending on the structure of the alkamides; this observation was also made by Dietz *et al.* (2001), who performed the first study on the absorption of *E. purpurea* alkamides in humans.

5. MICROBIOLOGY

A distinct antimicrobial activity of Echinacea species has not been described. Only pure echinacoside and some polyacetylenic compounds have been shown to exhibit low antibacterial activity *in vitro* (ESCOP 2003 a-c).
Extracts of the roots of pale and purple coneflower demonstrated *in vitro* antifungal activity against *Candida* ssp. and *Saccharomyces cerevisiae*; this was mainly attributed to the ketoalkenes and ketoalkynes (ESCOP, 2003 a, c).
Hydrophilic root extracts also showed a certain antiviral activity *in vitro*, and the antiviral activity of echinacoside against vesicular stomatitis virus in L-929 mouse cells was demonstrated in a plaque reduction assay (ESCOP, 2003 a,c). Also purple coneflower herb pressed juice as well as a decoction and a 30% ethanolic extract showed antiviral and viral resistance increasing activity in several *in vitro* models (ESCOP, 2003b).

6. EFFICACY

Echinacea preparations are regarded to be effective in humans in the adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common colds) and the derivative urinary tract, and externally as an adjuvant for the treatment of poorly healing superficial wounds (Blumenthal, 1998; ESCOP, 2003a-c; WHO, 1999). Full documentation of efficacy in these indications still remains problematic. Even though a considerable amount of clinical studies, especially concerning the efficacy for the prevention and treatment of common cold, have been performed, the outcome of these studies does not allow definite conclusions.
As studies concerning the efficacy of Echinacea as a growth promoter and immune enhancer in productive livestock are limited, no final conclusions are possible yet. In most of the studies no real growth promoting effect was demonstrated, but in some cases an enhanced feed conversion and also a positive influence on the immune system were found.
Generally it is difficult to interpret and compare study results concerning Echinacea, because the tested preparations widely differ in used species, extraction procedure and content of marker compounds.
No data exist concerning the potential of Echinacea preparations as a sensory and flavouring agent in animal feed. The plant parts used in medicine have a weak aromatic smell and their taste is sourly, bitter and in some cases they have a weak anaesthetic effect on the tongue due to their alkamide content (Bauer and Liersch, 1992). Thus it can be assumed that the plant is (at least for humans) not very well suited as a sensory and flavouring agent.

7. TOXICOLOGY

7.1. SAFETY

The toxicity of Echinacea species appears to be very low. For *E. purpurea* herb pressed juice there was found no toxicity in rats or mice at the maximum administrable dose. Also after a daily administration over a 4 week period, no toxic effects on rats were evident from laboratory or necropsy results. There was no evidence for any mutagenic potential of *E. purpurea* herb *in vitro* and *in vivo* (ESCOP, 2003b). For *E. pallida* and *E. angustifolia*, no toxicity studies are published. The pyrrolizidine alkaloids found in Echinacea are considered to be non toxic because in contrary to the hepatotoxic pyrrolizidine alkaloids from other plants they possess a saturated pyrrolizidine nucleus (Pearson, 2004).
Adverse effects of Echinacea preparations oberserved in humans seem rare and mainly comprise allergic reactions, which can be severe. Systematic studies of adverse effects of Echinacea have not been done (Ernst, 2002). No dose-dependent adverse effects have been

characterized, and no overdoses have been reported. Interactions are unknown. Contraindications are theoretical and comprise chronic progressive immuno-mediated diseases such as tuberculosis and multiple sclerosis; this is based on the potential harm that could result if the immune-mediated inflammatory components of these diseases were exacerbated by Echinacea´s immunostimulating properties (Barrett, 2003).
The safety of Echinacea in pregnancy has not been determined, and little relevant data is available. In one study, 206 women who used Echinacea during pregnancy were risk matched with 206 controls and assessed for adverse effects. Altough no evidence for increased risk was noted, the study lacked the statistical power and methodogical rigor to determine pregnancy associated risks with confidence (Barrett, 2003).

7.2 DOSAGE

E. purpurea herb pressed juice:
Adults: 6-9 ml of pressed juice daily; other equivalent preparations at comparable dosage
Children: Proportion of adult dose according to age or body weight.
The duration of continuous treatment should not exceed 8 weeks. No adverse reactions have been reported after long term oral administration (ESCOP, 2003b).
E. purpurea and E. pallida root tincture:
Adults: daily dose hydroethanolic extracts corresponding to 900 mg dried root; other equivalent preparations at comparable dosage.
Children: Proportion of adult daily dose according to age or body weight.
The duration of continuous treatment should not exceed 8 weeks (ESCOP, 2003a,c).

EMW

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EUCALYPTUS GLOBULUS

Eucalyptus globulus

1. INTRODUCTION

Even though Eucalyptus species are originally native to the Australian continent and Tasmania, their use for medical and flavouring purposes is nowadays common more or less all over the world. Both the medical and flavouring applications are mainly based on the essential oil content of the plant with 1,8-cineol (eucalyptol) as the main compound. Thus, in Europe mainly eucalyptol-rich species like *E. globulus* are used.
In medicine, the leaves and the volatile oil have traditionally been used to treat catarrh and coughs, inflammation of the respiratory tract, bronchitis, furthermore in the treatment of cystitis, diabetes, gastritis, kidney disorders, neuralgia, laryngitis, leucorrhoea, malaria, pimples, ringworm sinusitis, wounds, ulcers of the skin, urethritis and vaginits (WHO, 2002a, b). Nowadays, the leaves are used less often in the therapy of common cold, but the essential oil still plays an important role (Wichtl, 2002). It is stated to be effective internally as an adjuvant in the treatment of chronic obstructive respiratory complaints including bronchitis and bronchial asthma, and for the symptomatic relief of colds and catarrh of the upper respiratory tract, and externally for the symptomatic treatment of colds and rheumatic complaints (ESCOP, 2003, WHO, 2002a,b). Also in herbal veterinary medicine, the volatile oil is sometimes used as expectorant (Bentz *et al.,* 1989). The essential oil is also a component of certain dental root canal sealers (WHO, 2002a), and because of its antiseptic properties and its fresh taste it is often added to medical toothpastes and mouthwashes (Brand, 1993). Besides, eucalyptus preparations and eucapyptol are used as flavouring agents in many food products (Barnes *et al*., 2002).

2. DESCRIPTION OF THE PLANT

The genus *Eucalyptus* belongs to the Myrtaceae family, subfamily Myrtoideae, tribus Myrteae. The genus is extremely diverse, comprising about 400 species. The definite classification of the genus is still in discussion. At the moment, it is divided into 8 subgenera, which are further classified to sections, series and subseries (Brand, 1993). The genus is almost exclusively native to Australia and Tasmania, furthermore *Eucalyptus* species occur naturally in the

Malayan archipelago and New Guinea. Since the middle of the 18th century, many species have been cultivated all over the world in frost-free, Mediterranean, tropic and subtropic regions. *E. globulus* LABILL. is nowadays mainly grown in Spain, Portugal, southern France, Italy, the Caucasus, Algeria, Florida, California, Brazil, Mexico, Jamaica and India (Brand, 1993).
E. globulus is a large tree with smooth, pale or ash-grey bark, becoming 3 to 20 m high. The branchlets are quadrangular and grey-green. The leaves of young trees and the first leaves of young shoots are opposite, sessile, oval-oblong, with a cordate base, farinaceous-glaucous, and the older leaves are dangling, spirally arranged, lanceolate-falcate and up to 30 cm long. The flowers have very short pedicels, are almost umbellate, sometimes 2-3 in a fascicle. The fruits are turbinate, angular and 2-2.5 cm in diameter (WHO, 2002b).
The plant parts used are the leaves and the volatile oil, which is obtained from the leaves. Both materials comply to the respective monographs of the European Pharmacopoea (EAB): Eucalypti folium consists of the whole or cut dried leaves of older branches of *E. globulus* LABILL. Eucalypti aetheroleum is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus* rich in 1,8-cineole (eucalyptol). The species mainly used are *E. globulus* LABILL., *E. polybractea* R.T. BAKER and *E. smithii* R.T. BAKER (EAB, 2005a,b). Treatment with alkali and rectification is necessary for the further purification of the raw volatile oil in order to separate saponifiable compounds and aldehydes, and for the enrichment of 1,8-cineol. The different volatile oil grades on the market are not corresponding to a certain *Eucalyptus* species, but are only specified by their 1,8-cineol content. Adulterations of eucalyptus oil with camphor oil and with by-products of terpineol production have been reported. Due to the characteristic morphologic criteria of the leaves, adulterations of the leaves normally do not occur (Brand, 1993). According to the EAB, adulteration with the leaves of young branches of eucalyptus is not allowed (EAB, 2005a).

3. CONSTITUENTS

The leaves contain 1 to 3 % volatile oil, depending on provenience (Brand, 1993). The EAB specifies a minimum volatile oil content of 20 ml/kg for the whole drug and 15 ml/kg for the cut drug, with reference to the anhydrous drug (EAB, 2005a). The native oil contains 45 to 75% 1,8-cineole. Additionally, myrtenole, α-pinene, β-pinene, pinocarvone, γ-terpinene, α-phellandrene, several aldehydes and the sesquiterpenes alloaromadendrene, aromadendrene, globulole and α-grujunene have been described as minor constituents. The rectified essential oil has a 1,8-cineole content of up to 90%. Qualitative analysis of the volatile oil is performed by TLC with a reference solution containing 1,8-cineole. The chromatographic profile is obtained by GC and exactly specified by the EAB: for example less than 1.5% phellandrene, less than 0.1% camphor and at least 70% of 1,8-cineole have to be present in the volatile oil (EAB, 2005b).
Apart from the volatile oil, the leaves of *E. globulus* contain flavonoids, wax with 50% triacontane-16, 18-dione, tannins, ellagitannins and 2-4% triterpenes (ursolic acid derivatives). Also the presence of euglobales, compounds with acylphloroglucinol-monoterpene or -sesquiterpene structure, has been reported (Brand, 1993, WHO, 2002b).

4. PHARMACOLOGY

Many observed pharmacologic effects are caused by the volatile oil and its major constituent 1,8-cineole.

4.1: IN VITRO AND ANIMAL TESTING

Respiratory tract and antitussive effects
Oral administration of eucalyptus oil (50mg/kg b.w.) gave a 172% increased output in respiratory tract fluid in guinea pigs. Smaller increases were observed at similar dose levels in rabbits, cats, rats and dogs. Administration of non-lethal doses of the volatile oil by steam inhalation did not enhance the output of respiratory tract fluid in rabbits (WHO, 2002a, ESCOP, 2003).

Inhalation of the essential oil (5% emulsified in normal saline) led to a significant antitussive effect in guinea pigs, compared to codeine as a positive control. Intraperitoneal injection (50 mg/kg) of the volatile oil produced a less pronounced, but still significant effect in this model (WHO, 2002a).

Anti-inflammatory activity

Silva *et al.* (2003) tested the volatile oil of 3 eucalyptus species for their anti-inflammatory and analgesic acitivity in several animal models at concentrations between 0.1 and 100 mg/kg b.w. The oils exhibited both central and peripheral analgesic effects and produced anti-inflammatory effects as shown by inhibition of paw edema formation, inhibition of neutrophil migration into rat peritoneal cavities and decrease of vascular permeability. However, in some cases the results were inconsistent and the observed effects were not dose dependent in all assays.

Santos *et al.* (2004) found that pre-treatment, but not post-treatment with high doses of 1,8-cineole (200 and 400 mg/kg b.w.) significantly reduced colonic damage in rats on acute trinitrobenzenesulfonic acid-induced colitis. The authors suggest that the compound might be valuable as a dietary flavouring agent in the prevention of gastrointestinal inflammation and ulceration.

E. globulus volatile oil significantly reduced inflammatory cell infiltration and bronchiolitis severity in rats after elicitation of chronic bronchitis by lipopolysaccharide (LPS)-instillation (Lu *et al*., 2004). Especially after administration of high doses (300 mg/kg b.w.), there were significant decreases of mucin content in the bronchoalveolar lavage fluid and in some other parameters.

1,8-cineole was shown to inhibit the production of pro-inflammatory arachidonic acid derivatives and cytokines in several *in vitro* and *ex vivo* studies:

At a cineole concentration of 10 µg/ml, significant inhibition of the LPS-stimulated production of LTB_4, IL-1β, TXB_2 and a strong inhibition of TNFα production was observed in human blood monocytes *in vitro* (Juergens *et al*., 1998a). In a further in vitro study using human unselected lymphocytes and LPS-stimulated monocytes, Juergens *et al.* (2004) demonstrated that the compound had a stronger impact on TNFα and IL-1β production in monocytes compared to lymphocytes at a concentration of 10^{-6}M, and similar effects on monocytes and lymphocytes at a therapeutically relevant concentration of 10^{-5}M. The inhibitory effects on chemotactic cytokines were less pronounced. In an *ex vivo* experiment, LTB_4 and PGE_2 production was determined in isolated monocytes after stimulation with calcium ionophore A23187 before cineole treatment, after 3 days of treatment (3x daily 200 mg) and four days after discontinuation of treatment. Mediator production was significantly inhibited after 3 days of treatment in asthma patients as well as in healthy volunteers (Juergens *et al*., 1998b).

According to the authors, the strong inhibition of cytokine production observed in these studies makes the compound interesting in the treatment of airway inflammation, bronchial asthma sinusitis and COPD, where these mediators seem to play an important role (Juergens *et al.,* 2004).

In a different *in vitro* model, Vigo *et al.* (2004) showed that a 50% ethanolic *E. globulus* leaf bud extract possesses NO-scavenging activity, and that pretreatment with this extract decreases iNOS and mRNA levels and inhibits NO production in J774A.1 murine macrophages stimulated with LPS and interferone-γ. Testing concentrations ranged from 8.5 to 84 µg/ml. From their findings the authors conclude, that the extract possesses an anti-inflammatory activity without decreasing cell viability.

Antioxidant acitvity

Eucalyptus volatile oil inhibited the liberation of ethene from α-keto-γ-methiol-butyric acid by the Fenton oxidant in a concentration-dependent manner, starting with significant inhibition at 0.1% of oil. In another test, the degranulation of activated human neutrophilic granulocytes was quantified using a system which led to HOCl production. Eucalyptus oil strongly inhibited HOCl production at concentrations above 0.1%, with almost complete inhibition at 0.25% (ESCOP, 2003).

In a different study, *E. globulus* volatile oil was tested for free radical scavenging and antioxidant activity using three different *in vitro* models, which allow the primary and the secondary step of oxidation as well as the lipid soluble antioxidant capacity to be followed. The

oil was almost ineffective in the DPPH test and in the photochemiluminescence test and gave moderate results in the β-carotene-bleaching assay (Sacchetti *et al*., 2005).

Antidiabetic activity
In earlier investigations, a hot aqueous *E. globulus* leaf extract had been shown to suppress streptozotocin-induced hyperglycaemia in mice when added to the diet (6.25%) and drinking water (0.25%). The same extract did not stimulate pancreatic insulin production. However, in a different study, intragastric administration of aqueous or ethanolic leaf extracts at a dose of 1 g/kg b.w. did not suppress alloxan-induced hyperglycaemia in mice and rabbits (WHO, 2002b).
Also Jouad *et al.* (2003) found, that repeated p.o. administration of an aqueous *E. globulus* leaf extract exhibited a significant, dose-dependent hypoglycaemic effect in streptozotocin treated diabetic rats. The basal plasma insulin concentrations were significantly increased after repeated oral administration. In rats with a normally functioning pancreas however, no significant changes in blood glucose levels were observed. Extracts were administered at high doses (150 and 300 mg extract/kg b.w.). An eucalyptus leaf hot water extract was furthermore found to significantly decrease glucose diffusion in an *in vitro* dialysis based model of glucose absorption at a concentration of 50 g/l (Gallagher *et al*., 2003).

Other effects
Lahlou and coauthors (2002) found that bolus injections of 1,8-cineole (0.3-10 mg/kg b.w., i.v.) elicit hypotension in both conscious and anaesthesized normotonic rats. According to the authors, the effect seems to be due to an active vascular relaxation rather than to a withdrawal of sympathetic tone.
Soares *et al.* (2005) showed that 1,8-cineole reduces the contractile activity of isolated rat cardiac muscle *in vitro*. The compound at concentrations ranging from 0.003 to 0.3 mM reduced isometric tension, the rate of force development and time parameters. The authors suggest a possible calcium channel blocking action for the compound.
Eucalyptus volatile oil was shown to significantly inhibit bone resorption *in vivo* in rats, when added to the feed at a daily dose of 100 mg/animal. Also pure eucalyptol, which was added at a dose of 24 mg/animal, was effective. In an *in vitro* model on osteoclast inhibitory activity, it was shown that eucalyptol does not have a direct inhibitory effect. The authors suggest that the compound might be metabolized to active derivatives *in vivo* (Mühlbauer *et al*., 2003).

4.2 Veterinary studies

Varroa destructor Anderson and Trueman , the cause of varoosis, is the most damaging parasite in honey bees and represents a serious infestation of bee colonies. The use of synthetic acaricides is hazardous due to accumulation. So, among others, essential oil mixtures have been successfully tested as alternative treatment. Adamczyk *et al.* (2005) investigated, whether essential oil components accumulate in honey samples from hives treated with a commercial mixture of thymol, eucalyptol, menthol and camphor. They found thymol at concentrations ranging from 0.75 to 8.20 µg/g , low amounts of camphor and no eucalyptol or menthol residues. The authors conclude that the presence of these residues does not represent a sanitary risk or a risk for human health, but may change the taste of the honey. Thus, they suggest to conduct a sensorial analysis.

4.3 Human studies

A clinical trial without controls assessed the effects of Aetheroleum Eucalypti as a nasal decongestant in 31 healthy volunteers. Inhalation of the essential oil (10 ml) over a period of 5 minutes had no effect on nasal resistance to airflow. However, the oil had a stimulant or sensitizing effect on nasal cold receptors, and the majority of subjects reported a feeling of increased airflow. A single-blind, parallel clinical trial assessed the efficacy of vaporized essential oil in reducing nasal congestion in 234 patients with acute respiratory tract infections. The oil was significantly active only in the first hour following treatment. In other studies including patients with acute common colds, no significant differences in nasal decongestant activity were reported between the essential oil and vehicle only (WHO, 2002, ESCOP, 2003).
To compare the effects of secretolytics on lung function, a double-blind, placebo-controlled, three way cross-over study was carried out in 30 patients with chronic obstructive respiratory

complaints, with treating periods of 4 days separated by washout periods of 2-3 days. Subjects received either 3x daily 200 mg of cineole in capsules, 3x daily 30 mg of ambroxol.HCl in tablets, or placebo only. No concomitant medications were permitted. Significant improvements from treatment with cineole and ambroxol were observed with respect to forced vital capacity, forced expiratory volume in one second, and peak expiratory flow. Changes in cough score were not significant. In a second study, the same doses of cineole and ambroxol were administered for 7 day periods with 3-5 day washout periods, and concomitant medication was permitted. In lung function tests by body plethysmography, airway resistance and specific conductance significantly improved after cineole or ambroxol treatment, whereas inthrathoracic gas volume and residual volume were improved significantly only by cineol. The spirometric parameters were not significantly improved, only vital capacity increased significantly after cineole treatment. Scores of dyspnoea, coughing and amount of secretion showed improvement after both treatments without reaching significance. Based on the plethysmographic results, the authors conclude that cineole appears to have a bronchodilator and an expectorant effect (ESCOP, 2003).

In a randomized, double blind, placebo-controlled study, 32 patients suffering from steroid-dependent asthma (5-24 mg prednisolone as the lowest maintenance dose daily, inhaled steroids, other asthma medications) were additionally assigned to receive 3x200mg cineole or placebo daily for 12 weeks. Oral prednisolone dose was reduced by 2.5 mg increments every 3 weeks. A decresase of 36% of daily dose level was tolerated in the cineole group, as compared to only 7% in the placebo group (Juergens *et al*., 2003).

Studies documenting the efficacy of 1,8-cineole or eucalyptus oil in other inflammatory diseases do not exist so far. There is one further study investigating the analgesic effect of local treatment of headache with a combination eucalyptus oil and peppermint oil. Even though an improved cognitive performance and muscle and mental relaxation were observed, the preparation and also the single volatile oils had no effect on the sensitivity to headache (WHO, 2002a).

4.4 Absorption, distribution, metabolism, excretion

It is suggested, that eucalyptus oil is readily absorbed in the gastrointestinal tract after p.o. administration due to its lipophilic constituents. After local application, the volatile oil was rapidly absorbed in mice. After topical application of an ointment containing 66.5 mg cineole, 15% of the compound were found in rat skeletal muscle after 3 hours. When the same ointment was applied under occlusive conditions, the resorption quote increased to 60%. Concerning inhalative application, humans absorb about 20% of the provided cineole amount at a water bath temperature of 80°C. Rabbits were shown to absorb 5 to 10% of cineole applied as an aerosol (Brand, 1993).

In mice, 5 minutes after p.o. administration of 7 or 14 mg cineole, a maximum blood level of 6.6 and 16.2 ng/ml respectively was determined. As the compound was applied in emulsified rosmary oil, the validity of these results for pure cineole is questionable. Inhalation of a cineole-air-mixture over 80 minutes, equivalent to about 77 mg cineole, led to a linearly increasing cineole blood level with a maximum of 42 µg/ml in rabbits. Following intravenous administration of a solution of cineole in ethanol to rats, 1% of the administered amount was found in lung tissue and 4% in liver tissue after 1 hour. After the daily administration of 500 mg cineole/kg b.w. for 4 days, cineole passed the placenta barrier, but was not found in the milk of rats (Brand, 1993; ESCOP, 2003).After inhalative application, the elimination of cineole in mice was biphasic with a short half life of 6 minutes during the first phase and a half life of about 45 minutes during a second phase, indicating elimination in accordance with a two-compartment model. In humans, a plasma half-life of 35.8 minutes was determined after a 10-minute inhalation of cineole. After i.v. application, rats eliminate about 4% of the administered cineole dose within 5 hours via exhalation (Brand, 1993, ESCOP; 2003).

From the main constituents of eucalyptus oil, the following non conjugated metabolites have been found in brushtail possum (*Trichosurus vulpecula*): α-pinene was metabolized to myrtenic acid and trans-verbenol, β-pinene to myrtenic acid, p-cymene to p-cresole and cumic acid, and 1,8-cineole to p-cresole, 9-hydroxycineole and cineol-9-oic acid (Southwell *et al.*, 1980).

Miyazawa *et al.* (1989) isolated 2-*exo*-hydroxycineole as the main metabolite, as well as 2-*endo*-hydroxycineole, 3-*exo*- and 3-*endo*-hydroxycineole from rabbit urine after p.o.

administration of 1,8-cineole. Recently, a study of Miyazawa et al. (2001) provided evidence, that 1,8-cineole is oxidized at position 2 forming 2-*exo*-hydroxy-cineole by CYP3A enzymes in human and rat liver microsomes *in vitro*. CYP3A4 is suggested to be a principle enzyme in catalyzing this reaction in human liver microsomes. Also Unger and Frank (2004) found *in vitro* inhibitory activity of eucalyptus oil against several CYP 450 enzymes.

5. MICROBIOLOGY

Eucalyptus oil and leaves have been shown to possess antibacterial activity against a number of gram positive and gram negative organisms, as well as antifungal and antiviral activity.
The following MIC for eucalyptus oil against gram positive bacteria were determined: *Listeria monocytogenes* 0.25% (V/V), *Bacillus subtilis* 1% (V/V), *Staphylococcus aureus* and *Enterococcus* spp. 2% (V/V); For gram negative bacteria, the following MIC´s were found: *Shigella flexneri* 0.25% (V/V), *Klebsiella pneumoniae* 0.5% (V/V), *Salmonella choleraesuis*, *Proteus mirabilis* and *Enterobacter aerogenes* 2% (V/V). No inhibitory effect was found against *Escherichia coli* or *Pseudomonas aeruginosa* in this study, however, in another study *E. coli* was also inhibited. In this investigation, a good activity against a *Streptococcus* isolated from bronchial aspirates, as well as against pathogenic strains of *S. aureus*, *Klebsiella, Salmonella typhi* and *Proteus* ssp. was demonstrated (ESCOP, 2003).
An ethanol-water leaf extract inhibited the growth of *S. aureus* at a concentration of 25 µg/ml. An aqueous leaf extract inhibited the growth of *E. coli*, *S. aureus*, *B. subtilis* and *Enterococcus faecalis* (MIC 0.07-1.3 mg/ml) (WHO, 2002b).
Eucalyptus oil shows *in vitro* fungicidal activity against *Candida tropicalis, Rhizopus nigricans, Penicillium digitatum, Candida albicans, Aspergillus niger, Cryptococcus rhodobenhani, Saccaromyces cerevisiae, Cryptococcus neoformans, Mucor mucedo, Helminthosporium sativum, Alternaria solani, Nigrospora panici, Aspergillus fumigatus and Streptomyces venezuelae*, the organisms being inceasingly susceptible in the above order (agar diffusion method) (Brand, 1993). It also exhibited good antifungal activity against six *Trichophyton* species that commonly cause tinea infections, with MIC´s ranging from 0.125 to 1 mg/ml (Shin and Lim, 2004).
Methanolic and hydromethanolic leaf extracts inihibit *Candida albicans*, and an ethanol-water extract showed antimalarial activity, inhibiting the growth of *Plasmodium falciparum in vitro* at a concentration of 75 µg/ml. An aqueous leaf extract inhibited the replication of Influenza A virus A_2 (Mannheim 57), vaccinia virus and herpes simplex virus type 2 *in vitro* at a concentration of 0.1% (WHO, 2002b)

6. EFFICACY

The efficacy of 1,8-cineole, the main constituent of eucalyptus volatile oil, for the treatment of chronic obstructive respiratory complaints including bronchitis and bronchial asthma, and for the symptomatic relief of colds and catarrh of the upper respiratory tract is quite well established by several clinical studies. Despite promising *in vitro* and *in vivo* results concerning the anti-inflammatory potential of this compound, so far no clinical trials have been conducted in order to demonstrate the efficacy of cineole in other inflammatory disorders such as allergic rhinitis and inflammatory bowel diseases. However, it has to be considered that in some *in vivo* trials mentioned above, very high doses were administered, and that therefore the results may not be clinically relevant.
So far no studies have been performed to document a medical application of eucalyptus in productive livestock. Eucalyptus oil is occasionally used as an expectorant in herbal veterinary medicine (Bentz *et al*., 1989), but no data concerning efficacy and safety are available.
Concerning the use as a flavouring agent, eucalyptus is listed by the Council of Europe as a natural source of food flavouring (leaves, flowers and preparations in category N4, with limits on Eucalyptol as an active principle). In the USA, eucalyptus is used for food use and eucalyptol is listed as a synthetic flavouring agent (Barnes *et al*., 2002).

7. TOXICOLOGY

7.1 SAFETY

Externally, eucalyptus oil is stated to be generally non-toxic, non-sensitizing and non-phototoxic. Undiluted eucalyptus oil should not be taken internally, as high doses are toxic (Barnes *et al.,* 2002). In humans, the toxic dose is very difficult to determine, as the ingested doses given in the existing case reports differ tremendously. In some cases, the ingestion of only 4-5 ml was lethal, whereas in a different case, intoxication with 100 to 200 ml was survived. Intoxication symptoms are stomach ache, sickness and emesis, sometimes diarrhea. Sometimes breathing problems occur due to bronchospasm, and in most cases the CNS is affected (drowsiness, speech disorders, headache, in severe cases inconsciousness and coma). Severe intoxications are characterized by dyspnea, circulatory collapse and coma. Eucalyptus oil and preparations thereof should not be used for inhalation or applied in the face or nose region of babies or infants, because due to the intensive aroma glottis cramp, bronchospasm, asthma-like conditions and even apnoea can occur (Brand, 1993). Eucalyptus oil should not be taken internally during pregnancy, and the internal use should be avoided in case of inflammation of the gastrointestinal tract, gall bladder and decreased liver function. As the oil apparently influences liver enzymes, herb-drug interactions might be possible (ESCOP, 2003; WHO, 2002a).

Experimental data are available on the toxicity of eucalyptus oil and 1,8-cineole:

The oral LD_{50} of eucalyptus oil has been determined as 4.44 g/kg in rats and 3.32 g/kg in mice. The LD_{50} of cineole was determined as 2.48 g/kg b.w. in rats, >5 g/kg b.w. in rabbits after dermal application and 100 mg/kg in mice after i.m. application (Brand, 1993; ESCOP, 2003). In order to investigate the subacute toxicity of cineole, rats were fed 150-1200 mg/kg b.w. by stomach tube or cineole in encapsulated form with the diet, equivalent to 381-3342 mg/kg b.w./day for male and 353-3561 mg/kg/day for female rats. At dose levels of 600 mg/kg and higher, dose-dependent decrease in body weight gain and absence of a normal degree of hepatic centrilobar cytoplasmic vacuolization was observed in male rats. In addition, lesions in liver, kidneys and parotid salivary glands were found at all dose levels in male rats fed encapsulated cineole.

In a long term study, groups of 52 male, pathogen-free, CFLP mice were given cineole at 0, 8 or 32 mg/kg b.w. daily by gavage (in a toothpaste base) on 6 days per week for 80 weeks, followed by an observation period of 16-24 weeks. No treatment-related effects on body weight, food consumption, survival, weight of adrenals, kidneys, liver, lungs or spleen, or on the microscopic appearance of brain, lungs, liver and kidneys, or on tumor incidence, were observed. Studies in rats concerning the reproductive toxicity showed that 1,8-cineole penetrated placental tissue to give a fetal blood level sufficient to stimulate the hepatic enzyme activity at dose levels exceeding 500 mg/kg b.w. Mice treated with 135 mg eucalyptus oil/kg b.w. (s.c.) at day 6-15 of gestation (period of organ formation) showed no evidence of embryotoxicity or fetotoxicity. 1,8-cineole showed no mutagenic effects in the Ames test in various *Salmonella typhimurium* strains. In Chinese hamster ovary cells, the compound furthermore did not induce chromosome aberrations with or without metabolic activation. Sister chromatid exchanges were induced only without metabolic activation at cytotoxic doses that induced cell cycle delay, and sister chromatid exchanges induced by mitomycin C in cultured CHO K-1cells were not increased by post-treatment with cineole. In mice pretreated with subcarcinogenic doses of 9,10-dimethyl-1,3-benzanthracen, the compound appeared to be a weak promoter and cocarcinogen in papilloma formation (ESCOP, 2003; Brand, 1993).

The European Commission Scientific Comitee on Food concluded from the available animal data that the daily eucalyptol intake from food is not a cause of concern for humans. However, for a more precise risk characterization, further data on exposure and toxicity would be needed (EC, 2002). No experimental data are available on the safety of eucalyptus and eucalyptole in productive livestock.

7.2 DOSAGE

Volatile oil:
for internal use 0.05 to 0.2 ml per dose; 0.3 to 0.6 ml daily; in capsules 100 to 200 mg 2-5 times daily
As a lozenge containing 0.2 to 15 mg dissolved slowly in the mouth, repeated every 0.1 to 1 hour (ESCOP, 2003)

Leaf:
Daily dosage: 4-6 g crude drug or equivalent preparations (WHO, 2002b).

EMW

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FOENICULUM VULGARE



Foeniculum vulgare

1. INTRODUCTION

Foeniculum was the name given to this plant by the Romans and is derived from the Latin word *foenum*, hay. The *Anethum Foeniculum* of Linnaeus embraced two varieties, the Common or Wild Fennel and the Sweet Fennel. These are considered by De Candolle as distinct species named respectively *Foeniculum vulgare* (also called bitter fennel) and *Foeniculum dulce*. Fennel was well known to the Ancients and was cultivated by the ancient Romans for its aromatic fruits and succulent edible shoots. There are several varieties of Fennel fruit known in commerce: sweet or Roman Fennel, German or Saxon Fennel, wild or bitter Fennel, Galician Russian and Roumanian Fennel, Indian, Persian and Japanese. The fruits vary very much in length, width, taste and other characters, and are of very different commercial value. The medicinal parts are the Fennel oil extracted from the ripe fruit and the dried ripe fruit ('Fennel seeds') of *Foeniculum vulgare.* One of the major constituents include the phenylpropanoid anethole. Anethole and the monoterpene fenchone inhibit spasms in smooth muscles, such as those in the intestinal tract, and this is thought to contribute to fennel's use as a carminative (gas-relieving and gastrointestinal tract cramp-relieving agent).

2. DESCRIPTION OF THE PLANT

Fennel (*Foeniculum vulgare* Mill.) is a highly aromatic biennial or perennial herb, erect, glaucous green, and grows up to 2 m tall. The leaves grow up to 40 cm long; they are finely dissected, with the ultimate segments filiform, about 0.5 mm wide. The flowers are produced in terminal compound umbels 5—15 cm wide, each umbel section with 20—50 tiny yellow flowers on short pedicles. The fruit has an almost cylindrical shape with a rounded base and a narrower summit crowned with a large stylopod. It is generally 3 mm to 12 mm long and 3 mm to 4 mm wide.

2.1 SYSTEMATICS

Foeniculum vulgare Miller belongs to the Family *Apiaceae* (former *Umbelliferae*), the genus is represented by this species only, divided in two subspecies, ssp. *piperitum* and ssp. *vulgare,* the latter one forming three varieties, var. v*ulgare* (bitter or medicinal fennel), var. *dulce* (sweet fennel) and var. a*zoricum* (vegetable 'Florentine' fennel).
Fennel is native to Southern Europe and South-West Asia. Indigenous to the Mediterranean region, it has spread inter alias to England, Germany, and even Argentina. Fennel is also found today in Iran, India and China. It is large scale cultivated e.g. in Egypt, India, China, Australia and

within Europe in France, Germany, Hungary, Poland and the Balkan countries and many different annual, biennial and perennial cultivars are known distinguished by their size of the fruits, the content and the composition of the essential oil.

2.2 PLANT PARTS AND PRODUCTS USED

Apart from vegetable fennel bulbs, the ripe fruits and the essential oils are used commercially.
The fruits are frequently distinguished into shorts and longs in commerce, the latter being the most valued. The odour of fennel seed is fragrant, its taste is warm, sweet and agreably aromatic. Fennel seeds consists of the dried, ripe fruits of *Foeniculum vulgare.* For medicinal use the fruits of the cultivated fennel, especially the Saxonian, Galician, Roumanian and Russian varieties, yield up to 6% volatile oil and these varieties are suitable for pharmaceutical use.

The Fennel oil is the essential oil obtained from the dried, ripe fruits of *Foeniculum vulgare* by hydrodistillation.

3. CHEMICAL CONSTITUENTS (CHEMICAL ANALYSIS, STANDARDIZATION)

The principal constituents of *Foeniculi aetheroleum* (the oil obtained by steam distillation of the ripe fruits of *Foeniculum vulgare* Miller var. *vulgare)* are (Figure 1):
Trans-anethol (50 – 75%)
Fenchon (12 – 33%)
Estragole (2 – 5%)

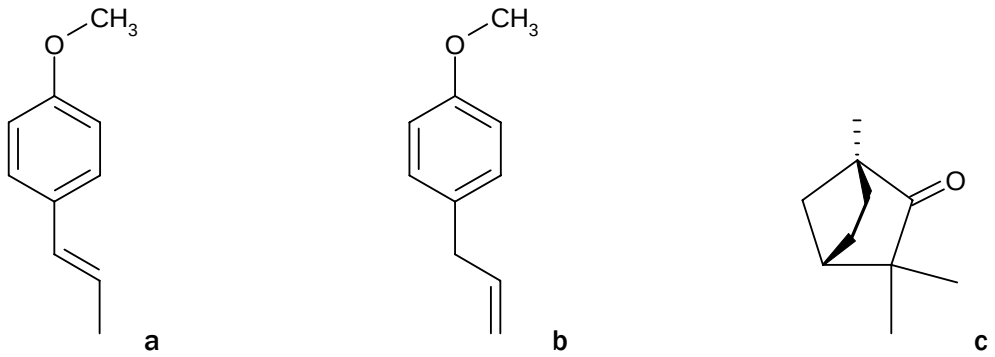

Figure 1 Chemical structure of trans-anethole (a), estragole (b) and fenchone(c)

Additional components are: trans-ocimene 20 - 330 ppm, alpha-pinene 200 - 8820 ppm, camphor 10 - 180 ppm, estragole 64000 ppm, limonene 200 - 9420 ppm, linalool 1 - 2050 ppm, myrcene 100 - 2700 ppm and other terpenes.
Trans-anethole counts for the anise taste, estragole provides the sweetness, while fenchone gives the bitter taste.
Fenchone is a colourless liquid possessing a pungent , camphoraceous odour and taste, and when present gives the disagreeable bitter taste to many of the commercial oils. It probably contributes to the medicinal properties of the oil, hence only such varieties of Fennel as contain a good proportion of fenchone are suitable for medicinal use.

3.1 CHEMICAL ANALYSIS

Separation and determination of the components of fennel oil can be achieved by either TLC or GLC (with mass spectrometry) (Ph.Eur. 5, 2005). International Standards specifications have been published for fennel seed (ISO 7927-1:1987) (Bilia A.R. et al., 2000).
A novel and rapid headspace solvent microextraction followed by gas chromatography-mass spectrometry (HSME-GC-MS) for the analysis of the volatile compounds of *Foeniculum vulgare* Mill is described. The HSME-GC-MS method was simple, inexpensive and effective and can be used for the analysis of volatile compounds (Fang L. et al., 2006).

3.2 STANDARDIZATION, STABILITY

Bitter fennel consists of the dry, cremocarps and mericarps of *Foeniculum vulgare* Miller sp. *vulgare* var. *vulgare*. It contains not less than 40ml/Kg of essential oil, calculated with reference to the anhydrous drug. The oil contains not less than 60.0 per cent of anethole and not less than 15.0 per cent of fenchone (Ph.Eur. 5, 2005).

Sweet fennel consists of the dry, whole fruits of *Foeniculum vulgare* Mill. subsp. *vulgare* var. *dulce.* It contains not less than 20 ml/kg essential oil, the oil contains not less than 80,0% anethole (ESCOP 2003).

Commercial Fennel varies largely in quality , this being either due to lack of care in harvesting or deliberate adulteration. It may contain much sand, dirt, stem tissues, weed seeds or other material, that it amounts to adulteration and is unfit for medicinal use or it may have had some of its oil removed by distillation.
Much of the commercial oil is adulterated with oil from which the anethol or crystalline constituent has been separated. Good oil will contain as much as 60% of anethol.
The *Foeniculum* oil is stored in a well-filled, airtight container, protected from light and at a temperature not exceeding 25°C (Ph.Eur. 5, 2005).

4. PHARMACOLOGY

Fennel oil and seeds are approved by ESCOP (2003) for the following indications:
Catarrh of the upper respiratory tract, cough, and dyspeptic complaints. In the traditional medicine, the herb was used for fish tapeworms, skin conditions and for various eye complaints, including conjunctivitis. Fennel honey is used for catarrh of the upper respiratory tract in children.

EFFECTS ON THE GASTROINTESTINAL TRACT

Fennel and its herbal drug preparations are widely used for dyspeptic complaints such as mild, spasmodic gastric-intestinal complaints, bloating and flatulence (Chakurski, I. et al., 1981). Different studies had shown that the extract of *Foeniculum vulgare* is effective in the treatment of colic in breastfed infant (Savino F. et al., 2005).

4.1 IN VITRO STUDIES

Antioxidant activity

The antioxidant activity of the aqueous extracts of fennel (*Foeniculum vulgare*) was investigated in comparison with the known antioxidant ascorbic acid in vitro studies. The amount of aqueous extract of fruits and ascorbic acid needed for 50% scavenging of superoxide radicals was found to be 205 µg (fennel) and 260 µg (ascorbic acid). The amount needed for 50% inhibition of lipid peroxide was 4600 µg (fennel) and 5000 microg (ascorbic acid). The quantity needed for 50% inhibition of hydroxyl radicals was 700 microg (fennel) and 4500 microg (ascorbic acid). The daily intake may be beneficial as food additives (Satyanarayana S. et al, 2004).

4.2 ANIMAL STUDIES

Antinflammatory, analgesic and anti-platelet activity

F. vulgare is claimed to have an effect in relieving inflammation. In an in vivo study with mice, oral administration of *Foeniculum vulgare* fruit methanolic extract exhibited inhibitory effects against acute and subacute inflammatory diseases and type IV allergic reactions and showed a central analgesic effect (Choi E.M. et al, 2004).
The extract has shown also antioxidant effects, reducing malondialdehyde (MDA) (as a measure of lipid peroxidation) level and increasing plasma superoxide dismutase (SOD) and catalase activities (Ruberto G. et al, 2000).
An effect of oil of *Foeniculum vulgare* on hemostasis has been evidenced, with a significant correlation with its phenylpropanoid content (Stashenko E.E. et al, 2002).

Bronchodilatatory effects

The relaxant effects of aqueous and ethanol extracts and an essential oil from *Foeniculum vulgare* were compared to negative controls (saline for aqueous extract and essential oil and ethanol for ethanol extract) and a positive control (diltiazem) using isolated tracheal chains of the guinea pig precontracted by methacholine and KCl. The results confirmed the bronchodilatory effects of ethanol extract and essential oil from *Foeniculum vulgare*. The effect doesn't appear to be related to a inhibitory effect of ethanol extracts and essential oil from *Foeniculum vulgare* on calcium, but suggest a potassium channel opening effect for this plant, which may contribute on its relaxant effect on guinea pig tracheal chains (Boskabdy M.H. et al, 2004).

Hepatoprotective effects

Hepatoprotective activity of *Foeniculum vulgare* essential oil was studied using carbon tetrachloride (CCl4) induced liver injury model in rats. The results of this study indicate that *Foeniculum vulgare* essential oil has a potent hepatoprotective action against CCl(4)-induced hepatic damage in rats with evidence of decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin (Ozbek H. et al, 2003).

Effects on vascular system

An intravenous administration of the lyophilized water extract of leaves produced a significant dose-related reduction in arterial blood pressure, without affecting the heart rate or respiratory rate. The hypotensive effect appeared not to be mediated via adrenergic, muscarinic, ganglionic or serotonergic receptors, while histamine antagonists inhibited the hypotensive effect in a dose-related manner (Abdul-Ghani).

In another study the hypotensive effects of the water extract of *Foeniculum vulgare* were investigated in hypertensive and normotensive rats. After oral administration Foeniculum increased water, sodium and potassium excretion, suggesting that it has not a vascular relaxant activity, but it appeared to act mainly as a diuretic and a natriuretic (El Bardai S. et al, 2001).

Effects on reproductive system

The main constituent of the essential oils of fennel and anise, anethole, has been considered to be the active estrogenic agent. *Foeniculum vulgare* has been traditionally used as estrogenic agent. It has been reputed to increase milk secretion, promote menstruation, facilitate birth, alleviate the symptoms of the male climacteric, and increase libido (Albert-Puleo M. 1980).

The estrogenic activity of the seed extract was confirmed in an in vivo study with male and female rats. Following the oral administration of acetone extract of *Foeniculum vulgare* seeds for 15 days is male rats, total protein concentration was found to be significantly decreased in testes and vas deferens and increased in seminal vesicles and prostate gland. In female rats, oral administration of the extract for 10 days led to vaginal cornification and oestrus cycle(Malini T. et al, 1985).

While moderate doses caused increase in weight of mammary glands, higher doses increased the weight of oviduct, endometrium, myometrium, cervix and vagina (Annusuya S. et al, 1988).

4.3 HUMAN STUDIES

Effects in infantile colic

Fennel seed oil has been shown to reduce intestinal spasms and increase motility of the small intestine. The use of fennel oil emulsion eliminated colic in 65% (40/62) of infants in the treatment group, which was significantly better than 23.7% (14/59) of infants in the control group ($P < 0.01$). Side effects were not reported for infants in either group during the trial. The study suggests that fennel seed oil emulsion is superior to placebo in decreasing intensity of infantile colic (Alexandrovich I. et al, 2003).

4.4 ADME

Few pharmacokinetic data are available for *Foeniculum vulgare*. Some information is found in literature on its main constituent. Trans-anethole is rapidly absorbed and distributed in the rat and in the mouse. The main metabolites found in urine are p-hydroxypropenylbenzene, p-methoxyhippuric acid, p-hydroxycinnamic acid, and p-methoxybenzoic acid, mainly as glucuronides and sulfates. *Trans*-anethole is hydroxylated at the 3'-carbon by hepatic

microsomes from rats and mice. A study carried out on five human subjects given a 500 mg dose the 24-hour metabolites were anisic acid (52%) and p-hydroxybenzoic acid (5%) and t*rans*-anethole was not detectable in the blood.
Few pharmacokinetic data are available for *Foeniculum vulgare*. Some information is found in literature on its main constituent. Trans-anethole is rapidly absorbed and distributed in the rat (Le Bourhis, 1973; Fritsch *et al.,* 1975) and in the mouse (Le Bourhis, 1968; Strolin-Benedetti & Le Bourhis, 1972; Le Bourhis, 1973). The main metabolites found in urine are p-hydroxypropenylbenzene, p-methoxyhippuric acid, p-hydroxycinnamic acid, and p-methoxybenzoic acid, mainly as glucuronides and sulfates. *Trans*-anethole is hydroxylated at the 3'-carbon by hepatic microsomes from rats and mice (Swanson *et al.,* 1981). A study carried out on five human subjects given a 500 mg dose the 24-hour metabolites were anisic acid (52%) and p-hydroxybenzoic acid (5%) and t*rans*-anethole was not detectable in the blood (Le Bourhis, 1973).

5. MICROBIOLOGY

Antibacterial activity
The antimicrobial properties of essential oils obtained from *Foeniculum vulgare* have been investigated in different trails. The oil has been shown to be effective against *Streptococcus haemolyticus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella species (Sigh G. et al., 2002), (Lo Cantore P. et al., 2004),* (Kwon Y.S. et al., 2004). Another study has shown an activity against the foodborne pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus (Dadalioglu I. & Evrendilek G.A., 2004).*
The methanol extract of fennel, as other plant species used traditionally to treat gastrointestinal disorders, were assessed for their effect on the growth of *Helicobacter pylori in vitro*. A MIC of 50 µg/mL was assessed (Mahady G.B. et al., 2005).

Antifungal activity
Fennel oil possesses a strong antifungal activity. According to the results of a study carried out on *Aspergillus* spp., *Penicillium* spp., *Trichoderma viride*, *Cladosporium cladosporioides*, *Fusarium tricinctum,* the observed antifungal activity of all the investigated oils can be attributed to the main compound, *trans*-anethole (Mimica-Dukic N. et al., 2003), (Abu-Jawdah Y. et al., 2002).

Repellent properties
The repellent activity of materials derived from the methanol extract of fruits from *Foeniculum vulgare* against hungry *Aedes aegypti* females was examined using skin and patch tests. Responses varied according to compound, dose, and exposure time, but fenchone was found as potential mosquito repellent agent (Kim D.H. et al., 2002).
Another study showed that fennel oil-containing aerosol and cream produced 84% and 70% repellency, respectively, at 90 min after exposure against mosquito belonging to the genera *Culex*, *Anopheles*, *Aedes* and *Armigeres* (Kim S.I. et al., 2004).
Acaricidal activities of components derived from *Foeniculum vulgare* fruit oil were found against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* after direct contact application (Lee H.S. 2004).

6. EFFICACY

6.1 QUALITY OF THE ANIMAL PRODUCT

In a study, three-hundred unsexed day-old Arbor Acres broiler chickens were fed with a commercial diet or diets enriched with fennel at 1%. At 35 days of age, each group was divided into 2 equal subgroups, where the first was placed under normal conditions (24 deg C) and the second was exposed to 38 deg C for 3 h to 6 days from 35 to 40 days of age. It was shown that adding fennel as natural feed additives to broiler diets under normal or high temperature

conditions increased (P<0.05) live body weight, liveweight gain, feed conversion, total plasma protein, serum albumin, globulin, plasma glucose and triiodothyronine (T3) values and decreased mortality rate, plasma cholesterol, plasma total lipids, alanine aminotransferase and aspartate aminotransferase (P<0.05). Creatinine and weights of the liver, spleen, bursa fabricii and intestine were not affected by the additives. Also, there was a significant (P<0.05) decrease in respiration rate and body temperature and a minimal increase in blood pH during heat exposure. There was a decrease (P<0.05) in body weight, liveweight gain, feed consumption, T3, plasma glucose and the total plasma proteins and an increase (P<0.05) in mortality rate, body temperature and respiration rate corresponding to high temperature conditions (Tollba A.A.H. 2003).

7. TOXICOLOGY

Concerning the acute toxicity of the main costituents of Foeniculum vulgare, the oral LD50 of trans-anethole are 3.05 g/kg bw, 2.09 g/Kg bw and 2.16 g/kg bw for mce, rats and rabbits respectively. For trans-anethole intraperitoneal LD50 values are 1.41 and 2.67 g/kg bw for mice and rats respectively. Cis-anethole is 10 to 20 times more toxic than trans-anethole but the content in the oil is very low. Rabbits and dogs tolerate 3 g anethole per Kg bw without any toxic manifestation. With respect to long-term toxicity, no data for the oil are available. A one year feeding study showed that the NOEL for rats is 125 mg anethole/Kg. (EMEA/MRL/418/98).
No data are available on acute, sub-acute and sub-chronic toxicity of estragole. In 2000 the Committee of Experts on Flavouring Substance (CEFS) of the Council of Europe evaluated estragole and recommended a limit of 0.05 mg/Kg (detection limit) (EMEA/HMPWP/338/03).
Long term studies were carried out on mice and rats. There was a reported statistically significant increase in the incidence of benign hepatocellular adenomas and hepatocellular carcinomas in female rats receiving 1% *trans*-anethole, but it was within the range of spontaneous incidence of similar lesions. In a series of experiments was performed to investigate the carcinogenic potential of *trans*-anethole in mice *trans*-anethole produced no different results from controls in necropsy histological evaluations .
The mutagenic activities of anethole and its metabolites were studied using different bacterial strains, and using a mouse micronucleus assay. A study indicated that trans-anethole showed mutagenic activity in the Ames S. typhimurium assay but was inactive in a B. subtilis Rec assay and was negative in an E. coli uvr A reversion test.
In another study no mutagenic activity was observed at concentrations of up to 50 µg trans-anethole/plate but the addition of 3'-phosphoadenosine-5'phosphosulphate to the microsomal assay markedly increased the mutagenicity of trans-anethole. Negative results were also obtained in a mouse micronucleus assay after oral or intraperitoneal administration.
No reproduction and teratogenicity studies are available for the compound.
The mutagenicity of estragole, another constituent of fennel essential oil is reported (De Vincenzi et al., 2000) as well as its carcinogenic potential in rodents (Smith et al., 2002). However, this flavouring agent has a clear threshold for carcinogenicity in animals that is well above the levels currently approved for use in foods (Waddell, 2002).

7.1 SAFETY

No data in literature suggest possible adverse effects in animals due to continuative assumption of *Foeniculum Vulgare* as flavouring agent in feed. In the United States of America trans-anethole and fenchone are included in the GRAS-list (generally considered as safe) by the FDA.
No safety concern at current levels of intake when used as a flavouring agent. An ADI of 0.6 mg/kg bw for trans-anethole has been established by JECFA. A fenchone concentration of 5 mg/kg in food is permissible.

Dosage

The oil of *Foeniculum vulgare* (Foeniculi aetheroleum) is contained in two veterinary medicinal products. One product is intended to be used as nose-spray to facilitate breathing in new born animals of all food producing species in a dose of 0.1 to 0.3 g (corresponding to a dose of 0.015

mg Foeniculi aetheroleum). The other product, containing 1% Foeniculi aetheroleum, is intended for the oral treatment of gut disorders, in a maximal dose of 15 mL (corresponding to 150 mg Foeniculi aetheroleum). The product is targeted to cattle, horses, pigs, sheep and goat.
Foeniculi aetheroleum is included in Annex II of Council Regulation (EEC) N. 2377/90 as a substance that does not need a MRL level.

Interaction
A significant interaction between fennel and ciprofloxacin was observed in a study. Absorption, distribution and elimination of ciprofloxacin were all affected. These changes might be because of the formation of a more lipophilic ciprofloxacin chelate in the presence of relatively large amounts of metal cations in fennel (Zhu *et al*., 1999).

DT/SG/LG/AT

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GAULTHERIA PROCUMBENS

Gaultheria procumbens

1. INTRODUCTION

Gaultheria procumbens is a plant native of the United States and source of an essential oil. Once a popular flavoring in candies and chewing gums, wintergreen has also enjoyed long use as a herbal remedy. The first record of the therapeutical use of this oil, as is often the case with valuable medicines, is to be found in empirical medicine. Indian people, including the Sioux, Penobscot and Nez Perce nations, use wintergreen tea to treat arthritis pain and sore muscles. Later, the settlers used the leaves of the herb for similar purposes, as well as to alleviate headaches and colds. The proprietary remedy, very popular about the beginning of last century under the name "Panacea of Swaim," or "Swaim's Panacea," introduced it. The essential oil of *Gaultheria procumbens* (oil of wintergreen) is marketed for the external treatment of neuralgia, myalgia, arthralgia and other pains. The oil is a colourless to yellowish liquid with a characteristic 'wintergreen' odour, slightly soluble in water, soluble in organic solvents and oils.

2. DESCRIPTION OF THE PLANT

Wintergreen is a small, procumbent, evergreen shrub, up to 15-20 cm high, with slender creeping stem giving rise to erect branches, bearing towards their ends 3-8 leaves. The leaves are alternate, thick, toothed, glossy, lanceolate, 2,5 – 5 cm long and dark green. The flowers are white, bell-like, often with a touch of pink, in 2 or 3 flower umbels. The fruit is a berry, reddish violet, globose, 6-9 mm long, minty, with a rather spongy texture. The seeds are orange-yellow, wedge shaped, 1-1.3 mm long, 0.8-1 mm wide, wingless.

2.1 SYSTEMATICS

Wintergreen, *Gaultheria procumbens*, belongs to the Ericaceae family.
The plant, native of North America, is distributed from Alaska to Newfoundland (Kron et al., 1999).
Other common names include Box Berry, Checkerberry, Deerberry, Eastern Teaberry, Ground Holly, Mountain Tea, Creeping Wintergreen, Ground Tea, Partridge-Berry, Petit the du bois (Quebec, "little tea of the woods"), Redberry Wintergreen, Spice Berry, Teaberry, Winisibugons (Ojibwe, "dirty leaf").

2.2 PLANT PARTS AND PRODUCTS

The leaves of *G. procumbens* are used for the extraction of the essential oil known as "wintergreen oil".
Almost the same oil can be obtained from sweet birch (*Betula lenta* L.).

3. INGREDIENTS/CONSTITUENTS (CHEM. ANALYSIS, STANDARDIZATION)

Gaultheria procumbens is a source of "wintergreen oil," which is used as a flavoring in candies, chewing gum, and some medicine. The oil is extracted from the leaves, previously macerated in water, by steam distillation. The leaves contain an odorous volatile oil, which may be obtained in the same manner as oil of peppermint. The specific gravity of the oil is 1.173 at 10° C. (50° F.). It is colorless at first, but subsequently becomes more or less of a pinkish color, has a hot and aromatic taste. The main chemical components of wintergreen oil are methyl salicylate and gaultherin.

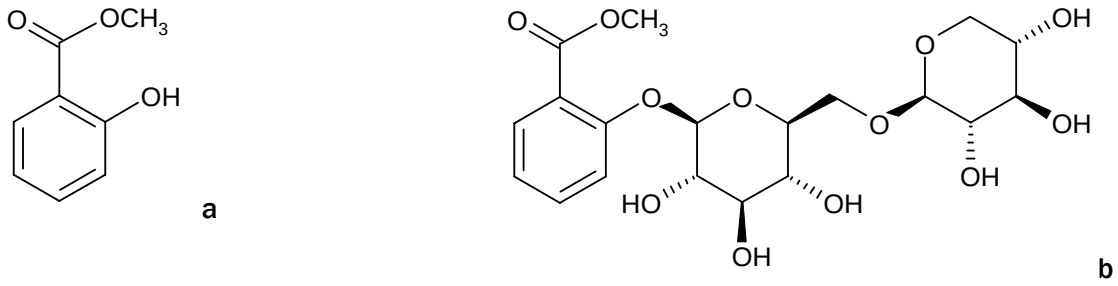

Figure 1. Chemical structure of methyl salicylate (a) and gaultherin (b).

The dried leaves are used as a tea or as flavouring agent.
According to Ribnicky et al. (2002) wintergreen contains 10 mg/g FW of total salicylate - the highest levels observed in plants. The level of free salicylate and methyl salicylate were significantly less in the intact leaves of wintergreen. High levels of total salicylate were also observed in flowers, stems and berries of the plant. The salicylate was present predominantly as a derived form of methyl salicylate called gaultherin.
The following species also contain gaultherin: *G. acuminata, G. fragrantissima, G. leucocarpa, G. punctata, G. rudis, G. cuneata, G. griffithiana, G. hookeri, G. itoana, G. yunnanensis.*
Methyl salicylate determination can be achieved by sodium hydroxide titration according to the European Pharmacopoiea (01/2005:0230).
The application of a 2H NMR method in determining the naturalness of an important flavor, methyl salicylate, has been proposed by Le Grand et al. (2005). Extraction was performed on dry wintergreen leaves. One kilogram of raw material was left to soak overnight with 10 L of warm water. Such maceration causes hydrolysis of the primeveroside of methyl salicylate (also known as gaultherin) and frees methyl salicylate, the chief constituent of wintergreen oil (95-99%). In fact, methyl salicylate does not occur in the plant in the free form, but as a glycoside. The distillate obtained over 6 h was separated by condensation. Distillation was ended by the addition of 40 mL of cyclohexane. The aqueous phase was extracted two times by 50 mL of cyclohexane. Cyclohexane phases were combined and concentrated under N_2 flow. The purity of samples was checked by $_1$H NMR.

4. PHARMACOLOGY

4.1 ANIMAL STUDIES

The pharmacological properties of *Gaultheria* are due to its content in methyl salicylate and its derivative gaultherin, that have antinflammatory and analgesic properties. The mechanism of action of the salicylates is based mainly on the inhibition of the cyclo-oxygenase enzyme that intervenes in the synthesis of prostanoids from arachidonic acid, just as all salicylic acid derivatives. They also inhibit the release of PGF2a and PGE2 from thrombin-stimulated platelets as well as the synthesis of thromboxanes and favour the production of prostacyclin PGI2 (Atkinson and Collier, 1980; Amann and Peskar, 2002; Vane and Botting, 2003; Zhang et al., 2006).
The glycoside gaultherin has also been studied for its analgesic and anti-inflammatory effects and it has less adverse effect then other salicylates. Gaultherin (200 mg/kg, orally) significantly

inhibited the abdominal contractions in the acetic acid-induced writhing test in mice. The anti-inflammatory effect of gaultherin was demonstrated in the croton oil-induced ear edema model in mice, after oral administration: gaultherin (400 mg/kg/day, for 3 days) and equimolar dose of aspirin (200 mg/kg/day, for 3 days) produced comparable inhibitory effects. Gaultherin has less negative effects on gastric mucosa with respect to aspirin: no lesion was observed in the stomach when gaultherin (100 mM; 330 mg/kg) was given orally to mice. The reason could be that gaultherin released salicylate in intestine slowly, not in stomach and it left the cyclooxygenase-1 unaffected, which was the source of cytoprotective prostaglandins in gastric epithelium (Zhang et al., 2006).
The main component of *Gaultheria procumbens*, methylsalicylate, is used in cattle, horses, sheep, goats and poultry. It is used topically in emulsion in the treatment of muscular and articular pain. The recommended dose is 600 µg/kg bw twice a day. The duration of treatment is usually less than one week (EMEA/MRL/696/99, 1999). It is included in Annex II of Council Regulation (EEC) N. 2377/90 as a substance that doesn't need a MRL level.
Gaultheria procumbens contains a glycoside that, when hydrolyzed, releases methyl salicylate. *Gaultheria procumbens* has not to be used as flavouring in pet food since salicylates are toxic to dogs and cats. Since cats metabolize salicylates much more slowly than other species they are more likely to be overdosed. Use of methylsalicylate in combination with anticoagulants such as warfarin can result in adverse interactions and bleedings (Chow et al., 1989, Ramanathan, 1995, Tam et al., 1995, Yip et al., 1990).

4.2 HUMAN STUDIES

Even though the use of wintergreen oil as a topical anti-inflammatory is quite common in human medicine, there's a lack of scientific literature on the efficacy on human patients. Human studies have been focused mainly on the toxicology and pharmacokinetics, and are here reported in the corresponding sections.

4.3 ADME

In rats, after oral administration of 500 mg methylsalicylic acid/kg bw, the drug is nearly completely hydrolysed to salicylate within 1 hour and at this time the concentrations of free salicylates in plasma and brain were 278 and 42 µg/ml. These levels are similar to those observed after the administration of acetylsalicylic acid. A similar pattern of hydrolysis has been also observed in dogs (EMEA/MRL/696/99, 1999).
The liver is the main site of methyl salicylate hydrolysis in rats, rabbits, dogs and monkeys (EMEA/MRL/696/99, 1999). In dogs 0.2 to 0.5% of an oral dose and 14% of an intramuscular dose are excreted as unchanged methylsalicylic acid in the urine. Rabbits excrete methylsalicylic acid mainly as glucuronate (12 to 55%) but also as sulphate (10%) conjugate (Behrendt et al., 1989).
Methyl salicylate is extensively metabolised to salicylic acid in dermal and subcutaneus tissues following topical administration.
In humans approximately 12 to 20% of topically applied methylsalicylic acid is systemically absorbed within 10 hours of application (Martin et al., 2004, Morra et al., 1996, Pratzel, 1990). When methylsalicylic acid was given orally to 6 healthy adults, about 21% of unhydrolysed ester was present in plasma after 90 minutes. In a case of an accidental oral intake of wintergreen oil 21% was present in the circulation after 1.5 hours (EMEA/MRL/696/99, 1999). After application of a topical formulation containing 20 % methylsalicylate on rat skin, the compound penetrated into underlying tissues (dermis, subcutaneous and superficial muscle) reaching a maximum concentration in the first hour after the treatment. Furthermore, salycilates were cleared in the systemic circulation where their concentration rose rapidly after 1 hour (Cross et al., 1999). No data on oral bioavailability are available.
Studies on the metabolism of gaultherin showed that after administration of a single dose of gaultherin (200 mg/kg in mouse, 140 mg/kg in rat), the parent compound was not detected in plasma of mice and rats, but it has been found that the concentration of a metabolite of gaultherin was increasing in the first few hours after administration. The metabolite was demonstrated to be salicylate in animals by HPLC and it was the only metabolic product of gaultherin. The maximum plasma concentration (C_{max}) of salicylate in mouse and rat was 60 and 70 µg/ml, respectively and the time to reach C_{max} (T_{max}) was 5 and 7.5 h after dosing.

However, it has been reported that the concentration of salicylate in the gastric mucosa and blood rose promptly after administration of aspirin and the peak was reached within 1 h before declining rapidly. The results showed that gaultherin was a pro-drug and could be metabolically converted to the active salicylate slowly (Zhang et al., 2006).
After incubation with intestinal microflora in anaerobic conditions, the concentration of gaultherin declined slowly and the metabolite was identified as methyl salicylate by HPLC. The results suggested that orally administered gaultherin was hydrolyzed by glycosidase from intestinal bacteria to produce methyl salicylate, the aglycone from which the sugars of the administrated compound had been removed, and then absorbed.
The ester linkage of gaultherin had to be hydrolyzed to give the final salicylate. Gaultherin was incubated with animal and human plasma which contained plenty of esterase to investigate whether it can be hydrolyzed by esterase. The results showed the ester linkage could be cleaved by esterase to produce the final salicylate metabolite. These results strongly suggested that orally administered gaultherin did not act by being directly absorbed and metabolized to salicylate in the liver, but rather only after it was transformed into methyl salicylate by intestinal bacteria and then the absorbed methyl salicylate was hydrolyzed rapidly by esterases in intestine, blood and liver to produce salicylate (Zhang et al., 2006).

5. MICROBIOLOGY

Essential oils and extracts from *Gaultheria spp.* have been studied for their antibacterial activity. A significant inhibitorial effect was found for extracts of *Gaultheria leucocarpa* var. *yunnanensis* against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Anti-bacteria test with extracts of water, acetic ester and n-butanol showed that 3 extracts from 22 samples had anti-*Staphylococcus aureus* action, and the extracts from root and stem showed the same result. 2 extracts could kill *Escherichia coli* and *Pseudomonas aeruginosa*, suggesting that not only essential oil but also other ingredients have antibacterial activity. Anti-fungi test of the same extracts was also performed but didn't indicate remarkable action (Ma et al., 2001a,b).

6. EFFICACY

Methyl salicylate is used as a flavouring agent for food, beverages and tooth powders, liquid dentifrices, etc. No safety concern at current levels of intake when used as a flavouring agent. The JECFA established ADI is 0,1-0.5 mg/kg bw (JECFA, 2001).
Other species of *Gaultheria* have been studied for their antioxidant properties, attributable to their content in polyphenols like catechins. Catechins are recognized to have antioxidant properties that can confere an antioxidant protection to meat products, thus improving their quality and *shelf life* (Tang et al., 2000, Tedesco et al., 2004).
It has to be taken into account that the use of methylsalicylate for topical application in combination with anticoagulants such as warfarin can result in adverse interactions and bleedings (Chow et al., 1989; Yip et al., 1990; Tam et al., 1985; Ramanathan, 1995).

7. TOXICOLOGY

The acute oral toxicity of methylsalicylic acid has been investigated in several animal species. Oral LD50 values of 1110 mg/kg bw in mice, 887 and 1250 mg/kg bw in rats, 2100 mg/kg bw in dogs, 1300 and 2800 mg/kg bw in rabbits have been reported (Davison et al., 1961, Taylor et al., 1964) Administration of 600 to 4700 mg/kg bw of methylsalicylic acid to dogs produced nausea, vomiting, intense hyperpnoea, excitation of the nervous system, diarrhoea and a hypermetabolic state as a result of uncoupling of oxidative phosphorylation (EMEA/MRL/696/99, 1999).
In dogs methylsalicylic acid given at doses of 0, 50, 150, 250, 500 and 800 mg/kg bw for up to 10 weeks, induced weight loss, liver fatty degeneration and death at doses of 500 mg/kg bw or higher. No adverse effects were noted after doses as high as 250 mg/kg bw. As only summary information was available no NOEL could be established (EMEA/MRL/696/99, 1999).

Chronic toxicity studies were performed in rats and dogs. In dogs, growth retardation, liver enlargment with enlarged hepatocytes were observed at doses of 150 mg/kg bw or more. In rats, the dose of 500 mg/kg bw induced signs of toxicity, such reduced growth rate, increase in relative eight of testes, heart and kidneys, while gross pituitary lesions were observed in animals treated with 250 mg/kg bw (EMEA/MRL/696/99, 1999).
In hamsters methylsalicylic acid was also found to provoke teratogenic effects at both high oral doses (1750 mg/kg bw) and high topical doses (1750 to 14000 mg/kg bw) (EMEA/MRL/696/99, 1999).

7.1 SAFETY

No informations are available in literature about quality of food products derived from animals fed/treated with *G. procumbens* or wintergreen oil respectively.

DT/SG/LG/AT

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GENTIANA LUTEA

Gentiana lutea L

1. INTRODUCTION

All species belonging to the genus *Gentiana* have intense bitter properties residing in the root and herbage. The most commonly used species is *Gentiana lutea* L., the Yellow Gentian. Gentian root has a long history of use as an herbal bitter in the treatment of digestive disorders and is an ingredient of many proprietary medicines. In some pharmacopoeias *Gentiana asclepiada* L., *Gentiana pannonica* SCOP., *Gentiana punctata* L. and *Gentiana purpurea* L. have been used as substitutes. Its name is derived from Gentius, King of Illyria (180-167 BC) who discovered the plant's healing value. It was used in the Middle Ages as an antidote to certain poisons. Gentian root contains some of the most bitter compounds known and is used as a scientific standard for measuring bitterness. Other names include Yellow Gentian, Bitter Root, Bitterwort, Centiyane, and Genciana.

2. DESCRIPTION OF THE PLANT

Gentaina lutea L. is a perennial plant growing at least up to 1 m. The adult plants have thick roots and rhizomes, which function as storage organs. The broad lanceolate to elliptic leaves are deeply veined, 12-25 cm long and 4-10 cm broad. Most of them belong to a basal rosette until flowering. It is important to realise that *Gentiana lutea* L. has opposite leaves differing from the highly toxic *Veratrum album* [Garnier et al., 1985; Zagler et al., 2005]. *Gentiana lutea* L. has brightly yellow trumpet-shaped flowers, which are arranged in terminal and axillary clusters. The terminal flowers are mostly pentamerous, i.e. with 5-7 corolla lobes (petals) and 5 sepals without folds between lobes. This is one of the few species in *Gentiana* that has rotated, very deeply divided corollas and no glandular disk at the base of the ovary.
Gentiana differs from *Gentianella* (see below 2.1) in that it has plicae (thin folds between corolla lobes), intracalycine membrane on the calyx (thinner areas between the calyx lobes) and a disk at the base of the ovary.

2.1 SYSTEMATICS

Gentiana lutea L. belongs to the genus *Gentiana* comprising more than 400 species. The description of this large genus has changed considerably, since various species have been

repeatedly excluded and included from the genus [Struwe et al., 2002]. Two genera that earlier belonged to *Gentiana* were separated into *Gentianella* and *Gentianopsis.*

2.2 Occurrence

Gentian species are wide-spread in the alpine habitats of Europe, Asia and North America. Some species also occur in northwest Africa, eastern Australia and New Zealand. *Gentiana lutea* L. grows in the mountains of central and southern Europe up to the altitude of 2500 m. In Italy, it is relatively frequent in the pastures of Western and Central Alpine and Subalpine plains [Buffa et al., 1991]. The most important crops originate from collections of wild plants in Spain and Switzerland. To a smaller extend *Gentiana* is cultivated in France and Germany.

2.3 Plant Parts and Products

The medicinal parts of *Gentiana lutea* L. are the dried, underground parts of the plant. Sometimes the fresh, above-ground parts are used. The best quality crude drug for medicinal use is harvested either in autumn, at the end of the vegetative cycle, or in spring before the plants start sprouting. After harvesting, the roots are quickly dried and then left to season for at least 6 – 8 months until they are yellow or yellow-brown in colour [Buffa et al., 1991].
Commercial gentian root extracts are normally obtained by percolation with about 60-70% ethanol in water, yielding extracts with the characteristic bitter taste and aroma. To facilitate solute diffusion and increase the extraction yield pre-treatment of the material with pectolytic enzyme preparations has been proposed [cited in: Arino et al., 1997].

3. Ingredients/Constituents

The roots and rhizomes accumulate a variety of compounds such as soluble carbohydrates (30-50% on dry weight), mainly gentianose and sucrose and a smaller percentage of glucose, fructose and gentiobiose [Franz et al., 1985; Rossetti et al., 1984], lipids (6-7% on dry weight) and insoluble carbohydrates, mainly pectin. Important constituents of gentian are the secoiridoid bitter constituents amarogentin (0.05-0.15%; bitter index: 58 x 10^6; Figure 1A), gentiopicroside (2.5-3.5%; Figure 1B), swertiamarin (0.15-0.20%; Figure 1C) and sweroside (< 0.1%; Figure 1D). The latter compounds have a bitter index of 12 x 10^3. Recently another bitter principle was identified as loganic acid in *Gentiana lutea* L. for the first time [Carnat et al., 2005]. Since the bitter compounds are located in the rhizoderm, their contents are highest in the younger, thinner roots, whereas in older roots gentiopicroside is dominating [Buffa et al., 1991].
The root and rhizome also contain small quantities of free amino acids [Rossetti et al., 1984], essential oil, mainly composed of limonene, linalool, carvacrol, *cis*-linalyl oxide and α-terpineol [Arberas et al., 1995; Arino et al., 1997; Chialva et al., 1986], polyphenolics, i.e. gentianine gentioflavin and the xanthones gentisin, isogentisin and gentioside. Gentian root is devoid of starch and ash content ranges from 3 to 4%.

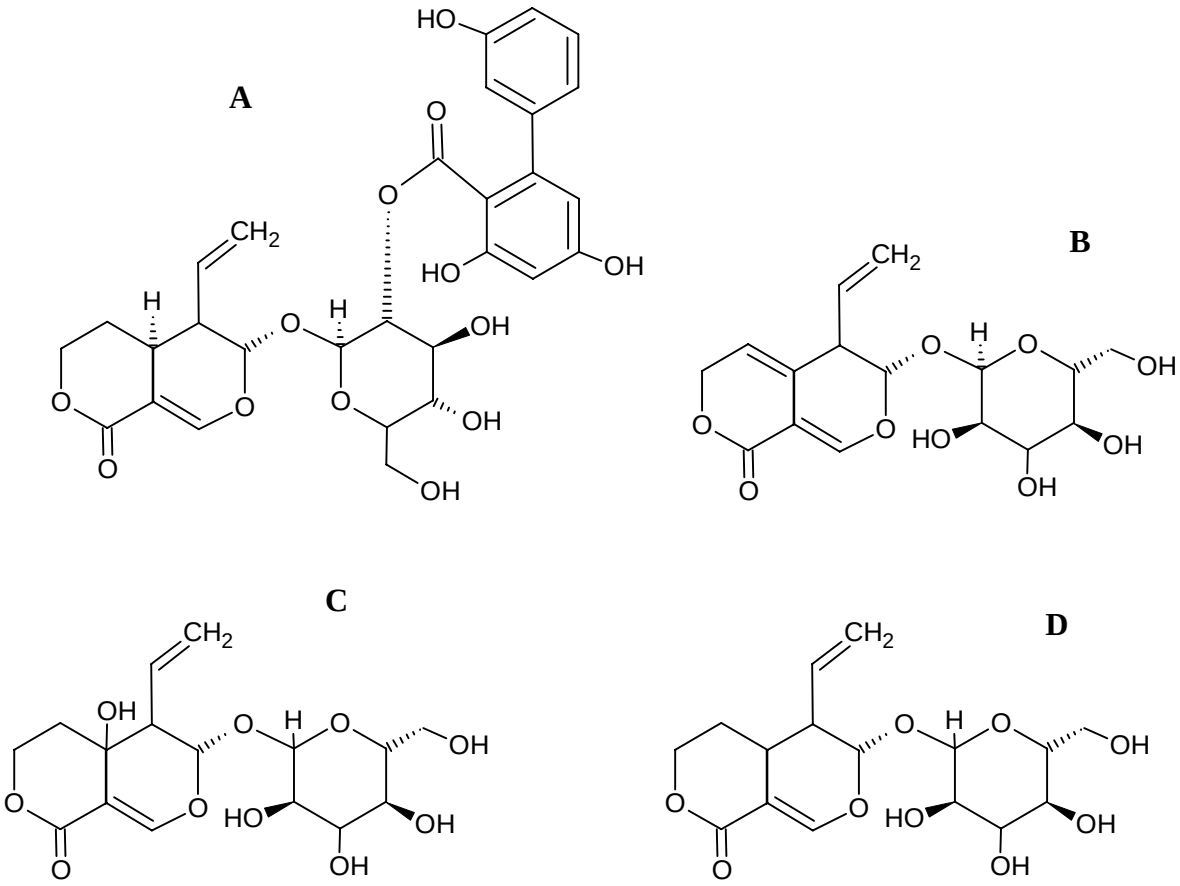

Figure 1 Structures of the bitter principle of gentian (A: amarogentin; B: gentiopicroside; C: swertiamarin; D: sweroside)

Several studies dealt with the composition of the aerial parts of *Gentiana lutea* L.. Hostettmann et al. (1973) reported the presence of isogentisin and two flavonic heterosides (isoorientin-4´-O-glucoside and isovitexin-4´-O-glucoside) in the leaves of *Gentiana lutea* L.. Mangiferin has been found in the aerial parts [Lubsandorzhieva et al., 1986] showing antioxidative, antidepressant and other pharmacological activities [Born et al., 1996; Ghosal et al., 1996, 1975]. In a recent study the chemical composition and seasonal variations in the amounts of secondary compounds in *Gentiana lutea* L. leaves and flowers has been investigated by means of HPLC [Menkovic et al., 2000]. Additionally, seasonal variations in the contents of the individual components were observed. It could be concluded that during flowering, leaves are rich in compounds possessing C-glycoside structures while O-glycoside structures accumulate mainly before flowering. The accumulation of secoiridoids (Gentiopicroside, Swertiamarin) more or less constantly increased during the vegetation period reaching the maximum in October.

3.1 Chemical Analysis

The bitter principles amarogentin and gentiopicroside are extracted with aqueous alcohol. Maximum concentrations of amarogentin and gentiopicroside were obtained with ethanol: water 55: 45 (v/v). With ethanol: propylene glycol: water 20: 20: 60 (v/v/v), the yields of bitter compounds were 5% lower for amarogentin and 16% lower for gentiopicroside. Using propylene glycol: water 30: 70 (v/v) the yields further decreased by 12% and 25%, respectively [Arino et al., 1997]. Identification and quantification is performed by HPLC and LC-MS at 233 and 270 nm, respectively, using methanol and water as the mobile phase [Arino et al., 1997; Sticher and Meier, 1980; Wolfender et al., 1997; Wolfender et al., 1993]. Additional methods, e.g. TLC

densitometry, micellar electrokinetic capillary chromatography, were used for the quantification of the bitter principles [Bodart et al., 1996; Glatz et al., 2000; Krupinska et al., 1991].
Essential oils are determined by steam distillation of gentian roots. After fractionation on a silica gel column, individual compounds were characterised by GC and GC-MS [Arberas et al., 1995; Chialva et al., 1986].

3.2 STANDARDIZATION, STABILITY

According to DAB 10 (Ph. Eur) the identity of gentian roots is proved by DC as described by Wagner and Vasirian (1974). Gentian tinctures and dry extracts are similarly analysed by DC detection of gentianin which emerges from gentiopicroside in alkaline media [DAB 10; Floss et al., 1964].
Dried gentian roots shall yield 30% of extract and have a bitter index of at least 10^3 according to DAB 10. DAB 10, ÖAB 90 and Helv VII require a bitter index of at least 10^2 and 30-40, respectively, for liquid preparations (tinctures, liquid extract). For dry extracts the bitter index ranges between 400-500 and at least 40,000, according to Helv II and ÖAB 90, respectively.
Studies regarding the stability of gentian extracts and the bitter principles, respectively, are scarce. Air-drying of the gentian roots resulted in considerable hydrolysis of gentiopicroside caused by native enzymes. The degradation could be reduced by gentiopicroside stabilisation using alcohol vapours [Beguin, 1929; Beguin, 1928; Beguine-Golovine, 1927], thus enabling to prepare stabilised extracts. The stabilising effect of alcohol on the bitter principle of *Gentianaceae* could be confirmed by Ishida et al. (1973) proving 6 months stability. The impact of drying was also studied by Carnat et al. (2005). Artificial drying at 40°C was found to minimise the loss of iridoids (gentiopicroside, longanic acid, swertiamarin) resulting in the retention of bitterness of the fresh gentian roots. In contrast, natural drying caused marked changes and might therefore affect the flavour of gentian roots.
Hayashi and Kosiro (1976) examined the stability of gentiopicroside and sweriamarin in 0.1 N $NaHCO_3$. In this study, both constituents were readily degraded and only 12% of gentiopicroside and 14% of swertiamarin were retained after 7 days.

4. PHARMACOLOGY

Gentian roots and other intensively bitter plants have been used for centuries by herbalists in Europe as digestive aids. Other folk uses included the topical application on skin tumours, treatment of fevers and diarrhoea. Nowadays, the Commission E and the British Herbal Compendium recognised the bitter substances contained in the herb, particularly amarogentin, the essential active principle. The bitter compounds stimulate gustatory receptors in the taste buds, causing a reflex promotion of saliva, gastric juice and bile secretion, thereby stimulating appetite [Hartke and Mutschler, 1987; Schmid, 1966]. Additionally, the ingredients of the gentian root are anti-inflammatory, antiseptic, emmenagogue, febrifuge and refrigerant.
The Commission E, the British Herbal Compendium and ESCOP approved the internal use of gentian root for digestive disorders, such as loss of appetite, flatulence, anorexia, atonic dyspepsia, gastrointestinal atony, and as a tonic and anti-emetic [Blumenthal et al., 1998; Bradley, 1992; ESCOP, 1997]. According to the German Standard Drug Authorisation digestive problems, such as insufficient production of gastric juice [Braun, 1997] are the accepted indication for infusions from gentian root. The external use or the use of the essential oils is not noted.

4.1 ANIMAL STUDIES

Gentiana lutea L. roots, particularly its bitter principles, caused an increasing secretion of saliva and digestive juices, thus alleviating digestive disorders. These pharmacological activities have been confirmed by animal studies. Gentian tincture orally administered to dogs in an experiment which prevented the tincture reaching the stomach, caused a 30% increase of gastric juice in comparison with untreated animals [Meier and Meier-Liebi, 1993]. Ethanol extracts of *Gentiana lutea* L. ssp. *symphandra* roots, containing 20.93% gentiopicroside, 5.27% swertiamarin and 2.55% swertioside, showed positive effects on bile production in rats. The bile

flow was decreased by CCl_4 treatment. A single dose of the lyophilised extracts (500mg/kg) administered intraduodenally normalised the bile flow compared to control rats [Oeztuerk et al., 1998]. Aqueous extracts of gentian directly stimulate acid production in cultured cells from rat gastric mucosa, causing a concentration-dependent 1.7-fold acid increase at 100 µg/mL [Gebhardt, 1997]. Gentian root extract perfused into the stomach of anaesthetised rats showed a dose-dependent increase of gastric secretion (12%, 27% and 37% higher than controls). At dosage of 0.5 mL/kg of the same gentian preparation the incidence of gastric ulceration in pyloric ligation tests in rats was not affected. Gentian root infusion orally administered to sheep at a daily dose of 5 g before feeding, produced a stimulant effect on secretion of enzymes in the small intestine [cited in: ESCOP, 2003]. Bronchosecretion in rabbits was elevated in comparison with control animals after administration of gentian root extract directly into the stomach over three days at a dosage equivalent of 12.6 mg/kg/day of dried root [Chibanguza et al., 1984].
Ozturk et al. (2002) investigated the effect of a methanol extract of *Gentiana lutea* L. ssp. *symphyandra* roots for its possible effect on the central nervous system of mice. They found that doses of 250 and 500 mg/kg (i.p.) of the methanol extract caused a significant increase in the swimming endurance test and exhibited slight analgesic activity, but no lethality in mice, suggesting some activity on the central nervous system. However, there was no evidence of sedation or muscular fatigue at the doses employed. The presence of gentiopicroside, swertiamarin and sweroside in the methanol extracts of *Gentiana lutea* L. roots, which were made responsible for the CNS, were proved by HPLC analyses.
Amarogentin showed induction of detoxification enzymes, which was accompanied by significant reduction in lipid peroxidation and inhibition of incidence as well as multiplicity of dimethylbenzanthracene (DMBA) induced papillomas. Purified amarogentin enriched extracts significantly inhibited cell proliferation and induced apoptosis, suggesting the chemopreventive potential of active constituents from *Gentianaceae* [Saha et al., 2004].
Gentiopicroside, assumed to be the major antispasmodic component, was shown to inhibit the spontaneous contractions of isolated guinea pig ileum. Contractions of the ileum induced by histamine, acetylcholine, $BaCl_2$ and KCl were also significantly blocked by this constituent which suggests that it might be interfering with calcium influx into the smooth muscle cells [Rojas et al., 2000]. Additionally, gentiopicroside treatment significantly suppressed the increase of the tumour necrosis factor (TNF), a major inflammatory mediator, in serum at the therapeutic doses (30-60 mg/kg/day for 5 consecutive days), suggesting that gentiopicroside protected against hepatitis by inhibiting the production of TNF [Kondo et al., 1994]

4.2 Human Studies

Gentian root tincture (2 x 20 drops/day) was administered for 8 days to patients with inflammatory conditions of the gastrointestinal tract, colitis ulcerosa, morbus Crohn and non-specific inflammatory disorders, all of whom had elevated secretory immunoglobulin (sIgA) levels (20-200 mg/dL) in their saliva. Additionally, a group of eight healthy individuals (3-25 mg/dL) was treated in the same way. With the exception of two patients, sIgA levels steadily decreased in both groups. [El-Sedawy et al., 1989].
Secretion of gastric juice in ten healthy individuals was stimulated after a single oral dose of alcoholic gentian root extract equivalent to 0.2 g of dried root. In the same experiment emptying of the gall bladder was increased and prolonged, which was interpreted as cholagogic effect [Glatzel and Hackenberg, 1967]. Chou et al. (2003) investigated a common herbal Chinese medicine, composed of Gentiana root, Scutellaria root, Gardenia fruit, Alisma rhizome and Bupleurum root, and its beneficial effect in patients with hepatocellular carcinoma. In a cytotoxicity test of the isolated major ingredients gentiopicroside had only little effect on induced cell apoptosis in Hep3B cells.
In an open study, 205 patients with various dyspeptic symptoms (heartburn, vomiting, stomach aches, nausea, loss of appetite, constipation, flatulence) were treated with a hydroethanolic dry extract (5:1) of gentian root at a dosage of 240 mg twice or three times daily (average daily dose 576 mg of extract equivalent to 2.9 g of dried root) for about 15 days. Improvements in symptoms were evident after 5 days in most cases, and by the end of the study the average level of improvement was 68%. Efficacy of the preparation was assessed by the doctors as

excellent (symptoms eliminated) in 31% of the patients, good in a further 55%, moderate in 9% and inadequate in 5% [ESCOP, 2003; Wegener, 1998].

4.3 ADME (ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION)

Only few studies regarding the absorption, distribution and metabolism of the bitter principle gentiopicroside were found. Its absorption and metabolism in rabbit blood for 24 h were monitored by Wang et al. (1994) observing a recovery of 99.2%.

The pharmacokinetics and tissue distribution of gentiopicroside was studied following oral and intravenous administration in mice fitted to a 2-compartments open model. The blood serum half-life of gentiopicroside was 6.1 h and 2.8 h for intravenous and oral administration, respectively. The T_{max} of gentiopicroside after oral administration was 0.50 h, and the bioavailability was 39.6%. The AUC (area under curve) gradient in individual tissues following intravenous administration was kidney >serum >liver >spleen >lung >thymus >fat >heart >muscle >stomach >intestinal >brain. The MRT (magnetic resonance tomography) gradient was muscle >serum >lung >spleen >lung >intestinal>heart >stomach >brain >liver >thymus >kidney >fat. Overall, the data show that gentiopicroside was absorbed rapidly in mice, but with a low bioavailability, and was extensively distributed to tissues, but was generally cleared quickly with short MRTs. The study demonstrated the need for repeated dosage, or better, a slow release formulation as an ideal means of administering gentiopicroside [Wang et al., 2004].

Metabolic conversion of gentiopicroside was demonstrated *in vitro*, using human intestinal bacteria. Gentiopicroside anaerobically incubated with *Veillonella parvula* ssp. *parvula*, produced five metabolites which were identified as erythrocentaurin, gentiopicral, 5-hydroxymethyl-isochroman-1-one, 5-hydroxmethyl-isochromen-1-one and trans-5,6-dihydro-5-hydroxymethyl-6-methyl-1 H, 3 H-pyrano[3,4-c]pyran-1-one [El-Sedawy, 1989].

Studies including excretion data could not be found.

5. MICROBIOLOGY

In a patent application the extract of *Gentiana lutea* L. obtained from roots, leaves and stems was shown to be useful as a drug in the treatment of bacterial infections including Gram-positive bacteria, particularly Clostridia, *Streptococcus*, *Staphylococcus aureus*. Its antimicrobial effect corresponded to that of ampicillin [Stierna et al., 2005]. The patented preparation is claimed to be particularly suitable in the treatment of infections of the upper and lower respiratory tracts, such as rhinosinusitis and bronchitis.

Additionally, several selective antifungal activities of gentian root extracts have been reported [Meier and Meier-Leibi, 1993].

6. EFFICACY

At the date of write no information available.

7. TOXICOLOGY

7.1 SAFETY

Gentian root extracts showed no toxicity and were generally well tolerated [Meier and Meier-Liebi, 1993]. The acute oral LD_{50} in mice of 25 mL/kg of gentian extract was the same as that of 30% ethanol [cited in: ESCOP 2003].

Dosage/Administration

Unless otherwise prescribed an average daily dosage of 1-3 g of tincture, 2-4 g of fluid extract and 2-4 g of roots, respectively. The comminuted drug and dried extracts are used for infusions; bitter-tasting forms of medications are used for oral administration.

Side effects/Adverse Reactions/Contradications/Precautions

People sensitive to bitter substances may experience headaches occasionally after using gentian root preparations. Mutagenic activity has been noted for gentian in animal studies and therefore, large dosages are not recommended. The European Council listed gentian root as a natural source of food flavouring (category N2). This category indicates that gentian can be added to foodstuffs in small quantities, with a possible limitation of an active principle (as yet unspecified) in the final product. Gentian root is contraindicated in cases of stomach and intestinal ulcers and also in cases of high blood pressure, although no rationale is given to this restriction. Gentian is also contraindicated during pregnancy and lactation, in view of the documented mutagenic activity of the herb and also due to an effect of the herb on the menstrual cycle.

Interactions with other drugs

None known.

US

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LAVANDULA SP.



Lavandula sp..

1. INTRODUCTION

Lavandula angustifolia, formerly known as *L. vera* or *L. officinalis,* (Common Lavender, English Lavender, French lavender, Garden Lavender, Lavender) is a flowering plant in the family *Lamiaceae*, native to the western Mediterranean region, primarily in the Pyrenees and other mountains in northern Spain and is cultivated extensively (Lis Balchin et al. 2002).
Other *Lavandula* species have similar ethnobotanical properties and major chemical constituents. The main species are: *Lavandula latifolia* (Spike Lavender), a Mediterranean grass-like lavender; *Lavandula stoechas*, which has butterfly-like bracts on top of the flowers and is sometimes known as Spanish Lavender; and *Lavandula intermedia* or *Lavandula hybrida*, which is a sterile cross between *L. latifolia* and *L. angustifolia* largely cultivated in many varieties in mediterranean countries and also outside Europe.
The medicinal parts are the essential oil from the fresh flowers and/or the inflorescence, the flowers collected just before opening and dried, the fresh flowers and the dried flowers. The primary active constituents are monoterpenes, expecially linalool and linalyl acetate. In Europe, Lavender is used in phytomedicine preparations for digestive and mild nervous disorders as an antispasmodic, carminative and mild tranquilizer. Lavender oil is used as a fragrance component in products such as antiseptic ointments, creams, lotions and jellies. All types of lavender products are used as a fragrance ingredients in cosmetics products, including soaps, detergents, creams, lotions and perfumes.

2. DESCRIPTION OF THE PLANT

Lavandula angustifolia is a strongly aromatic shrub growing up to 0,5 -0,8 m tall. The leaves are evergreen, 2-6 cm long and 4-6 mm broad. The flowers are pinkish-purple (lavender-coloured), produced on spikes 2-8 cm long at the top of slender leafless stems 10-30 cm long.

2.1 SYSTEMATICS

Lavender belongs to the family Lamiaceae (Labiatae), the Genus *Lavandula* comprises of more than 30 species of which only very few are commercially used: *Lavandula angustifolia* Mill. (syn.

L.vera, L.officinalis) , *L.latifolia* Medik., *L.hybrida* (*L.angustifolia x L.latifolia*), *L.dentata* Boiss. and *L.stoechas* Ging.

2.2 OCCURRENCE

The plant is indigenous to the mountainous regions of the countries bordering the western half of the Mediterranean, and cultivated extensively for its aromatic flowers in various parts of France, in Italy , Hungary, Bulgaria and in England - and even as far north as Norway. Outside Europe it is large scale cultivated in Australia (Tasmania), New Zealand and China.

2.3 PLANT PARTS USED AND PRODUCTS

'English lavender flower' consists of the dried flower of *Lavandula angustifolia*, gathered shortly before fully unfolding as well as its preparation. Flowering shoots are harvested when the middle section of the spike is flowering; it is cut 10 cm beneath the insertion of the spike.
The most common product is the essential oil being distilled from fresh or wilted flower spikes and stems of hybrid *Lavandula* called 'lavandin' and its varieties/cultivars.

3. INGREDIENTS/CONSTITUENTS (CHEM. ANALYSIS, STANDARDIZATION)

Lavender oil has been reported to contain more than 100 components.
The essential oil (1 to 3%) of *Lavandula angustifolia* is rich in linalool (30 to 55%) and linalyl acetate (20 to 35%). Further aroma components are β-ocimene, cineol, camphor and caryophyllene epoxide. Linalyl acetate is the major compound found in flowers (240 to 7200 ppm). The plant contains also rosmarinic acid (12000 ppm), and coumarin (1000 to 1500 ppm). The same compounds are found in the other *Lavandula* species: in *Lavandula latifolia* linalyl acetate (1-198 ppm), linalool (1490 - 5104 ppm), rosmarinic acid (7000 ppm), coumarin (22 ppm).

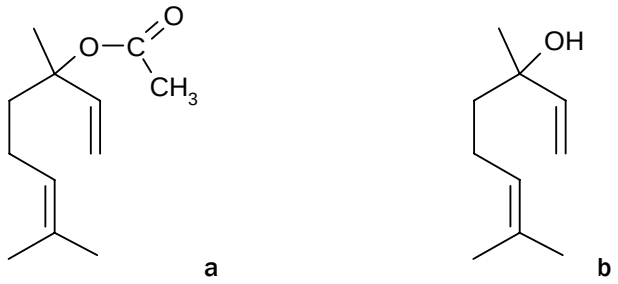

Figure 1. Chemical structure of linalyl acetate (a) and linalool (b).

3.1 CHEMICAL ANALYSIS: METHODS TO DETERMINATE THE COMPOUND

The major distinction among the three oils is in their relative contents of linalyl-acetate, linalool, 1,8-cineole and camphor. The linalool concentration is usually higher in Spike lavender oil than in English lavender oil. The amounts of linalool, linalyl-acetate, cineole and camphor in Lavandin oil are between those of the other two oils. The quantitative standards of *Lavandula angustifolia* (DAC 1986) are reported: volatile oil not less than 1,3%; foreign matter, entirely grayish brown or grey flowers not more than 10%; leaves and steams not more than 5%; other foreign matter, not more than 12%. Ash, not more than 7,0%.
Separation and determination of the components of *Lavandula* oil can be achieved by gas chromatography with mass spectrometry or infra-red spectrometry. According to Ph.Eur. 5 (2005) identity is tested by TLC and the purity by GLC

3.2 STANDARDISATION, STABILITY

International Standards specifications have been published for *Lavandula angustifolia* oil (ISO 3515:2002). *Lavandulae aetheroleum* is also included into the Ph.Eur. 5 (2005) with standard contents of 20 – 45% linalool, 25 – 46% linalyl acetate, terpinene-4-ol up to 6,0% and Cineol less than 2,5%. Lavender oil, stored in a cool and dark place, is stable up to six month.

4. PHARMACOLOGY

4.1 ANIMAL STUDIES

Spasmolytic effect

Oral administration of lavender has been shown to have antispasmodic effect on intestinal and uterine smooth muscle in animal studies. The mechanism of this action has not been fully explained, but it doesn't seem to be related to an action on adrenergic or cholinergic receptors, nor action at calcium and potassium channels. It has been observed that linalool and linalyl acetate can increase intracellular cAMP (Lis-Balchin andHart, 1999).

Antimutagenic activity

A concentration-dependent antimutagenic activity of *Lavandula angustifolia* oil has been found by the bacterial reverse mutation assay in *Salmonella typhimurium* TA98 and TA100 strains and in *Escherichia coli* WP2 uvrA strain (Evandri *et al.*, 2005).

Anticancer

Preliminary data from animal studies suggest that perillyl alcohol (the hydrogenated form of d-limonene) and other monoterpenes found in lavender can have an antineoplastic effect (Liston *et al.*, 2003). Studies on rats have demonstrated the regression of mammaty tumors after oral administration of limonene and perillyl alcohol (Haag and Gould, 1994). Perillyl alcohol has shown to inhibit the growth of pancreatic adenocarcinoma in hamsters (Burke *et al.*, 1997).

Anticholesterolhaemic effect

Cineole, a cyclic monoterpene found in lavender, lowers cholesterol in rats via inhibition of the HMG-CoA enzyme (Clegg *et al.*, 1982); the lavender constituent perillyl alcohol has been shown to inhibit the conversion of lathesterol to cholesterol (Ren and Gould, 1994).

4.2 HUMAN STUDIES

Lavender oil is widely used in aromatheraphy due to the sedative effects of the inhaled volatile compounds (Buchbauer *et al.*, 1991; Lewith *et al*., 2005). The mechanism of action is unknown but some authors have suggested that *Lavandula angustifolia* can have a similar action to the benzodiazepines and to enhance the effects of gammaminobutyric acid in the amygdale (Salah and Jager, 2005). It has been found that linalool inhibits acetylcholine release and alters ion channel function at the neuromuscular junction (Re *et al.*, 2000). Human trials dealing with human patients suggest that aromatherapy with lavender have a small positive effect in reliving anxiety (Cavanagh and Wilkinson, 2002).

In vitro and in vivo studies had shown that linalyl acetate and linalool have local anaesthetic activity following topical application (Ghelardini *et al.*, 1999; Barocelli *et al.*, 2004).

Internally, English Lavender is used for mood disturbances such as restlessness or insomnia and functional abdominal complaints (nervous stomach irritation, meteorism, nervous intestinal discomfort). Externally, English Lavender is used for treatment of functional circulatory disorders.

Lavandula is approved by the German Commission E monographs (BAnz no. 228, dated 05.12.1984) in following condition: nervousness and insomnia, loss of appetite, circulatory disorders, and dyspeptic complaints.

4.3 ADME

Lavender oil is quickly absorbed by the skin. The main constituents, linalool and linalyl acetate, are detectable in the blood five minutes after topical application, peak at 19 minutes, and largely disappear from the blood within 90 minutes (Jager *et al.*, 2002).

No data are available on kinetics following oral administration of lavender. The constituents limonene and perillyl alcohol (POH) are metabolized into perillic acid (PA) and dihydroperillic acid (DHPA) (Boon *et al.*, 2000). In rats fed a diet containing POH or limonene, peak levels of PA can be seen at 1-2.5 hours, peak levels of DHPA are noted at 2-3.5 hours, and half-lives for each metabolite are 1-2 hours (Haag and Gould, 1994). POH, PA, and DHPA are detectable in subjects' urine following high doses of POH ingestion. Approximately 9% of the total dose can be recovered in the first 24 hours. PA is the major metabolite found, with <1% of recovered POH.

The absorption of POH does not appear to be affected by concomitant ingestion of foods (Ripple *et al.*, 1998).

5. MICROBIOLOGY

An in vitro trial with a batch culture of rumen microrganisms the ethanol extract of *Lavandula angustifolia* have shown to increase the production of volatile fatty acids and to increase the fermentation yield up to 50% (Brodiscou *et al.*, 2000)

Antimicrobial properties

Lavender oil (primarily *L. angustifolia*) has been found to be active against many species of bacteria and fungi. For example, *L. angustifolia* oil was demonstrated to have in vitro activity against both MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant *Enterococcus faecalis*) at a concentration of less than 1% (Edward-Jones *et al.*, 2004). Antimicrobial effects of oil *Lavandula angustifolia* and Lavandula latifolia essential oils have been found against *Staphylococcus aureus*, *Salmonella Typhimurium*, and *Listeria monocytogenes* (Larrondo *et al.*, 1995; Dedalioglu and Evrendilek, 2004; Rota *et al.*, 2004).

Concerning the antifungal effect, *L. angustifolia* (1% and 10%) inhibited conidium germination and germtube growth of the fungus *Botrytis cinerea* (Antonov *et al.*, 1997), although conidial production of *Penicillium digitatum* was not affected by *L. angustifolia* at concentrations up to 1000 µg/mL (Daferera *et al.*, 2000). Lavender oil has shown both fungistatic and fungicidal activity against *Candida albicans* strains. At lower concentrations, it inhibits germ tube formation and hyphal elongation, indicating that it is effective against *C. albicans* dimorphism and may thus reduce fungal progression and the spread of infection in host tissues (D'Auria *et al.*, 2005).

Several papers have demonstrated that both linalool and *L. angustifolia* oil have ascaricidal and repellent activity (Perrucci *et al.*, 1994; Cavanagh and Wilkinson, 2002; Choi *et al.*, 2002; Traboulsi *et al.*, 2002, Pavela *et al.*, 2005).

6. EFFICACY

Lavandula angustifolia leaves, flowers and essential oil are used as flavouring agent for food preparations and beverages. No information is available about the quality of animal products following *Lavandula* administration.

7. TOXICOLOGY

Linalyl acetate is of very low acute toxicity to mammals, the acute oral LD50 is 13360 mg/kg body weight (bw) in mouse and 14550 mg/kg bw in rat (Taylor *et al.*, 1964). In a 12-week feeding study on 15 males and 15 females, using mixed esters, retardation of growth of female rats occurred at a level of 24.3 mg/kg/day. Linalyl acetate is an ester that is expected to be hydrolysed to linalool and acetic acid in the gastro-intestinal tract (FAO, 1967).

The main effects of the hydrolysis product, linalool, in a 28-day oral rat study were increased liver and kidney weight, thickened liver lobes and pale areas on the kidneys and in females only hepatocellular cytoplasmic vacuolisation. Other findings were related to local irritation of the gastro-intestinal tract. Based on the effects on liver and kidney a NOAEL of 160 mg/kg bw/day was derived for linalool (equivalent to 148 mg/kg bw/day linalyl acetate). In this study no effects on male and female gonads were found (OECD SIDS, 2002).

Linalool was tested in a reproduction screening test. The NOAEL for maternal toxicity based on clinical signs and effects on body weight and food consumption was 500 mg/kg bw/day for linalool (equivalent to 464 mg/kg bw/day linalyl acetate). The NOAEL on reproduction toxicity and developmental toxicity is 500 mg/kg bw/day (equivalent to 464 mg/kg bw/day linalyl acetate) based on the decreased litter size at birth and pup morbidity/mortality thereafter.

Linalyl acetate does not induce gene mutations or chromosomal effects in vitro (OECD SIDS, 2002).

7.1 SAFETY

No data in literature suggest possible adverse effects in animals due to continuative assumption of *Lavandula* spp. as flavouring agent in feed. In the United States of America *Lavandula* spp. has been included in the GRAS-list (generally considered as safe) by the FDA.
No safety concern at current levels of intake when used as a flavouring agent. An ADI of 0-0.5 mg/kg bw for citral, geranyl acetate, citronellol, linalool, and linalyl acetate, expressed as citral, has been established by JECFA. Linalyl acetate is commonly use ad food additive at a dose of 1-15 ppm.

Administration - Dosage

The essential oil of *Lavandula angustifolia* (*Lavandulae aetheroleum*) is used in veterinary medicinal products for topical use together with other plant extracts or essential oils for antiseptic and healing purposes. The product is used in horses, cattle, sheep, goats, rabbits and poultry. It is included in Annex II of Council Regulation (EEC) N. 2377/90 as a substance that does not need a MRL level (EMEA, 1999).
The essential oil is also used in human medicinal products to induce sleep and to treat functional disorders of the upper gastrointestinal tract at oral doses of 20 to 80 mg/person. The whole drug is used for infusions, as an extract and as a bath additive.

Interactions with other drugs

Due to its sedative properties, essential oil of Lavandula spp. can show interaction with other sedating drugs: in rats, concomitant use of lavender and pentobarbital or chloral hydrate has significantly increased sleeping time and narcotic effects (Gilani *et al.*, 2000). Lavender contains varying amounts of coumarins and may therefore theorically increase the effect of anticoagulant medications (Basch *et al.*, 2004).

DT/SG/LG/AT

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MACLEAYA/SANGUINARIA

Macleaya, Plume Poppy

1. INTRODUCTION

Macleaya cordata, *Sanguinaria canadensis* and *Chelidonium majus* have been used for centuries as alternative medicines. Currently the extracts from these medicinal plants are components of veterinary and human phytopreparations, and of oral-hygiene agents such as dentifrices mouthrinses and chewing gums. Sanguinarine (SA) and chelerythrine (CHE) are biologically active components of these extracts. They display distinct antibacterial and antiinflammatory properties, but, on the other hand, they have been reported as having adverse effects - genotoxicity and hepatotoxicity (Kosina et al., 2004).

Macleaya has sometimes been merged with neotropical *Bocconia* (Hutchinson, 1920), which differs in having perennial stems, long-stipitate ovaries, fleshy, single-seeded capsules dehiscing from the base, and much larger seeds.

Synonym: *Bocconia cordata (Willd.), Bocconia japonica,* Federmohn, Plume-poppy, tree-celandine

Varieties: *Macleaya microcarpa* = *Macleaya kewensis* Turrill, *Macleaya cordata* var. yedoensis, Family: *Papaveraceae*

The caryotype of *Sanguinaria canadensis* (x=9) shows that this species may constitute an independent tribe. *Macleaya* (x=10) is similar to *Bocconia* and differs from the other genera of subfamily (Safonova, 1994).

2. DESCRIPTION OF THE PLANT

Macleaya cordata is a fast growing deciduous perennial that grows to 2.0 m high by 1.0 m wide. Stems simple or branching distally, hollow, leafy. Leaves: 10-30 cm long and wide, alternate, petiolate; blade 1-2 subpalmately or pinnately lobed, margins irregularly and coarsely toothed; abaxial surface usually whitish and densely short-pubescent, adaxial glabrous. Plumelike inflorescences to 30 cm or more, terminal, paniculate, many-flowered; bracts present. Flowers: pedicels 4-10 mm, sepals 2, distinct, white to cream colored, spatulate, 5-10 mm; style to ca. 1 mm; petals absent; stamens 25-30; pistil 2-carpellate; ovary substipitate, 1-locular; style short; stigma 2-lobed. Capsules nodding, substipitate, 2-valved, dehiscing from apex. Seeds 4-6, dark brown, reticulate arillate (Chittendon, 1959), x = 10.

It is hardy to zone 3. It is in flower from July to August. The flowers are hermaphrodite (have both male and female organs).
The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil with a pH ranging from acid to alkaline and partial (light woodland) to full sun with moderate moisture (grassy places, open meadows and the grassy floors of Cryptomeria plantations (Phillips and Rix, 1991)). This plant might do well located in cultivated beds.
It might be found almost anywhere in temperate North America east of the Mississippi River at elevations below 1000 m. Native to temperate eastern Asia (China and Japan).

Sanguinaria canadensis L. is a perennial plant, one of the earliest and most beautiful spring flowers. It has a lovely white flower and produces only a single leaf and a flowering scape about 15 cm high. When the leaf first appears it is wrapped round the flower bud and is a greyish-green colour covered with a downy bloom. The leaves are palmately 5 to 9 lobed, the lobes either cleft at the apex or having a wavy margin, and are borne on leaf stems about 12 to 35 cm long. After flowering the leaves increase in size, the underside paler showing prominent veins. The flower measures about 2.5 cm across, is white, rather waxlike in appearance, with numerous golden-yellow stamens in the center. The petals soon fall off, and the oblong, narrow seed pod develops, attaining a length of about 2.5 cm. The rootstock is thick, round and fleshy, slightly curved at ends, and contains an orange-red juice, and is about 2 to 10 cm long, with orange-red rootlets. When dried it breaks with a short sharp fracture, little smell, taste bitter acrid and persistent, powdered root causes sneezing and irritation of the nose. The root is collected in the autumn, after leaves die down; it must be stored in a dry place or it quickly deteriorates.

3. INGREDIENTS / CONSTITUENT

Macleaya cordata contains several alkaloids: Allocryptopine, angoline, berberine, bocconine, bocconoline, chelerythrine, chelilutine, chelirubine, coptisine, cyptopine, dehydrocheilanthifoline, ethoxychelerythrine, ethoxysanguinarine, heleritrine, homochelidonine, macarpine, oxysanguinarine, protopine, protopine-N-oxide, sanguinarine (Duke, 1992; Kiryakov et al., 1967). Active Ingredients: Sanguinarine, chelerythrine.
It was shown that a suspension culture of Macleaya cordata accumulated benzo(c)phenanthridine alkaloids in the cell biomass. By the composition of the alkaloids and their content the cell culture markedly differed from the intact plant and callus tissue. The benzo(c)phenanthridine compounds in the cell culture were mainly represented by the reduced forms: dihydrosanquinarine and dihydrochelirubine. In the green plant they were mainly represented by the oxidized forms: sanquinarine and chelerythrine. The cell culture practically contained no chelerythrine characteristic of the intact plant (Fonin et al., 1995).

M. kewensis is recommended as source of raw materials for the manufacture of the drug Sanguinarine. The highest content of the alkaloids sanguinarine and chelerythrine was found in the leaves (Abizov et al., 2003).

In *Sanguinaria canadensis* and *Chelidonium ma*jus similar alkaloids have been found. The rhizomes also contain red resin and an abundance of starch.

3.1 CHEMICAL ANALYSIS

Sanguinarine forms colourless crystals. Chelerythrine is also colourless and crystalline. Protopine (also found in opium) is one of the most widely diffused of the opium alkaloids.
A high-performance liquid chromatographic method is described for the analysis of the benzophenanthridine alkaloid, sanguinarine, found in plant extracts. The method is demonstrated to be applicable to analyzing samples such as saliva and gingival crevicular fluid for sanguinarine following a simple acidified methanolic extraction step. The method utilizes an

ethyl silane column with acidic and basic ion-pairing reagents in the mobile phase with a limit of detection of 3 ng of sanguinarine in a sample (Reinhart et al., 1991).
Furthermore, a method for quantitative determination of sanguinarine is described, whereby the content of the alkaloid can be measured in the biomass of *M. cordata*. For this, the biomass is treated with ammonia, after which the alkaloids are extracted with chloroform and separated by TLC (standard silica plates), using a 25 : 25 : 3 (v/v/v) mixture of diethyl ether, petroleum ether, and methanol, respectively, as the solvent system. The extracts thus obtained are then subjected to spectrophotometry (Fonin et al., 1999).
Sanguinarine, the main compound of Sangrovit®, can be determined by HPLC-analysis in the feed, in the feed additive (Krause, 1996) and after oral application to pigs in the blood and faeces (Tschirner, 2004). Different procedures of extraction and systems of ion-pair chromatography were described.
Capillary electrophoresis was employed to determine the principal quaternary benzo(c)phenanthridine alkaloids, sanguinarine and chelerythrine, in plant extracts and an oral hygiene product. Phosphate-Tris buffer of pH 2.5 was used as a background electrolyte, limits of detection were 3 mumol L-1 (sanguinarine) and 2.4 mumol L-1 (chelerythrine) using UV detection at 270 nm. The method, which correlated well with HPLC, is suitable for serial determination of sanguinarine and chelerythrine in plant products and pharmaceuticals (Sevcik et al., 2000).
The metabolism of the phenolic 1-benzyltetrahydroisoquinoline alkaloids was studied in cell cultures of *Macleaya* and *Corydalis* species. The crude alkaloid fraction obtained from feeding experiments was investigated by application of the combined LC/NMR and LC/APCI-MS (/MS) techniques. Several metabolites were detected, and their structures were identified. Bioconversion of the phenolic 1-benzyltetrahydroisoquinoline into the pseudoprotoberberine was demonstrated. LC/APCI-MS and LC/CD experiments carried out on a chiral column permitted the deduction of the major enantiomeric form of the chiral metabolites. Thus, the combination of NMR, MS, and CD data permitted the structural elucidation and stereochemical analysis of the metabolites in the extract matrix solution, without isolation and sample purification prior to the coupling experiments (Iwasa et al., 2005).

3.2 STANDARDIZATION

Sangrovit® is produced from the rhizomes or herbs of bloodroot plant (*Sanquinaria canadensis*), *Chelidonium majus* or *Macleaya cordata* with a standardized amount of sanguinarine and recommended as feed additive for rearing pigs, broilers and dairy cattle (www.phytobiotics.com). It leads to a secretion of enzymes in the intestine, has bacteriostatic, mucolytic and anti-inflammatory effects, inhibits aminoacid degrading enzymes and enhances the availability of lysin and tryptophan (Lindermayer, 2005).

M. cordata extract (YIXIN HERBS TECH) mainly constains sanguinarine and chelerythrine. Specifications: Total Alkaloids 40%-60%.

4. PHARMACOLOGY

The whole plant of *Macleaya cordata* is analgesic, antioedemic, carminative, depurative and diuretic (Duke and Ayensu, 1985). The juice from the stems of the leaves is used to treat insect bites (Grieve, 1984). A decoction of the leaves and stems is used in the treatment of ringworm (Duke and Ayensu, 1985). The poisonous sap is used to counter poisonous sores (Duke and Ayensu, 1985).
Other Uses: The dried hollow stems can be used as whistles (Stuart, 1911; Stuart, 1979). *M. cordata* kills insects and mosquito larvae. The flowers are used to kill maggots whilst the whole plant is used to kill larvae and insects (Duke and Ayensu, 1985; Yuan and Zhang, 1999).

In isolated, isometrically contracting left guinea pig atria, sanguinarine, a benzophenanthridine alkaloid from the papaveracea Sanguinaria canadensis, produced a concentration-dependent

positive inotropic effect. Between 2.3 x 10(-6) M and 6.5 x 10(-5) M, sanguinarine increased contractility by 108% which was comparable to the maximal inotropic effect of ouabain. Within the same concentration range, sanguinarine caused inhibition of Na+,K+-ATPase isolated from guinea pig myocardium. 100% inhibition of Na+,K+,ATPase activity occurred at 1 x 10(-4) M sanguinarine. The I50 for enzyme inhibition and the ED50 for the inotropic action of sanguinarine were the same (6-6.5 x 10(-6) M) indicating that both effects may be causally related (Seifen et al., 1979).

Quarternary Benzophenanthridine alkaloids possess antiinflammatory activity (Lenfeld at al., 1981).

In assays carried out in vitro with rat liver postmitochondrial supernatant, sanguinarine and chelerythrine were found to possess an inhibitory effect on the activity of the alanine and aspartate aminotransferases. A more pronounced inhibitory effect on the activity of alanine aminotransferase was observed. The measurements of the concentration dependency of the inhibitory effects of sanguinarine, chelerythrine and nitidine revealed that alanine aminotransferase activity was inhibited only by alkaloids with substituents in position 9 and 10 of ring D. Sanguinarine was a more powerful inhibitor than chelerythrine. Nitidine, a structural isomer of chelerythrine had no inhibitory effect even when a tenfold concentration compared to that of sanguinarine and chelerythrine was used (Walterova et al., 1981).

Chelidonine, sanguinarine, and chelerythrine are natural benzophenanthridine alkaloids that inhibit taxol-mediated polymerization of rat brain tubulin in the micromolar range. Chelidonine is a weak, competitive inhibitor of colchicine binding to tubulin but does not inhibit podophyllotoxin binding. On the other hand, sanguinarine inhibits both colchicine and podophyllotoxin binding to tubulin with I50 values of 32 and 46 microM, respectively, and chelerythrine inhibits with I50 values of 55 and 60 microM, respectively. The inhibition by these two agents is of the mixed type. Tubulin forms an acid-reversible pseudobase with the imminium ion of sanguinarine, probably through several of its sulfhydryl groups, as shown by the loss of the yellow color of sanguinarine and its 596-nm fluorescence emission peak. Chelidonine, on the other hand, cannot undergo such pseudobase formation, and we conclude that it acts by a different mechanism. A number of previously described pharmacologic effects of these agents may be due to their inhibition of microtubule function (Wolff and Knipling, 1993).

The inhibitory activity of the quarternary benzophenanthridine alkaloid, sanguinarine (isolated from *M. cordata*), on rat brain glutamate decarboxylase (GAD) was studied in vitro. The Ki value for sanguinarine was 7 x 10-4 mol/L. The inhibition was irreversible and increased during the period of preincubation of sanguinarine with the GAD preparation. The results suggest that reaction of the iminium bond in the benzophenanthridine molecule with thiol groups of the enzyme participates in GAD inhibition. As GAD catalyses the rate-limiting step of GABA [gamma-amino butyric acid] synthesis, its inhibition by sanguinarine may contribute to its physiological action in vivo (Netopilova et al., 1996).

Starting with an extract derived from the stem of *M. cordata* (Papaveraceae) that was active in the process of inhibiting phorbol 12,13-dibutyrate binding to partially purified protein kinase C (PKC), the benzophenanthridine alkaloid angoline was isolated and identified. This discovery appeared in context, as a related benzophenanthridine alkaloid, chelerythrine, has been reported to mediate a variety of biological activities, including potent and selective inhibition of protein kinase C (PKC). However, angoline was not observed to function as a potent inhibitor of PKC. Moreover, the authors were unable to confirm the reported inhibitory activity of chelerythrine. In a comprehensive series of studies performed with various PKC isozymes derived from a variety of mammalian species, neither chelerythrine nor angoline inhibited activity with high potency. To the contrary, chelerythrine stimulated PKC activity in the cytosolic fractions of rat and mouse brain in concentrations up to 100 microM. In addition, chelerythrine and angoline did not inhibit [3H]phorbol 12,13-dibutyrate binding to the regulatory domain of PKC at concentrations up to 40 microg/ml, and no significant alteration of PKC-alpha, -beta, or -gamma translocation was observed with human leukemia (HL-60) cells in culture. Further, chelerythrine did not inhibit 12-O-tetradecanoylphorbol 13-acetate-induced ornithine decarboxylase activity with cultured mouse 308 cells, but angoline was active in this capacity with an IC50 value of 1.0 microg/mL. A relatively large number of biological responses have been reported in studies conducted with chelerythrine, and alteration of PKC activity has been

considered as a potential mechanism of action. In light of the current report, mechanisms independent of PKC inhibition should be considered as responsible for these effects (Lee et al., 1998).

The nuclear factor NF-kappaB is a pleiotropic transcription factor whose activation results in inflammation, viral replication, and growth modulation. Due to its role in pathogenesis, NF-kappaB is considered a key target for drug development. It was shown that sanguinarine (a benzophenanthridine alkaloid), a known anti-inflammatory agent, is a potent inhibitor of NF-kappaB activation. Treatment of human myeloid ML-1a cells with tumor necrosis factor rapidly activated NF-kappaB, this activation was completely suppressed by sanguinarine in a dose- and time-dependent manner. Sanguinarine did not inhibit the binding of NF-kappaB protein to the DNA but rather inhibited the pathway leading to NF-kappaB activation. The reversal of inhibitory effects of sanguinarine by reducing agents suggests a critical sulfhydryl group is involved in NF-kappaB activation. Sanguinarine blocked the tumor necrosis factor-induced phosphorylation and degradation of IkappaBalpha, an inhibitory subunit of NF-kappaB, and inhibited translocation of p65 subunit to the nucleus. As sanguinarine also inhibited NF-kappaB activation induced by interleukin-1, phorbol ester, and okadaic acid but not that activated by hydrogen peroxide or ceramide, the pathway leading to NF-kappaB activation is likely different for different inducers. Overall, it was demonstrated that sanguinarine is a potent suppressor of NF-kappaB activation and it acts at a step prior to IkappaBalpha phosphorylation (Chaturvedi et al., 1997). For further explanations on NF-kappaB see page 13.

The results of a pharmacodynamic study showed that *M. cordata* could improve liver function of acute hepatic injuries in rats caused by tetrachloromethan or galactosamine. *M. cordata* could lessen the level of serum LDH and mortality of rats, increase ratio of serum A/G, protect cellular membrane effectively and inhibit fibrosis in rats with chromic heptic injury caused by tetrachloromethane. *M. cordata* still enhanced the function of T and B lymphocytes (Yang et al., 1999).

M. cordata extract (YIXIN HERBS TECH) mainly contains sanguinarine and chelerythrine. It was shown to be a reversible inhibitor of the enzymatic hydrolysis of acetylthiocholine. Specifications: Total Alkaloids 40% - 60% (http://www.organic-herb.com).

The possible inhibitory action was investigated on the enzymatic hydrolysis of acetylthiocholine by human erythrocyte acetylcholinesterase of principal alkaloids isolated from *Chelidonium majus* L. and *Macleaya (Bocconia) cordata* and *M. microcarpa* (namely sanguinarine, chelidonine, berberine), and of drugs "Ukrain" (thiophosphoric acid derivative of a sum of the alkaloids isolated from *Chelidonium majus* L.) and "Sanguirythrine" (a mixture of unseparated closely related to benzo[c]phenanthridine alkaloids sanguinarine and chelerythrine, isolated from *Chelidonium majus* L. and other plants of Papaveraceae family). All agents under study have been shown to be reversible inhibitors of the enzymatic hydrolysis of acetylthiocholine. On the basis of the kinetic data it has been determined that chelidonine belonged to reversible inhibitors of a competitive type. All other examined agents have been demonstrated to be inhibitors of a mixed competitive-noncompetitive type, and a greater contribution to the inhibition was made by the competitive constituent. Among all examined agents berberine, sanguinarine and "Sanguirythrine" were the strongest inhibitors of this reaction (the values of generalized inhibitory constants being 0.23, 0.23 and 0.29 microM, respectively) and cheliodonine and "Ukrain" were much weaker (2.0 and 2.5 microM, respectively). Judging from the data obtained, sanguinarine and chelerythrine exert similar inhibitory effects on the reaction of enzymatic hydrolysis of acetylthiocholine, since sanguinarine and "Sanguirythrine" have nearly equal generalized inhibitory constants (Kuznetsova et al., 2001).

Chelerythrine, sanguinarine and an alkaloid extract from *M. cordata*, sanguiritrin, were found to be inhibitors of aminopeptidase A and dipeptidyl peptidase IV, while fagaronine inhibited dipeptidyl peptidase IV only. At 50 microM, chelerythrine, sanguinarine and sanguiritrin inhibited aminopeptidase N by 82%, 85%, 88%, DPP IV by 38%, 62%, 57%, and fagaronine by 34%, respectively. When bovine serum albumin (500 microg/mL) was added, the inhibition of both proteases by quaternary benzo[c]phenanthridine alkaloids (QBA) (50 microM) was significantly diminished. Strong interaction of chelerythrine and sanguinarine with bovine and human serum albumin was proved by electrophoretic determination of their respective conditional binding constants (Sedo et al., 2002).

The quarternary benzo(c)phenanthridine alkaloid extract from *M. cordata* and the alkaloids fagaronine, sanguinarine and chelerythrine exerted differential inhibitory effect on the hydrolytic activity of particular dipeptidyl peptidase-like enzyme isolated from human blood plasma and from human and rat glioma cell lines. The alkaloid inhibitory effect was concentration-dependent in the range 25-150muM and directly pH-related (Sedo et al., 2003).
In a further study it has been shown that the major alkaloids from plants *Chelidonium majus* L. and *Macleaya (Bocconia) cordata* and *M. microcarpa*, namely, berberine, sanguinarine, chelidonine, and drugs "Ukrain" (thiophosphoric acid derivative of a sum of the alkaloids isolated from *Ch. majus* L.) and "Sanguirythrine" (a mixture of the alkaloids sanguinarine and chelerythrine, w/w 3:7, isolated from *Macleaya*), are irreversible inhibitors of oxidative deamination reaction of serotonin and tyramine as substrates, catalyzed by rat liver mitochondrial monoamine oxidase (MAO). At the same time these substances do not influence the oxidative deamination reaction of benzylamine as substrate (in concentration 1 mM or less). The substrate specificity of this inhibition manifests that mainly the oxidative deamination reactions catalyzed by MAO form A are inhibited by the agents studied. Among the examined agents, alkaloid chelidonine and drug "Ukrain" are the strongest inhibitors of the reaction. Alkaloids berberine and sanguinarine and drug "Sanguirythrine" exhibit a weaker action. Judging from the data obtained, sanguinarine and chelerythrine appear to exert similar inhibitory effects in this reaction, since sanguinarine and "Sanguirythrine" have similar values of bimolecular rate constants of their interaction with mitochondrial MAO. As it is well known, the MAO inhibitors appear to be, as a rule, pronounced antidepressants. The combination of malignotoxicity and antidepressive activity in drug "Ukrain" seems to be favourable for its clinical applications (Iagodina et al., 2003).
250 mu M sanguinarine and 250 mu M chelerythrine caused complete inhibition of 1.9 nkat alpha-amylase from porcine pancreas and 23.9% and 7.5% inhibition, respectively, from human saliva using a biosensor method which utilises a flow system equipped with a peroxide electrode (Zajoncova et al., 2005).
Benzo(c)phenanthridine and diisoquinoline alkaloids isolated from *Chelidonium majus* L. and *Macleaya (Bocconia) cordata* and *M. microcarpa* (berberine, sanguinarine, chelidonine) and drugs “Ukrain” and “Sanguirythrine” inhibited the enzyme activity of acetylcholinesterase from human erythrocyte and monoamine oxidase from the rat liver. All agents have been shown to be reversible inhibitors of the enzymatic hydrolysis of acetylcholine. Chelidonine is a reversible inhibitor of a competitive type, all others are inhibitors of a mixed competitive-noncompetitive type. Berberine, sanguinarine and “Sanguirythrine” were stronger inhibitors and as chelidonine and “Ukrain”. All tested agents acted as irreversible inhibitors of the oxidative deamination reaction of serotonine and tyramine and did not influence the oxidative deamination reaction of benzylamine as a substrate (Kuznetsova et al., 2005).
To observe the cytotoxic effects in vitro and antitumor effects in vivo of total alkaloid of *M. cordata* a MTT assay was used to assess the proliferation of Hep3B and H22 cells in vitro treated with the alkaloid. The inhibitory effects of the alkaloid on H22 and S180 tumors were observed in mice with subcutaneous inoculation of the tumor cells. The total alkaloid significantly inhibited the proliferation of human Hep3B cells and murine H22 cells in a dose-dependency in vitro. IC(50) of the alkaloid in 3 repeated experiments was 3.04, 3.98 and 2.98 mug/mL respectively in Hep3B cells, and was 2.89, 2.21 and 2.34 mug/mL in H22 cells. In the tumor-bearing mice, the alkaloid inhibited the development of H22 tumor and prolonged the survival of S180 tumor-bearing mice. At the daily dose of 1, 2, and 4 mg/kg.b.w (i.p.) and 4 mg/kg.b.w. (i.g.) for 10 days, the inhibition rates of the alkaloid on H22 tumor in 3 repeated experiments were 18.6%, 35.1%, 44.9% and 7.9% respectively, and the rates of survival prolongation of the tumor-bearing mice were 24.8%, 48.9%, and 52.7%, respectively. The alkaloid possesses antitumor effects both in vitro and in vivo (Pang et al., 2005).
The quaternary benzo[c]phenanthridine alkaloids (QBA) produce a plethora of species- and tissue-specific effects but the molecular basis of their biological activities remain mysterious.

4.1 BIOAVAILABILITY

4.1.1 ANIMAL STUDIES

Pharmacokinetic studies of sanguinarine after oral application of Sangrovit® to pigs showed that the alkaloid was marginally absorbed out of the intestine and excreted for the most part unaltered in the faeces (Tschirner, 2004).
Adult rats were orally administered with a single dose of sanguinarine (10mg SA per kg body weight in 1 mL water). In the plasma and the liver, dihydrosanguinarine (DHSA) was identified as a metabolite of SA by high performance liquid chromatography-electrospray ionization mass spectometry (HPLC/ESI-MS). Significantly higher levels of DHSA were found in both the plasma and the liver in comparison with those of SA. SA and DHSA were not detected in the urine. The formation of DHSA might be the first step of SA detoxification in the organism and its subsequent elimination in phase II reactions. Benz(c)acridine, in the literature cited SA metabolite, was found neither in urine nor in plasma and liver (Psotova et al., 2006).

5. MICROBIOLOGY

5.1 ANTIMICROBIAL ACTIVITY

The colonization on rat molars of S. sobrinus OMZ 176, S. mutans OMZ 376, or a combination S. sanguis OMZ 9 and S. sobrinus OMZ 176 after a short exposition to various fluoride solutions and disinfectants was tested in vitro. The test solutions included Act, Candida, Veadent (sanguinaria extract), sodium fluoride, amine fluoride, stannous fluoride, zinc fluoride hexetidine, stannous fluoride/amine fluoride solutions, chlorhexidine and water. The sterilized rat molars were immersed in the above test solutions for 60 seconds, then incubated with streptococci in broth for 8 hours, again dipped into the same test solutions for 60 seconds and reincubated for an additional 30 hours. The streptococcal suspension contained 14C labelled sucrose solutions. The deposits on the molars were dissolved in 6N potassium hydroxide during 16 hours. Finally, the beta rays emitted by the dissolved radiolabelled suspension were counted using a scintillation counter. The sodium fluoride containing solutions exerted no or a very limited effect on the bacterial deposits. In contrast to the other test solutions, sanguinaria extract (Veadent mouth rinse) mildly inhibited the S. sobrinus and S. mutans deposits, but plaque formation by the combination of streptococci was not hampered. Zinc fluoride/hexetidine, amine fluoride and stannous fluoride/amine fluoride solutions had a distinct and significant inhibitory effect on S. sobrinus and S. mutans deposits, but only a weak effect when mixed cultures were used for plaque formation. Chlorhexidine significantly inhibited the deposits of the three bacterial strains used in these experiments (Altenhofen et al., 1989).
Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC values) for sanguinarine were determined for common and etiologically important plaque bacteria. Because the efficacy of sanguinarine is believed to be enhanced by zinc, isobolograms were assessed to determine their mode(s) of interaction. Hydrogen ion concentration influenced the inhibitory activity of both sanguinarine and zinc. For sanguinarine, at the optimum pH (6.5), MIC values were 4 or 8 micrograms/ml for Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguis, Actinomyces viscosus and Actinomyces naeslundii. MIC values were 0.125-0.50 mmol Zn/ml. MBC values ranged from 1 to 8 mmol Zn/ml at pH 5.5. Isobologram data revealed that sanguinarine and zinc interacted synergistically. Viadent oral rinse, which contained 300 micrograms sanguinaria extract/ml and 0.2% zinc chloride (14.9 mmol Zn/l), was inhibitory to all strains tested. MIC values were 1 or 2% (ml Viadent oral rinse/100 ml aqueous solution) for all strains except A. viscosus for which the MIC value was 12% (vol/vol) (Eisenberg et al., 1991).
Sanguinarine and chelerythrine display distinct antibacterial properties (Kosina et al., 2004).
Sanguinarine has bacteriostactic effects (Lindermayer, 2005).

6. EFFICACY

6.1 HUMAN STUDIES

The sap of *Macleaya* has been used in traditional Chinese medicine as an antiseptic for wounds (Grey-Wilson, 1993).

6.2 ANIMAL STUDIES

In a feeding trial the effect of 50 ppm extractives of *Sanguinaria canadensis* as growth promotor on pig performance was tested. Supplementation of sanguinarine showed a significant improvement of daily weight gain of 7.5% in the first period till 60kg average body weight and an improvement of 6.9% over the whole test period with a finishing weight of 95kg. Continuing the trial to a body weight of 106kg showed a difference of 4.5% for daily weight gain in comparison with the control group (Neufeld K., 1992).

Sangrovit®, a phytogenic feed additive is produced of dryed and grounded herbs of *Macleaya cordata, Sanguinaria canadensis* or *Chelidonium majus*. All plants contain alkaloids. The main active compound is sanguinarine. It leads to a secretion of enzymes in the intestine, has bacteriostatic, mucolytic and anti-inflammatory effects, inhibits aminoacid degrading enzymes and enhances the availability of lysin and tryptophan (Lindermayer, 2005).
Sangrovit®, derived from *Sanguinaria canadensis* and *Chelidonium majus*, was investigated for its properites as phytogenic feed additive concerning fattening efficiency and carcass performance in pigs. The daily weight gain of the pigs was found high (826g) but no influence on fattening data could be observed. Positive effects could be stated in the quality of the carcasses. Muscle meat and the thickness of the bacon were found improved (Seskeviciene et al., 2003).
Sangrovit®, derived from *Sanguinaria canadensis*, was tested on the performance of lactating pigs and weaners. Supplementation of 30 ppm Sangrovit® tended to results in an enhanced weight of new born pigs. Though the results of the aminoacid-analysis in the blood indicated that Sangrovit® causes a higher intermediar availability of the essential aminoacids Lys and Trp, in an N-bilanz-study no effect of the feed additive on the N-retention in growing pigs could be observed. Feed intake and daily weight gain of the pigs in the Sangrovit®group were found 3 % higher than in the controls ($p > 0.05$) (Tschirner, 2004).
Furthermore, the effects of daily administration of the extract from *Macleaya cordata* (2 mg and 100 mg in 1 kg feed, sanguinarine:chelerythrine 3:1) were evaluated in the diet on the health status of swine. After 90-day administration, alkaloids were retained to a different extent in tissues. The highest SA/CHE retention was detected in the gingiva (0.55 micro g/g) and liver (0.15 micro g/g), no SA/CHE were detected in muscles. Plasma SA levels attained 0.11 micro g/ml. Treated animals did not display any results of haematological, biochemical or histological assay different from controls. A 32P-postlabelling assay proved that no DNA-adducts with SA/CHE were detected in pig livers. Not any symptom was observed linked to epidemic dropsy syndrome often attributed to sanguinarine. In conclusion, an average daily oral dose of alkaloids up to 5 mg per 1 kg animal body weight was proved to be safe (Kosina et al., 2004).
In investigations with rearing pigs Sangrovit®, derived from *Macleaya cordata*, was tested as growth promotor. The weight gain was 2-4% enhanced compared with the controls. Feed consumption was reduced by about 4% (Lindermayer, 2005).

6.3 PREPARATIONS AND DOSAGES

Fluid extract of Sanguinaria, U.S.P., dose 1 1/2 minims. Tincture of Sanguinaria, U.S.P., 15 minims. Powdered root, 10 to 30 grains. Sanguinarin, 1/4 to 1 grain. Fluid extract, 10 to 30 drops (www.botanical.com).

7. TOXICOLOGY

The sap of *Macleaya cordata* is very poisonous (Stuart, 1979).

Sanguinarinchloride, mouse, i.v., LD_{50} (mg/kg LW): 15.9
Sanguinarinchloride, mouse, s.c., LD_{50} (mg/kg LW): 102.5
Sanguinarinchloride, rat, i.v., LD_{50} (mg/kg LW): 29.0
Sanguinarinchloride, rat, oral, LD_{50} (mg/kg LW): 1658.0
Sanguinaria-extract A, rat, oral, LD_{50} (mg/kg LW): 1440.0
Sanguinaria-extract B, rat, oral, LD_{50} (mg/kg LW): 1250.0
Sanguinaria-extract B, rabbit, dermal, LD_{50} (mg/kg LW): > 200
(Becci et al., 1987; Schwartz, 1986; Walterova et al., 1981).

Reproductive and developmental toxicology studies were conducted with orally administered sanguinaria extract in rats and rabbits. Groups of animals which received no test article served as controls in all studies reported. No adverse effects on estrous cycling, male or female copulatory and fertility indices or gestation/lactation parameters were observed in rats given 10-100 mg/kg/day. Reduced body weights in the offspring during lactation were observed at the 100 mg/kg/day treatment level concomitant with maternal toxicity. No developmental toxicity, including teratogenicity, was observed on the fetuses of rats following maternal administration of 5-60 mg/kg/day. An increase in post-implantation loss was observed at maternally toxic dosage levels of 50 and 75 mg/kg/day in rabbits. Oral administration of sanguinaria extract in a perinatal and postnatal study in rats caused no adverse effects on litter size, parturition, or lactation of female rats nor on survival and growth of their offspring at dosage levels of 5-60 mg/kg/day. Maternal oral toxicity thresholds were 60 mg/kg/day in rats and 25 mg/kg/day in rabbits. It was concluded that the oral intake of sanguinaria extract has no selective effect on fertility, reproduction or fetal and neonatal development in rats or rabbits (Keller and Meyer, 1989).

The effects of benzo[c]phenanthridine alkaloids sanguinarine (SA), chelerythrine (CHE), fagaronine (FA) and their dihydroderivates were tested on human leukocytes and lymphocytes, rat peritoneal mastocytes and primary cultured hepatocytes. The cytotoxicity of SA and CHE on hepatocytes is dose (35-100 microM) and time (1-3 h) dependent. Both alkaloids decrease chemiluminiscence of leukocytes and inhibit a creation of active E-rosets. The degranulation of mastocytes is inhibited only by CHE. Dihydroderivates of SA and CHE did not display any effect on studied cells, dihydrofagaronine exhibits a hepatotoxicity after 3 h (Vavreckova et al., 1994).

The hepatotoxic effects of sanguinarine and chelerythrine (isolated from *Macleaya cordata*) and fagaronine were examined in rats. Daily administration of sanguinarine or chelerythrine (10 mg/kg, i.p.) produced marked liver degeneration and necrosis not observed in fagaronine-treated rats. Degradation and/or necrosis were not observed following chronic administration of sanguinarine (daily dose of 0.2 mg/kg, i.p., for 56 days). The hepatotoxic effects of the alkaloids were tested against isolated rat hepatocytes. Sanguinarine was the most cytotoxic followed by chelerythrine; fagaronine exhibited no toxicity. Sanguinarine increased plasma membrane permeability; chelerythrine was less potent. Alkaloid treatment did not increase lipid peroxidation or damage to mitochondrial membranes (Ulrichova et al., 1996).

The antitumor activities of a synthetic benzo[c]phenanthridine, NK109 (7-hydroxy-2, 3-methylenedioxy-5-methyl-8-methoxybenzo[c]phenanthridinium hydrogensulfate dihydrate), and of natural benzo[c]phenanthridines were tested in vitro and in vivo. NK109 had the highest activity among them. NK109 is similar in structure to fagaronine and fagaridine; however, it has a phenolic-OH at C-7. NK109 exists as a resonance hybrid, the keto-amine and zwitterionic forms in neutral media. The resonance hybrid is cationic and has molecular planarity; these have been considered to be essential for the antitumor activity of the benzo[c]phenanthridinium salts. On the other hand, the structurally similar benzo[c]phenthridine alkaloids, chelerythrine and sanguinarine, exist as pseudobases under the same conditions. The latter do not exhibit antitumor activity in vivo, probably because they lose both the immonium region and molecular planarity. Thus, NK109 may be considered to be novel category of benzo[c]phenanthridinium salt from the viewpoint of its structure under biological conditions (Nakanishi et al., 1999).

In a further study the cytotoxicity of QBA alkaloids, sanguinarine (SA), chelerythrine (CHE), fagaronine (FA), and the extract from *Macleaya cordata* in primary cultures of human and porcine hepatocytes was investigated. The cellular damage was assessed by the MTT assay, lactate dehydrogenase (LDH) leakage and the determination of intracellular glutathione (GSH) levels. The results are summarised as follows: (i) The alkaloids tested in doses 0.1 and 10 microM did not display statistically significant cytotoxicity for 0-3 h incubation; (ii) SA and CHE showed the dose- and time-dependent toxicity within the range 25-100 microM whereas FA was not toxic; (iii) the LDH leakage into the medium was higher for SA than for CHE, thus revealing a potent potential of SA to disturb cell-membrane integrity; (iv) after 3 h incubation with 100 microM SA/CHE, mitochondrial dehydrogenase activity (MTT assay) and the cellular GSH levels decreased to residual values of about 40% suggesting that mitochondria are unlikely to be a primary target for SA/CHE in the cell; (v) no differences were found in the response to QBA application in human versus porcine hepatocyte (Ulrichova et al., 2001).

No toxic effects were observed in rats fed up to 150ppm sanguinarine in the diet for 14d and in rats treated by gavage with up to 0.6mg/kg body weight for 30d (Becci et al., 1987).
No toxic effects were found with a mixture of 60% sanguinarine and 30% chelerythrine at a dose of 2 and 100 ppm, respectively, (0.1 and 5 mg/kg LW) in the feed of pigs, which were observed for 90 days. For that reason an average daily oral dose of alkaloids up to 5 mg per 1 kg animal body weight is proved to be safe (Kosina et al., 2004).

7.1 SAFETY

All rats survived when sanguinarine was administered in the feed to the animals over 14 days in a dose of 15, 45 and 150 ppm, respectively (Becci et al, 1987).
In some studies a liver toxicity was observed, when high doses (10mg/kg LW/day, i.p.) were administered (Dalvi, 1985; Ulrichová et al., 1996; Williams et al., 2000). With small amounts of sanguinarine (0.2 mg/kg LW/day, i.p.) no liver toxic effects could be found even when administered chronically (for 56 days) (Ulrichová et al., 1996).
An Expert Panel on the safety on Sanguinaria extract used in Viadent oral rinse and toothpaste products stated that Sanguinaria extract is safe in its present use in Viadent products based on a large margin of safety between levels of human exposure and levels found to produce minimum effect or to be without adverse effect in animals. The panel further concluded that published literature suggesting an association between human exposure to Sanguinaria extract and potential reproductive, cardiovascular, or ocular toxicity, or carcinogenicity is largely anecdotal, unfounded, and not corroborated by or consistent with the substantial data base that was subjected to peer review (Frankos et al., 1990).

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9. EXPLANATION OF NUCLEAR FACTOR KAPPA B

Nuclear factor kappa B (NF-kB) is a transcription factor found in a great variety of immune cells that plays an important role in the inflammatory and immune response by regulating pro-inflammatory cytokines (interleukin-1, -2, -6, -8) and proinflammatory mediators (ICAM-1, iNOS and COX-2). NF-kB is activated by a protein known as IKKs. IkBa is a strong inhibitor of NF-kB but is rapidly degraded in response to a proinflammatory stimulus. This degradation allows the NF-kB to be free to translocate into the nucleus.

Recent breakthroughs in the transcription and translation of a variety of mediators have led to increased interest in therapeutic approaches directed at the level of gene transcription (e.g. COX2, iNOS, IL1beta, TNFalpha, ICAM, etc.). One of the most important mediators is NF-kappaB which plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as IL-6 and IL-8, cell adhesion molecules, such as ICAM and VCAM, and inducible nitric oxide synthase (iNOS). These Proinflammatory mediators are known to play a role in inflammation and in the case of iNOS, may lead to organ destruction in some inflammatory and autoimmune diseases.

The importance of NF-kappaB in inflammatory disorders is further strengthened by studies of airway inflammation including asthma, in which NF-kappaB has been shown to be activated. This activation may underlie the increased cytokine production and leukocyte infiltration. Recent findings show that glucocorticoid also works by inhibition of NFkappaB. Further evidence for a role of NF-kappaB in inflammatory disorders comes from studies of rheumatoid synovium. Although NF-kappaB is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF-kappaB is present in the nuclei, and hence active, in the cells comprising rheumatoid synovium. Furthermore, NF-kappaB has been shown to be activated in human synovial cells in response to stimulation with TNF-alpha. This signifies increased cytokine and eicosanoid production. NF-kappaB is also likely to play a key role in neoplastic transformations, which are seen in 20-25% of certain human lymphoid tumors. Sodium salicylate and its semi-synthetic derivative, aspirin, and a number of new natural products of different chemical classes have demonstrated NF-kB inhibitory activity. Recently, an important mechanism of the inhibition of NF-kappaB suggests the possible activation of the receptors for peroxisomes.

9.1 RESEARCH ON NFKB

9.1.1 Inhibition of Nuclear Factor Kappa B (NFkB): An Emerging Theme in Anti-Inflammatory Therapies. Fulvio D'Acquisto, PhD, Michael J. May, PhD and Sankar Ghosh, PhD. Section of Immunobiology and Department of Molecular Biophysics and Biochemistry Howard Hughes Medical Institute Yale University School of Medicine New Haven, CT 06510. Molecular Interventions 2:22-35 (2002)

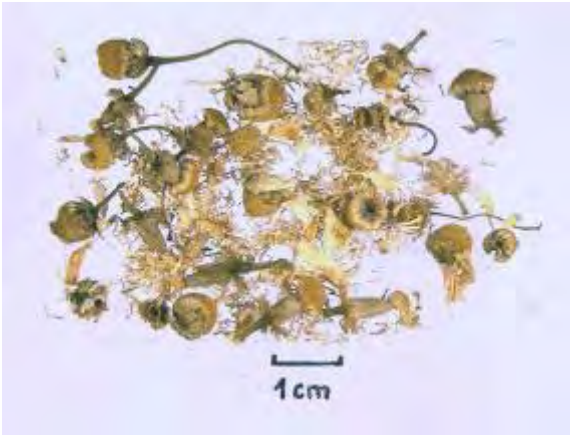
The application of anti-inflammatory therapies began thousands of years ago with the use of readily available natural resources. It is only recently, however, that the cellular and molecular mechanisms of inflammation have been appreciated sufficiently to design anti-inflammatory strategies with limited side effects. For example, salicylates and glucocorticoids, two widely used anti-inflammatory drug classes, are now known to inhibit the activation of NF- B, a transcription factor that regulates the inducible expression of a wide range of proinflammatory mediators. New generations of NF- B–targeting anti-inflammatory agents that are specific, efficacious, and cost-effective may therefore complement or replace current therapies. In this review, we describe various classes of NF- B inhibitors and discuss important unresolved issues regarding their use.

9.1.2 Andrographolide attenuates inflammation by inhibition of NF-kappa B activation through covalent modification of reduced cysteine 62 of p50. Xia YF, Ye BQ, Li YD, Wang JG, He XJ,Lin X, Yao X, Ma D, Slungaard A, Hebbel RP, Key NS, Geng JG. J Immunol. 2004 Sep 15;173(6):4207-17.

NF-kappaB is a central transcriptional factor and a pleiotropic regulator of many genes involved in immunological responses. During the screening of a plant extract library of traditional Chinese herbal medicines, we found that NF-kappaB activity was potently inhibited by andrographolide (Andro), an abundant component of the plant Andrographis that has been commonly used as a folk remedy for alleviation of inflammatory disorders in Asia for millennia. Mechanistically, it formed a covalent adduct with reduced cysteine (62) of p50, thus blocking the binding of NF-kappaB oligonucleotide to nuclear proteins. Andro suppressed the activation of NF-kappaB in stimulated endothelial cells, which reduced the expression of cell adhesion molecule E-selectin and prevented E-selectin-mediated leukocyte adhesion under flow. It also abrogated the cytokine- and endotoxin- induced peritoneal deposition of neutrophils, attenuated septic shock, and prevented allergic lung inflammation in vivo. Notably, it had no suppressive effect on IkappaBalpha degradation, growth rates. Our results thus reveal a unique pharmacological mechanism of Andro's protective anti-inflammatory actions.

9.1.3 Nuclear factor-kappaB: the enemy within. Aggarwal BB. Cytokine Research Laboratory, Department of Experimental Therapeutics, Unit 143, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA. aggarwal@mdanderson.org Cancer Cell. 2004 Sep;6(3):203-8. Numerous lines of investigation suggest that nuclear factor NF-kappaB, a proinflammatory transcription factor, could promote tumorigenesis. Various inflammatory agents, carcinogens, tumor promoters, and the tumor microenvironment activate NF-kappaB. NF-kappaB proteins themselves and proteins regulated by it have been linked to cellular transformation, proliferation, apoptosis suppression, invasion, angiogenesis, and metastasis. Constitutively activated NF-kappaB is common in wide variety of tumors. Furthermore, there exists genetic evidence that NF-kappaB mediates tumorigenesis. Thus, suppression of NF-kappaB activation should be effective in the prevention and treatment of cancer.

MATRICARIA RECUTITA



Matricaria recutita L

1. INTRODUCTION

Chamomile flowers belong to those drugs that experienced a wide range of medical applications in ancient times. The curative effect of chamomile has been known by physicians since 2500 years. Hippocrates described chamomile as a medicinal plant, and Galen and Asclepios recommended the use of infusions prepared from chamomile flowers. Furthermore, Mathiolus propagated the use of essential oil from chamomile as a antispasmodic. Today, chamomile ranks among the most important medicinal plants and has therefore already been monographed in many modern pharmacopoeias. However, studies regarding its application in animal nutrition are scarce.
Synonyms are *Matricaria chamomilla L., Chamomilla recutita (L.)* Rauschert

2. DESCRIPTION OF THE PLANT

True chamomile is an annual plant with thin spindle-shaped roots only penetrating flatly into the soil. The branched stem is erect, heavily ramified and grows to a height of 10-80 cm. The long and narrow leaves are bi- to tripinnate. The flower heads are placed separately, they have a diameter of 10 to 30 mm, and they are pedunculate and heterogamous. The golden yellow tubular florets with five teeth are 1.5 to 2.5 mm long, ending always in a glandulous tube. The 11 to 27 white plant flowers are 6 to 11 mm long and 3.5 mm wide and arranged concentrically. The receptacle is 6 to 8 mm wide, flat in the beginning and conical, cone-shaped later, hollow - the latter being a very important distinctive characteristic of *Matricaria* - and without paleae. The fruit is a yellowish brown achene.
True chamomile is very often confused with plants of the genera *Anthemis.* Special attention has to be paid to confusions with *Anthemis cotula* L. a poisonous plant with a revolting smell. In contrast to true chamomile *A. cotula* just as *A. arvensis* L. and *A. austriaca* Jacq. has setiform, prickly pointed paleae and a filled receptacle. Latter species are nearly odorless [Franke, 2005].

2.1 SYSTEMATICS

Although the systematic status is quite clear nowadays, there are a number of inaccuracies concerning the names. Apart from misdeterminations and confusion, the synonymous use of the names *Anthemis, Chamomilla,* and *Matricaria* leads to uncertainty with regard to the botanical identification. Moreover, the nomenclature is complicated by the fact that Linneaus made mistakes in the first edition of his ´Species Plantarum´ that he corrected lateron.
The best known botanical name for true chamomile is *Matricaria recutita* (syn*. Matricaria chamomilla*, *Chamomilla recutita* (L.) Rauschert, belonging to the genus Chamomilla S. F. Gray).

2.2 Occurrence

Indigenous to southern and eastern Europe and western Asia chamomile is nowadays found throughout found in Europe, North America, and also Australia. The commercial product is mainly cultivated in Argentina, Chile, Egypt (not always of pharmacopoeial quality, often for the food industry), Bulgaria, Slovakia, Hungary, and to a small extent from Spain, Czech Republic, and Germany [Franke et al., 2005].

2.3 Plant Parts and Products

Chamomile is widely cultivated and used all over the world. Interest in these drugs extends far beyond their role as medical plant but also for their great importance in food and cosmetic industries. Generally, extracts, the drug itself and tea mixtures were used for the pharmaceutical industry and foodstuff sector, whereas cosmetic industries preferred the essential oils. Therefore, different specifications are required depending on their field of applications [Franke, 2005].

Chamomile flowers – Matricariae flos

The flowers are usually used medicinally. Since the quality demands are arranged by the pharmacopoeias of the different countries, they can vary in a wide range. Pharmaceutical chamomile includes complete flowers, sometimes fallen apart to a very small extend with little amounts of fines.

Chamomile fines

The fines contain the seeds, the petals and the tubular flowers, which are separated during the mechanical cleaning-up procedure. Depending on the essential oil contents, the fines are used for pharmaceutical and foodstuff purposes.

Chamomile herb with flowers

The material includes flowers with higher amounts of stems due to mechanically harvesting. Therefore, quality parameters usually do not confirm with pharmacopoeias´ requirements, but can easily be used as food teas.

Chamomile herb

After harvest the flowering Chamomile herb is minced and dried without any further cleaning-up or processing.

Chamomile for extraction, Industrial Chamomile

This material includes chamomile flowers, which quality standards are in accordance with the pharmacopoeia. Because of the mechanical harvest and its preparation, ist external structure is in such a condition that this material is not suitable as pharmaceutical chamomile. From this quality medicinally used extracts are produced. Because of their high oil content flower heads fallen apart should not be regarded as worthless. If used in tea mixtures/blends a limitation of the fines is, however, justified due to the tendency of showing inhomogeneities.

Chamomile root

The roots are used for pharmaceuticals of the anthroposophical therapy. The essential oil differ considerably from the aerial parts [Reichling *et al.,* 1984].

Chamomile oil, *Matricariae aetheroleum*

The oil is produced by steam distillation of fresh or dried flower heads including the stems of chamomile flowers. The essential oil from fresh plant material results in higher yield and a better quality.

3. INGREDIENTS/CONSTITUENTS

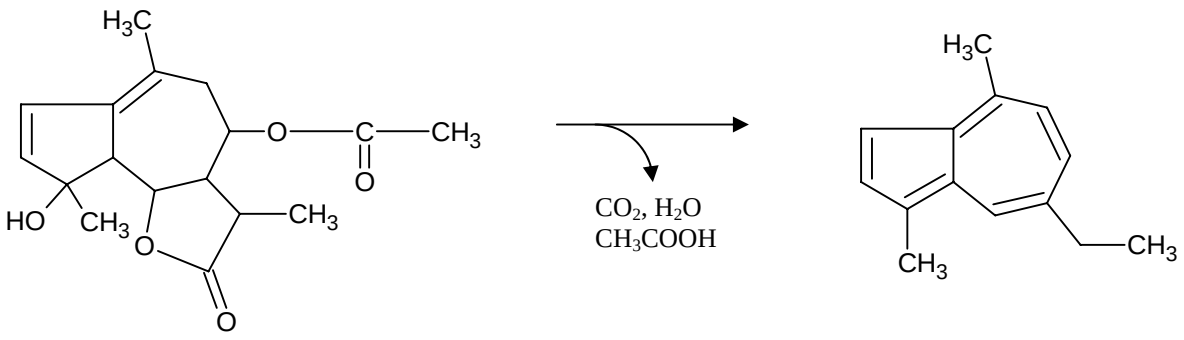
Chamomile contains a large number of therapeutically interesting and active compound classes. The most important ones are the components of the essential oil and the flavonoid fraction. Apart from them, the following compound classes were detected and characterized: mucins, coumarins, phenol carboxylic acids, amino acids, phytosterols, choline, and mineral substances. The chamomile constituents are classified in a lipophilic fraction containing individual components of the essential oil, coumarins, methoxylated flavone aglyca, phytosterols, lipidic and waxy substances, and a hydrophilic fraction including flavonoids, mucilage, phenyl carboxylic acids, amino acids, and choline.
The essential oil comprising the lipophilic compounds is present in the roots and in the flowers, whereas the latter are the main plants part for essential oil production. Furthermore, the composition of the essential oil in roots differs from that in flowers. Thus, therapeutic relevant compounds like chamazulene and bisabolol are absent in the roots. The oil content changes during ontogenesis, reaching a maximum of 0.3-1.5% in flowers just before full blossom [Franz 1981; Schilcher, 1985]. The approximate ratio of the main components in the essential oil is as follows:

- Up to 15% chamazulene and its precursor matricin [Stahl, 1954a-c]
- Up to 50% bisabolols and bisabolol oxides [Franz 1981; Schilcher, 1985]
- Up to 45% trans-ß-farnesene [Carle *et al.,* 1987; Schilcher, 1985]
- Up to 25% cis- and trans-isomers of the spiroethers [Schilcher, 1985]

The individual lipophilic and hydrophilic components in chamomile are described in the following sections.

Matricin/Chamazulene

Matricin is the precursore of chamazulene and present in ligulate florets and tubular florets of chamomile, but not in the bottom of the flower heads. Chamazulene is formed from matricin by the elemination of water and acetic acid and following decarboxylation. The structure of matricin and chamazulene is given in Figure 4.1..

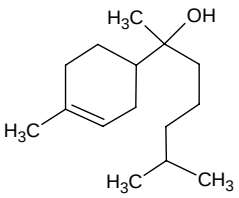


Matricin Chamazulene

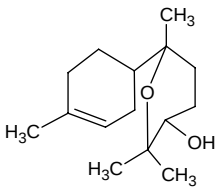
Figure 4.1. Structure of matricin and chamazulene

Bisabolols and bisabolol oxides

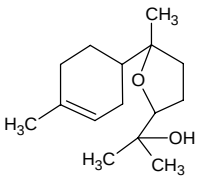
(-)-α-bisabolol, bisabololoxid A, B and bisabolonoxid A are important constituents of chamomile oil. Bisabololoxide C was only found in small quantities in flowers and is synthetically obtained from (-)-α-bisabolol by oxidation. The structures of (-)-α-bisabolol and its oxidation products are given in Figure 4.2. Depending on the main constituent of the essential oil (-)-α -bisabolol, bisabololoxid A, bisabololoxid B and bisabolonoxid A the species can be classified in four different chemotypes [Franz 1981;Schilcher, 1973]. With regard to the significantly higher pharmacological activity of (-)-α-bisabolol, only the first chemotype should be used for pharmaceutical products.



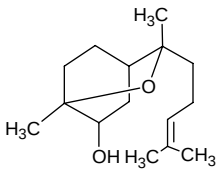
Bisabolol



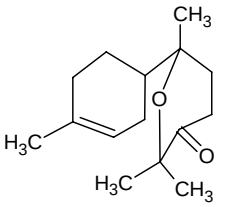
Bisabolol oxide A



Bisabolol oxide B



Bisabolol oxide C



(-)-α-Bisabolone oxide A

Figure 4.2. Bisabolol and its oxidation products

Trans-ß-farnesene

Beside trans-ß-farnesene, which can represent up to 45% of the essential oil, trans-α-farnesene was present only in traces [Lembercovics, 1979].

Spiroethers

Two spirocyclic polyines, the isomeric cis and trans enyne dicycloether, were found in chamomile flowers [Bohlmann *et al.,* 1961]. The cis spiroether is the major component in most plant specimens. The trans spiroether was predominant only in certain commercially available chamomile flowers [Schilcher, 1985].

Coumarins

Both the 7-methoxy-coumarin herniarin and the 7-hydroxy-coumarin umbelliferone are of pharmacological interest. In chamomile flowers from different origins a range of 37.4-98.5mg/100g of herniarin and 6-17.8mg/100g of umbelliferone were determined. Both were detected in ligulate and tubular florets. The average content in ligulate florets was significantly higher [Schilcher, 1985, 1987].

Flavonoids

Flavonoids represent the major fraction of the water-soluble components in chamomile. The most abundant derivatives thereof are apigenin 7-glucoside and its acetylated derivatives, as well as luteolin, quercetin and their glycosides. Beside hydroxylated aglyca, methoxylated flavonoids like chrysosplenol, jaceidin, chrysoeriol, patuletin, spinacetin, 6-methoxykaempferol, axillarin, chrysosplenetin, eupatoletin and eupalitin were identified in chamomile flowers [Carle and Isaac, 1985].

3.1 Chemical Analysis

Quality control methods for chamomile flowers and preparations thereof are contained in Ph.Eur. 5. The essential oil is extracted by steam distillation (Ph. Eur. 5) or dichlormethane (quantitative determination of single components) and analyzed by GC, headspace GC and TLC, respectively [Schilcher, 1987; Wagner and Bladt, 1986; Reichling *et al.,* 1979; Carle *et al.,* 1987; Hölzl *et al.,* 1975; Stuppner *et al.,* 1993].

The analysis of flavonoids is usually performed by extraction and subsequent identification and quantification by TLC and HPLC coupled to mass spectrometry. Adulterations of chamomile oil with (-)-α-bisabolol from other sources were detected using stable isotope and ^{2}H-NMR methods [Hörhammer *et al.,* 1963; Kunde and Isaac, 1979; Reichling *et al.,* 1979; Wagner and Bladt, 1986; Redaelli *et al.,* 1981; Dölle *et al.,* 1985; Carle *et al.,* 1993; Miething and Holz, 1989; Schmidt and Ness, 1993; Schmidt and Vogel, 1992; Tschiersch and Hölzl, 1992; Carle *et al.,* 1990; Carle *et al.*, 1992; Carle *et al.,* 1993].

The solvent system applied for the extraction of the essential oil can be used for the separation of the coumarins. Identification and quantification is performed by HPLC [Schilcher, 1985].

3.2 Standardisation, Stability

Matricin is very unstable and decomposes visibly to chamazulene by turning blue after a short time, particularly in aqueous solution [Schmidt *et al.,* 1991].

4. Pharmacology

Chamomile and its preparations are mainly used because of their anti-inflammatory, spasmolytic, and carminative activity. Moreover, chamomile showed bacteriostatic and fungistatic properties. The data given below are based on the ESCOP monograph (2003).

4.1 In vitro *Experiments*

Anti-inflammatory effects

Ethanolic (48% V/V) and isopropanolic (48% V/V) extracts of *Matricaria* flower inhibited 5-lipoxygenase, cyclooxygenase and the oxidation of arachidonic acid with IC_{50} values of 0.05-0.3%, while a supercritical carbon dioxide extract had an IC_{50} of 6-25 µg/mL for these activities. Investigations of individual constituents revealed that apigenin inhibited 5- and 12-lipoxygenase (IC_{50}: 8 and 90 µM respectively); chamazulene and (-)-α-bisabolol inhibited only 5-lipoxygenase (IC_{50}: 13 and 40 µM respectively); apigenin, *cis*-spiroether and (-)-α-bisabolol inhibited cyclooxygenase (IC_{50}: 70-80µM); only chamazulene showed antioxidative activity (IC_{50}: 2 µM) [Ammon and Sabieraj, 1996].

Trans-spiroether inhibited the provoked degranulation of rat mast cells in concentrations above 0.1 mM [Miller et al., 1996].

Apigenin markedly inhibited the transcriptional activation of cyclooxygenase (IC_{50}: 8.7 µM) and of nitric oxide synthase (IC_{50}: 3.1 µM) in lipopolysaccaride-activated macrophages [Liang et al., 1999].

Spasmolytic effect

The Chamomile flavonoids especially the aglyca are responsible for the spasmolytic effects, whereas musculotropic effects were stronger than the neurotopic ones [Hörhammer, 1961; Hörhammer and Wagner, 1962; Hörhammer *et al.,* 1963a; Salfner, 1963]. Extracts, in particular alcoholic extracts obtained from ligulate flowers (in contrast to tubular florets) seemed to exert stronger activity. Beside flavonoids, components of the essential oil were assumed to contribute to the spasmolytic effects. (-)-α-bisabolol, (+)-α-bisabolol and to a lower amount their oxides, the chamomile oil itself, *cis*-spiroether, a standardised hydroalcoholic extract (Kamillosan®) and the coumarin derivatives umbelliferone and herniarin showed spasmolytic activity. The hydrophilic (flavonoids) and the lipophilic components (essential oil) contributed to the musculotropic antispasmodic effect [Achterrath-Tuckermann *et al.,* 1980; Carle and Gomaa, 1992].

Sedative effects

Apigenin competitively inhibited the binding of flunitrazepam to the central benzodiazepine receptor (K_i = 4 µM) but had no effect on muscarinic receptors, α_1-adrenoreceptors or on the binding of muscimol to $GABA_A$ receptors [Viola et al., 1995].
HPLC fractions of a methanolic extract of *Matricaria* flower were able to displace flunitrazepam from its receptors in rat cerebellar membranes, the ligand Ro 5-4864 from 'peripheral' benzodiazepine receptors in rat adrenal gland membranes and muscimol from GABA receptors in rat cortical membranes. This last activity is mainly due to GABA present in the fractions [Avallone et al., 1996].
Apigenin inhibited the binding of Ro 15-1788, a specific ligand for central benzodiazepine receptors with an IC_{50} of 0.25 mM. Apigenin also reduced GABA-acticated Cl^- currents on cultured cerebellar granule cells dose-dependently by 15 ± 3% (0.1d µM apigenin), 24 ± 2% (1 µM apigenin) and 32 ± 4% (10 µM apigenin). This effect was blocked by coapplication of Ro 15-1788 [Avallone et al., 2000].

4.2 Animal Studies

Anti-inflammatory effects

The anti-inflammatory principles of chamomile were identified as chamazulene, matricin, chazulene carboxylic acid, guiazulene, (-)-α-bisabolol and the bisabololoxide [Pommer, 1942; Nöcker and Schleusing, 1958; Kristen and Schmidt, 1957; Roeckerath, 1950; Isaac, 1979; Goeters, 2001]. However, bisabololoxide show a lower activity than (-)-α-bisabolol [Jakovlev *et al.,* 1979]. The cis-enynedicycloether have less antiphlogistic activity as compared to the components mentioned above, but show edema inhibition in rats [Breinlich and Scharnagel, 1968; Verzár-Petri *et al.,* 1979]. Since these compounds are chemically unstable, their contribution to the total activity may be low, but their participation is apparent in certain test models. Additionally, the flavonoids provide antiphlogistic activity, which was examined recently [Baumann *et al.,* 1980; Carle and Gomaa, 1992; Della Loggia, 1985; Della Loggia *et al.,* 1986]. In particular, apigenin and luteolin exert an antioedematous effect similar to that induced by the non-steroidal anti-inflammatory drug indomethacin.

Anti-ulcerogenic effect

The development of ulcers induced in rats by indometacin, stress or ethanol was inhibited by an orally administered extract of *Matricaria* flower with an ED_{50} of 1 mL per rat and by (-)-α-bisabolol with an ED_{50} of 3.4 mg/kg body weight. These substances also reduced healing times for ulcers induced in rats by chemical stress (acetic acid) or heat coagulation [Szelenyi et al., 1979].

Wound healing effect

The wound healing activity of azulene has been demonstrated in studies on the thermally damaged rat tail and of *Matriacaria* flower constituents in accelerated healing of experimental injuries [Deininger, 1956; Zita, 1955].

Sedative effects

A sedative effect of *Matricaria* flower was demonstrated trough prolongation of hexobarbital-induced sleep, reduction of spontaneous mobility and reduction of explorative activity in mice [Dell Loggia et al., 1981; 1982].
Restriction stress-induced increases in plasma ACTH levels in normal and ovariectomised rats were decreased by administration of diazepam and inhalation of *Matricaria* flower oil vapour. Inhaling the vapour induced greater decreases in plasma ACTH levels in ovariectomised rats then treatment with diazepam; this difference was not observed in normal rats. Furthermore, the inhalation of *Matricaria* flower oil vapour induced a decrease in plasma ACTH level that was blocked by pre-treatment with fumazenil, a potent and specific benzodiazepine receptor antagonist [Yamada et al., 1996].
Apigenin (25 and 50 mg/kg) significantly reduced the time of latency ($p<0.05$) in the onset of picrotoxin-induced (6 and 8 mg/kg) convulsions [Avallone et al., 2000]. Apigenin also reduced locomotor activity after interperitoneal injections in rats (minimal effective dose: 25 mg/kg) but showed no anxiolytic, myorelaxant or anti-convulsant activity [Zanoli et al., 2000].

4.3 Human Studies

Anti-inflammatory effects

In a comparative open study involving 20 healthy volunteers with chemically-induced toxic dermatitis, the smoothing effect on the skin of an ointment containing *Matricaria* flower extract was significantly superior ($p<0.01$) to that of 0.1% hydrocortisone acetate or the ointment base [Nissen et al., 1988].
In an open study on 12 healthy subjects, a cream containing *Matricaria* flower extract (20mg/g) did not suppress UV-induced erythema but it reduced visual scores of skin redness in the adhesive tape stripping test ($p=0.0625$) [Korting et al., 1993]. In an analogous study, the cream produced 69% of the effect of a hydrocortisone-27-acetate ointment [Albring et al., 1983].
In a randomized, double-blind study, 25 healthy volunteers with UVB light-induced erythema were treated with various *Matricaria* flower preparations, hydrocortisone cream or the respective vehicle. Ranking the preparations according the visual assessment scores and mean values from chromametry, a cream containing a hydroalcoholic extract of *Matricaria* flower gave the best result [Kerscher, 1992].
In a bilateral comparative study 161 patients with inflammatory dermatoses, who had been treated initially with 0.1% of diflucortolone valerate, were treated during maintenance therapy with a cream containing *Matricaria* flower extract or one of three alternatives: 0.25% hydrocortisone, 0.75% fluocortin butyl ester of 5% bufexamac. The therapeutic results with the extract were quivalent to those of hydrocortisone and superior to those of fluocortin butyl ester and bufexamac [Aertgeerts et al., 1985].
In an open study involving 98 cancer patients, a *Matricaria* flower extract preparations containing 50 mg of α-bisabolol and 150-300 mg of apigenin 7-glucoside per 100 g , applied three times daily, reduced oral mucositis caused by localized irradiation or systemic chemotherapy [Carl, 1994].
In a phase III double-blind, placebo-controlled study involving 164 patients, mouth-wash containing *Matricaria* flower extract did not decrease 5-fluorouracil-induced stomatits [Fidler et al., 1996].
In a randomized, partially double-blind, comparison study, 72 patients with medium-degree atopic eczema were treated with a cream containing a *Matricaria* flower extract, of a 0.5% hydrocortisone cream or a placebo cream. After 2 weeks of treatment the *Matricaria* cream proved superior to the hydrocortisone creame and marginally superior to the placebo cream

with respect to the symptoms pruritus, erythema and desquamation [Patzelt-Wenczler and Pone-Pöschl, 2000].

Anti-inflammatory and antispasmodic effects

In an open multicentric study, 104 patients with gastrointestinal complaints such as gastritis, flatulence or minor spasms of the stomach were treated orally for 6 weeks with *Matricaria* flower extract preparation (standardised to 50 mg of α-bisabolol and 150-300 mg apigenin 7-glucoside per 100 g) at a daily dose of 5 mL. Subjectively evaluated symptoms improved in all patients and disappeared in 44.2% of patients [Stiegelmeyer, 1978].

Wound healing effects

In an open study, 147 female patients episistomized during childbirth were treated for 6 weeks with either an ointment containing *Matricaria* flower extract or a 5% dexpanthenol cream. The healng effect of the two preparations was comparable [Kaltenbach, 1991].
In a randomised, open placebo-controlled study on 14 patients, weeping dermatoses following dermabrasion of tattoos were treated topically with a *Matricaria* flower fluid extract preparations (standardised to 50 mg of α-bisabolol and 3 mg of chamazulene per 100 g). After 14 days the decrease in weeping wound area and the improvement in drying tendency were significant in the *Matricaria* flower group ($p<0.05$) [Glowania et al., 1987]. In another study with 120 patients with second degree haemorrhoids were treated with rubber band ligature alone, rubber band ligature with anal dilator and vaseline, or rubber band ligature with anal dilator and an ointment containing *Matricaria* flower extract. The last group showed the best results in amelioration of haemorrhage, itching, burning and oozing [Förster et al., 1996].

4.4 ADME (Absorption, Distribution, Metabolism and Excretion)

After cutaneous administration of [^{14}C](-)-α-bisabolol on mice, 82% of the radioactivity was found in the urine [Hölz and Hahn, 1985; Hahn and Hölzl, 1987].
Apigenin and luteolin are also readily absorbed by the skin. Skin preparation studies using hydroethanolic solutions of apigenin and luteolin on the upper arms of 9 healthy female volunteers gave steady state fluxes of 10.31 ng/min/cm² and 6.11 ng/min/cm² respectively [Merfort et al.,1994]. After oral administration of apigenin 7-glucoside to rats, free apigenin was detected in the urine [Griffiths and Smith, 1972].
After oral administration of 40 mL of a hydroethanolic *Matricaria* flower extract (containing 225.5 mg of apigenin 7-glucoside, 22.5 mg of apigenin and 15.1 mg of herniarin per 100 mL) to a female volunteer, no flavones could be detected in blood plasma nor in 24-hour urine, while herniarin was found in both (maximum plasma concentration of ca. 35 ng/mL; 0.324 mg in 24-hour-urine) [Tschiersch and Hölzl, 1993].
In germ-free rats no hydolysis of flavone glycosides could be observed; obviously intestinal microflora can effect the cleavage of the glycosidic bonds [Griffiths and Smith, 1972, Griffiths and Barrow, 1972]. Furthermore, orally administred Apigenin was detected in the blood serum of animals [Redaelli et al., 1980].

5. Microbiology

Antibacterial and antimycotic effects

The essential oil showed bacteriostatic and bactericidal activity, including gram positive, gram negative bacteria and fungi [Aggag and Yousef, 1972; Cinco *et al.,* 1983; Schilcher, 1987]. Again, (-)-α-bisabolol exhibited strongest antibacterial, fungistatic and fungicidal activity compared with the oxides and enyne dicycloethers. Chamazulen was shown to have also fungistatic activity, but at higher concentrations [Szabo-Szalontai and Verzár-Petri, 1976]. In a recent study has observed chamomile oil extracts inhibited the production of urease of Helicobacter pylori. This enzyme is known to be very important for the survival of this

microorganism under acid condition. Therefore, chamomile oil extract is supposed to inhibit the adhesion of H. pylori and is promising for the application in complex therapy of stomach ulcer and duodenal intestine [Shikov *et al.,* 1999].

6. EFFICACY

Kraszewki (2003) studied herbal mixtures, including chamomile, due to their efficiency in the nutrition of high-yielding cows. The milk from the cows was characterised by significantly better technological suitability for processing into hard cheese. Herb feeding also exerted a beneficial influence on the composition of fatty acids in milk, decreasing the amount of saturated acids and increasing that of unsaturated acids, including mono- and polyunsaturated acids that have a hypocholesterolemic effect. In contrast to this study, El-Nor, S. A. H. Abo, Fathy, F., Abdou, M. M. A. (2004) could not observe significant changes in the fatty acid profile, but significant increase in fat, total solids, total proteins, lactose and ash contents in milk from those cows getting herb supplemented feed (ratio of Chamomile 15mg/kg cow). This made milk more valuable in terms of cheese production and human nutrition.
Feeding a herbal mixture, containing chamomile flowers was effective in promoting weight gain compensation in weaned pigs suffering from runting-stunting syndrome [Kolacz *et al.,* 1997].
Ground chamomile flowers (50 mg/rat/day – 100 mg/rat/day) and the essential oil (0.2-0.35%) stimulated liver regeneration in partially hepatectomised rats when added to the diet [Gershbein, 1977].
Furthermore, aqueous extracts (decoctions, infusions and macerates) of dried flower heads of chamomile were tested *in vitro* against the mite *Psoroptes cuniculi* Delafond, responsible for otoacariosis in domestic animals. All extracts tested showed highly significant acaricidal activity when compared to the controls [Macchioni *et al.,* 2004]

7. TOXICOLOGY

Chamomile oil has been granted the GRAS status in the Unites States, and is admitted for foods and cosmetics by the FDA. The LD_{50} of chamomile oil exceeds 5g/kg weight for oral toxicity in rats and acute dermal toxicity in rabbits [Moreno, 1973; Opdyke, 1974]
The acute toxicity of orally administered (-)-α-bisabolol in mice and rats was very low with an LD_{50} of about 15 mg/kg weight. Subacute toxicity studies showed lowest toxic dose of (-)-α-bisabolol to be between 1 and 2 ml/kg oral for rats and dogs. Oral doses up to 1 ml/kg of (-)-α-bisabolol produced no discernible effects on the prenatal development of rats or rabbits. None of the tested dosages caused any deformations [Habersang *et al.,* 1979].

7.1 SAFETY

DOSAGE/ADMINSTRATION

Undesirable effects were observed only at high doses. Doses of 12.6-15.9 ml/kg of (-)-α-bisabolol caused retching and vomiting. Considering prenatal development of rats and white New Zealand rabbits intolerance was only observed with doses that were toxic for the adult animal by itself (approx. 3.0ml/kg) [Habersang *et al.,* 1979].
Topical exposure of guinea pigs to extracts from the bisabolol oxide B-chemotype of *Ch. recutita* was found to cause a moderate allergenic reaction. However, evidence was given that chamomile allergy is due to anthecotulid, a linear sesquiterpene lactone, which is detectable in this chemical strain at variable but low levels [Hausen *et al.,* 1984].

Side effects/Adverse Reactions/Contraindications/Precautions

Rare cases of contact allergy have been reported in persons with known allergy to *Artemisia* species [Schilcher, 1987].

Interactions with other drugs

None.

US/KS

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MENTHA SP

Mentha x piperita

1. INTRODUCTION

The true **mints** (genus *Mentha*) are perennial herbs in the Family Lamiaceae. There are about 25-30 species in the genus, seven from Australia, one in North America, and the others from Europe and Asia; several hybrids also occur. In the following list, the most important species and hybrids are in **bold types.**

Selected species

Mentha aquatica – Water mint, or Marsh mint, ***Mentha arvensis,*** **Corn Mint, Wild Mint and Japanese Peppermint,** *Mentha asiatica, Mentha australis, Mentha canadensis* (syn. *M. arvensis* var. *canadensis*), *Mentha cervina, Mentha citrata* (syn. *M. odorata*) – Bergamot mint (smells like Bergamot) *Mentha crispata, Mentha cunninghamii, Mentha dahurica , Mentha diemenica, Mentha gattefossei, Mentha grandiflora, Mentha haplocalyx, Mentha japonica*, *Mentha kopetdaghensis , Mentha laxiflora Mentha longifolia*, *Mentha micrantha , Mentha microphylla*, ***Mentha pulegium* – Pennyroyal,** *Mentha requienii* – Corsican mint, *Mentha sachalinensis Mentha satureioides****Mentha spicata*** **– Spearmint, Curly mint,** *Mentha suaveolens* (syn. *M. rotundifolia*) – Apple mint (smells like apples) and Pineapple mint (a variegated cultivar of Apple mint), *Mentha sylvestris* – Horsemint, Wild mint *Mentha vagans*

Selected hybrids

Mentha × *dalmatica* [= *M. arvensis* × *M. longifolia*], *Mentha* × *dumetorum* [= *M. aquatica* × *M. longifolia*], *Mentha* × *gracilis* [= *M. arvensis* × *M. spicata*], *Mentha* × *maximilianea* [= *M. aquatica* × *M. suaveolens*], *Mentha* × *muelleriana* [= *M. arvensis* × *M. suaveolens*], ***Mentha* × *piperita* [= *M. aquatica* × *M. spicata*] – Peppermint, Chocolate mint**, *Mentha* × *rotundifolia* [= *M. longifolia* × *M. suaveolens*], *Mentha* × *verticillata* [= *M. arvensis* × *M. aquatica*], *Mentha* × *villosa* [= *M. spicata* × *M. suaveolens*]

(http://en.wikipedia.org/wiki/Mentha)

The genus name *Mentha* is from the Greek *Mintha,* the name of a mythical nymph who metamorphosed into this plant; its species name *piperita* is from the Latin *piper,* meaning pepper, alluding to its aromatic and pungent taste (Tyler et al., 1988). Mint leaves have been used in medicine for several thousand years, according to records from the Greek, Roman, and ancient Egyptian eras (Briggs, 1993; Evans, 1991). The origin of peppermint cultivation is disputed, though there is some evidence that it was cultivated in ancient Egypt. Roman naturalist Pliny the Elder (ca. 23–79 C.E.) wrote of its uses by the Greeks and Romans. Peppermint was first recognized as a distinct species by botanist John Ray in his *Synopsis Stirpium Britannicorum* (second edition, 1696), and his *Historia Plantarum* (1704). It became official in the *London Pharmacopoeia* in 1721 (Briggs, 1993; Grieve, 1979; Tyler et al., 1988). Today, peppermint leaf and/or its oil are official in the national pharmacopeias of Austria, France, Germany, Great Britain, Hungary, Russia, and Switzerland, and the *European Pharmacopoeia* (Ph.Eur. 5*,* 2005; BP, 1988; Bradley, 1992; DAB 10*,* 1991; AB*,* 1981; Ph.Fr.X, 1990; Ph.Helv.VII*,* 1987; Ph.Hg.VII*,* 1986; USSR X*,* 1973; Wichtl and Bisset, 1994).

In Germany, peppermint leaf is one of the most economically important individual herbs, demonstrated by the fact that in 1993 nearly four thousand tons were imported, and in 1994 almost five thousand tons. It is also one of Germany's own most important medicinal plant crops (Lange and Schippmann, 1997). It is licensed as a standard medicinal tea, is official in the *German Pharmacopoeia,* and approved in the Commission E monographs (leaf and oil). It is used as a monopreparation and also as a component of many cholagogue, bile-duct, gastrointestinal, and liver remedies, and some hypnotic/sedative drugs (BAnz*,* 1998; Bradley, 1992; Braun et al., 1997; DAB 10*,* 1991; Wichtl and Bisset, 1994). In German pediatric medicine, peppermint leaf (67%) is combined with chamomile flower (33%) as an herbal tea to treat gastric upset in children. It is also used as a component of various "kidney and bladder" teas for children. Peppermint oil is used as a component of *Inhalatio composita* (45% eucalyptus oil, 45% pumilio pine oil, 10% peppermint oil) specifically indicated for coryza and nasal catarrh in children (Schilcher, 1997). In the United States, peppermint leaf is used singly and as a main component of a wide range of digestive, common cold, and decongestant dietary supplement and OTC drug products, in fluid and solid dosage forms. Peppermint leaf and peppermint oil are official in the U.S. *National Formulary.* Peppermint oil is used in the United States as a carminative in antacids, a counterirritant in topical analgesics, an antipruritic in sunburn creams, a decongestant in inhalants and lozenges, and as an antiseptic or flavoring agent in mouthwashes, gums, and toothpastes (Briggs, 1993; Leung and Foster, 1996; Tyler et al., 1988).

The historical use of peppermint is not dramatically different than its use in modern herbal medicine. Classified as a carminative herb, peppermint has been used as a general digestive aid and employed in the treatment of indigestion and intestinal colic by herbalists.

Most modern human studies have investigated peppermint oil rather than peppermint leaf as a treatment for stomachache (May et al., 1996), spastic colon syndrome (Somerville et al., 1984), postoperative nausea (Tate, 1997), relief of colonic muscle spasm during barium enema (Sparks et al., 1995), irritable bowel syndrome (Carling et al., 1989; Dew et al., 1984; Fern ndez, 1990; Koch, 1998; Lawson et al., 1988; Lech et al., 1988; Liu et al., 1997; Nash et al., 1986; Pittler and Ernst, 1998; Rees et al., 1979), prevention of abdominal distension in postoperative gynecological patients (Feng, 1997), and headaches (Gobel et al., 1994; Gobel et al., 1996). The use of peppermint oil for irritable bowel syndrome is based on preparations in enteric-coated capsules, causing a spasmolytic activity on smooth muscles of the gut. In animal tests, the probable mechanism of action has been shown to be the inhibition of smooth muscle contractions by blocking calcium influx into muscle cells (Forster et al., 1980; Giachetti et al., 1988).

Pharmacopeial grade peppermint leaf must be composed of the dried whole or cut leaf with not more than 5% stem fragments greater than 1 mm in diameter and not more than 10% leaves with brown spots caused by *Puccinia menthae.* The whole leaf must contain not less than 1.2% (ml/g) and the cut leaf must contain not less than 0.9% volatile oil. Botanical identity must be confirmed by macroscopic and microscopic examinations and organoleptic evaluation (Ph.Eur.3*,* 1997; Wichtl

and Bisset, 1994). The ESCOP peppermint leaf monograph requires that the material comply with the *European Pharmacopoeia* (ESCOP 2003, Ph.Eur. 5, 2005).

The herb **Pennyroyal** (*Mentha pulegium*, family Lamiaceae), is also a member of the mint genus; an essential oil extracted from it is used in aromatherapy. Pennyroyal has a traditional folk medicine use in inducing abortions and is an abortifacient. These oils are high in pulegone, a highly toxic volatile, which can stimulate uterine activity. The oil of Mentha pulegium is also used as a flea repellant for pets. This latter usage is the origin of the plant's Latin species name, the flea being *Pulex irritans* (http://en.wikipedia.org/wiki/Pennyroyal)

Pulegone is a naturally occurring organic compound obtained from the essential oil of Pennyroyal. It is a clear colorless oily liquid with pennyroyal odor. It is classified as a monoterpene and has the molecular formula $C_{10}H_{16}O$. Synonyms for pulegone are *R*-(+)-5-methyl-2-(1-methylethylidene)cyclohexanone, p-menth-4(8)-en-3-one and delta-4(8)-p-menthen-3-one.

2. DESCRIPTION OF THE PLANT

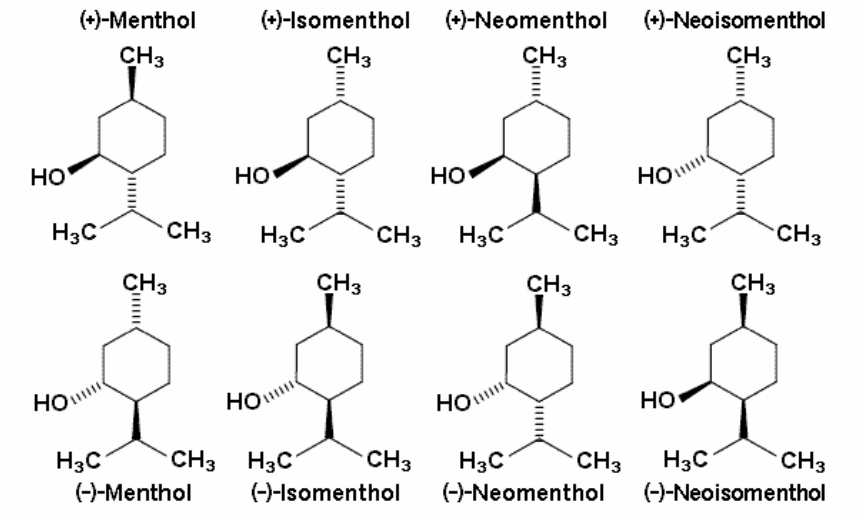
Mentha x piperita is a perennial herb, which is 30-90 cm high. Stems are square, erect or ascending, branched and the upper portion is always quadrangular.
Leaves are arranged opposite to each other and are petiolate, ovate-oblong to oblong-lanceolate, serrate and pointed. The upper surface of the leaves is dark green.
The flowers of *Mentha x piperita* are purplish and occur in thick, terminal, spicoid racemes of verticillasters. Each flower shows a tubular calyx with 5 sharp, hairy teeth, a purplish, irregular, 4-cleft corolla, 4 short stamens, a 4-celled ovary and a projecting style ending in a bifid stigma.
The fruit consists of 4 ellipsoidal nutlets.
Plant material of interest are the dried leaves and the essential oil. The leaves are whole, broken or cut.

Nowadays, the therapeutic indication for *Menthae piperitae folium* is the symptomatic treatment of digestive disorders such as dyspepsia, flatulence and gastritis. The internal use of the essential oil of Menthae piperitae is also the symptomatic treatment of digestive disorders, such as flatulence. But it is also used in the treatment of irritable bowel syndrome and the symptomatic treatment of coughs and colds. Menthae piperitae aetheroleum is also used in external treatments, such as the relief of coughs and colds, the symptomatic relief of rheumatic complaints and tension-type headache, pruritus, urticaria and pain in irritable skin conditions. (ESCOP)

Pennyroyal (*Mentha pulegium*) is a perennial mint with a variable habit, ranging from low-growing, spreading plants to lanky, upright subshrubs. The pale or deeper pink, blue, or violet flowers are clustered in dense whorls at the upper nodes. The plant has a powerful and pungent minty odor. The stems are square in cross-section, ascending from rhizomes. Branches and simple leaves are opposite on stems. Unlike many mints, the flowers are not strongly bilateral, but only slightly two-lipped. (California Invasive Plant Council database: http://ucce.ucdavis.edu/datastore/detailreport)

3. INGREDIENTS / CONSTITUENT

The main active compound of the leaves is the essential oil (1-3%), of which the principal contituent is usually menthol, in the form of (-)-menthol (usually 35-55%) with smaller amounts of stereoisomers such as (+)-neomenthol (ca. 3%) and (+)-isomenthol (ca. 3%), together with menthone (10-35%) menthyl acetate, menthofuran, cineole, limonene and other monoterpenes. The structure of Menthol and the other seven stereoisomers:



Over 100 components have been identified in the oil including numerous other monoterpenes and small amounts of sesquiterpenes, notably viridoflorol (ca. 0,5%), which is characteristic of oil from *Mentha x piperita*.
To comply with the European Pharmacopoeia the oil must contain menthol (30-55%), menthone (14-32%), isomenthone (1,5-10%), menthyl acetate (2,8-10%), menthofuran (1-9%), cineole (3,5-14%), limonene (1-5%), not more than 4% of pulegone and not more than 1% of carvone, with a ratio of cineole content to limonene content greater than 2.
Various flavonoids are present in the leaves including luteolin and its 7-glycoside, rutin, hesperidin, eriocitrin and highly oxygenated flavones. Other constituents include phenolic acids and small amounts of triterpenes. (ESCOP)

Peppermint oil (Ph Eur) contains maximum 4.0 % pulegone and between 1.0 and 9.0 % menthofuran. Mint oil, partly dementholised (Ph Eur) contains maximum 2.0 % pulegone, but no limit is given for menthofuran. There is no Ph Eur monograph on pennyroyal or pennyroyal oil. It is known from the literature that pennyroyal herb contains 1-2% essential oil of which pulegone is the principal component (60-90%) (Barnes et al., 2002). (EMEA, 2005)

Other mints may contain rather different constituents: *Mentha pulegium* (pennyroyal) contains 80% pulegone, and *M. crispa* (crispate mint) contains 50% carvone. Another famed cultivar, spearmint, owes its aroma to carvone, limonene, dihydrocarvone, menthone, pulegone, 1,8-cineol and β-pinene. (www.uni-graz.at/~katzer)

3.1. Chemical Analysis

European pharmacopeial grade peppermint oil is the volatile oil distilled with steam from the fresh aerial parts of the flowering plant. Its relative density must be between 0.900 and 0.916, refractive index between 1.457 and 1.467, optical rotation between –10 and –30?, among other quantitative standards. Identity must be confirmed by thin-layer chromatography (TLC), organoleptic evaluation, and quantitative analysis of internal composition by gas chromatography.

3.2. Standardisation

Peppermint leaves consist of the whole or cut dried leaves of Mentha x piperita L. The whole drug contains not less than 12 ml/kg essential oil, the cut drug contains not less than 9 ml/kg.

The essential oil consists of 35-55% (-)-menthol, 10-35% menthone, menthyl acetate and other monoterpenes (Ph.Eur. 5, 2005; ESCOP 2003). Minimum requirements for food are lower.

4. PHARMACOLOGY

Carminative: The exact mechanism of this action is not known. However, one proposition is relaxation of the esophageal sphincter leading to release gas pressure into the stomach.
Antispasmodic: Inhibits contraction of smooth muscles by blocking the influx of Ca+2 into muscle cells.
Choleretic: Stimulates the flow of bile. May also improve the solubility of bile.
Analgesic: Stimulates nerves which perceive cold, while depressing those that perceive pain. The body reacts to this sensation quite strongly by increasing blood flow to the area of application, thus producing a period of warmth. (http://www.geocities.com)

4.1. HUMAN STUDIES

Most modern human studies have investigated peppermint oil rather than peppermint leaf as a treatment for stomachache (May et al., 1996), spastic colon syndrome (Somerville et al., 1984), postoperative nausea (Tate, 1997), relief of colonic muscle spasm during barium enema (Sparks et al., 1995), irritable bowel syndrome (Carling et al., 1989; Dew et al., 1984; Fern ndez, 1990; Koch, 1998; Lawson et al., 1988; Lech et al., 1988; Liu et al., 1997; Nash et al., 1986; Pittler and Ernst, 1998; Rees et al., 1979), prevention of abdominal distension in postoperative gynecological patients (Feng, 1997), and headaches (Gobel et al., 1994; Gobel et al., 1996). The use of peppermint oil for irritable bowel syndrome is based on preparations in enteric-coated capsules, causing a spasmolytic activity on smooth muscles of the gut. In animal tests, the probable mechanism of action has been shown to be the inhibition of smooth muscle contractions by blocking calcium influx into muscle cells (Forster et al., 1980; Giachetti et al., 1988).
In one double-blind, placebo-controlled multicenter trial, Enteroplant®, consisting of peppermint oil (90 mg) and caraway oil (50 mg) in an enteric-coated capsule, was studied in 45 patients with non-ulcerous dyspepsia. After four weeks of treatment both the intensity of pain and the global clinical impression were significantly improved for the group treated with the peppermint/caraway combination compared with the placebo group (p=0.015 and 0.008, respectively) (May et al., 1996).
Against headache:
The effectiveness of peppermint oil was studied for use against tension-type headaches. The liquid test preparation contained 10 g of peppermint oil and ethanol (90%). The external application of peppermintoil reduced the headache-intension significantly compared with Paracetamol (Schulz and Hänsel, 2004).
Carminative and spasmolytic activity:
In human, during endoscopy, peppermint oil injected into the lumen of the colon relieved colonic spasms (Leicester and Hunt, 1982). The *Indian Pharmacopoeia* reported carminative activity (IP, 1996). The volatile oil of peppermint leaf is carminative and a potent spasmolytic, acting locally to produce smooth muscle relaxation (Bradley, 1992; Leicester and Hunt, 1982). The spasmolytic activity of the essential oil is stronger than of the single components (Wichtl, 1997).
Secretolytic, mucolytic and antibacterial Effect:
The essential oil shows secretolytic, mucolytic and antibacterial effects in the area of the respiratory tract. (Wagner and Wiesenauer, 2003).
The Commission E approved the internal use of peppermint oil for spastic discomfort of the upper gastrointestinal tract and bile ducts, irritable colon (in enteric-coated capsules), catarrhs of the respiratory tract, and inflammation of the oral mucosa; and external use for myalgia and neuralgia.

ESCOP indicates its internal use for flatulence, irritable bowel syndrome, and coughs and colds. Its external use is indicated for coughs and colds, rheumatic complaints, pruritus, urticaria, and pain in irritable skin conditions (ESCOP, 1997). Peppermint oil is used in Western and Eastern cultures to treat indigestion, nausea, sore throat, diarrhea, colds, and headaches (Leung and Foster, 1996).

Topical use

Antiseptic and anaesthetic effect:

The cooling effect of menthol is because of the affection of the cold-receptors in skin and mucous membranes. Menthol also has an antiseptic effect and in higher dosages an anaesthetic effect. (Braun, 1994).

4.2. VETERINARY STUDIES

Menthol is often used as a repellent against insects and in lotions to cool legs (especially for horses).

Treatment of mastitis:

Mentha piperita is used in a pharmaceutical preparation for treatment of mastitis in animals and humans, which consists of a mixture of a decoction and infusion, in ammonia spirit, of 16 medicinal herbs. (Deryabin AM; PCT International Patent Application 1990; -13305)

Ruminants:

Effect on digestibility, ruminal fermentation and protozoa:

A study showed the positive effect on the digestibility by feeding peppermint. The authors constitue that peppermint has a great potential as a natural manipulator of rumen fermentation by depressing ammonia-nitrogen concentration or numbers of protozoa. (Ando et al., 2003).

Antioxidative activity:

An experiment was done to examine the effects of peppermint feeding on antioxidative activity in milk of cows and showed positive results (Uegaki R. *et al.,* 2001)

Pigs:

Peppermint leaves are used in herbal mixtures for piglets to enhance weight gain (Rekiel A., 1998).
A herbal mixture with Peppermint as one component was successfully used as an alternative for an antibiotic substitute. Pigs obtained good average body weight gains (Urbanczyk J. *et al*., 2002).
Another herb mixture, Peppermint as one component, represented good results in feeding on rearing performance of calves (Wawrzynczak S. *et al*., 2000).

5. MICROBIOLOGY

Antimicrobial activity

Aetheroleum Menthae Piperitae inhibited the growth in vitro of *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Enterococcus faecalis* and *Escherichia coli*, but did not affect the growth of *Bacillus cereus, Penecillium cyclopium* or *Aspergillus aegyptiacus*. The essential oil inhibited the growth in vitro of *Trichophyton equinum* and *T. rubrum* (at a concentration of 0,4µg/ml), *Aspergillus flavus, A. fumigatus* and *A. niger*. (WHO Monographs, 2002)
Extracts of Folium Menthae Piperitae have antibacterial and antiviral activity in vitro. Addition of ground leaves to the agar medium inhibited the growth of *Salmonella typhimurium*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* at concentrations of 0.1–2.0%. Aqueous and ethanol extracts

of the leaves reduced the number of plaques of the rinderpest virus at concentrations of 4–8mg/ml (*17*). Aqueous extracts of the leaves demonstrated activity against
the following viruses in egg and cell culture: Newcastle disease, herpes simplex, vaccinia, Semliki Forest and West Nile (WHO, 2002)

Essential oil of peppermint induced inhibitory effect on the growth of Aeromonas isolates (Zaki HM. *et al*., 2001)

6. EFFICACY

The leaves and the essential oil of peppermint, Mentha × piperita L., have traditionally been used as carminative and antispasmodic herbal drugs. Spasmolytic effects of the essential oil have been demonstrated both in vitro on isolated gut segments and in vivo on healthy volunteers and in gastrointestinal endoscopy. In short-term studies, peppermint oil was revealed to be superior to a placebo in the treatment of irritable bowel syndrome. Due to the lack of well designed and carefully executed long-term studies, the significance of peppermint oil in the treatment of irritable bowel syndrome has not yet been elucidated. Menthol, the main component of peppermint oil, contributes to its spasmolytic effect. In human plasma and urine, mentholglucuronide has been detected as its main metabolite. Aqueous extracts from peppermint leaves show antiulcerogenic and cholagogic effects. In combination with the spasmolytic properties of the essential oil, hydrophilic compounds may contribute to the efficacy of peppermint leaves on spasmodic discomfort in the gastrointestinal and biliary system. (van Rensen, 2004)

7. TOXICOLOGY

Intragastric administration of the essential oil (100mg/kg body weight) to rats daily for 28 days induced histopathological changes (scattered cyst-like spaces) in the white matter of the cerebellum. No behavioural or clinical symptoms due to the encephalopathy were observed. (ESCOP)
Pulegone is mainly metabolised through pathways involving menthofuran and these two substances show similar toxicity. Evaluation of their toxicity should be made together. The Committee noted that only a limited database was available on pulegone and menthofuran and considered that these data were inadequate for the derivation of an ADI (acceptable daily intake). To establish a NOEL for pulegone and menthofuran, at least a further 90-day study is required together with studies on genotoxicity at the gene and chromosome level, and probably also reproductive and developmental toxicity studies. (EMEA, 2005).
The interest in toxicity of pulegone, menthofuran and peppermint oil appears to have been provoked by three reports in the literature. It was reported that pulegone, when given to rats for 28 days, caused histopathological changes in the liver (vacuolisation) and the brain ("cystlike spaces") (Thorup et al. 1983a,b; Olsen and Thorup, 1984). The histopathological changes were seen in rats receiving 80 and 160 mg/kg/day of pulegone. However, all hematological and clinical chemical parameters were found to be within the normal range in all groups. There were neither obvious signs of clinical symptoms due to encephalopathy. Based on these studies the NOEL (no effect level) of pulegone was considered to be 20 mg/kg bw/day.
Later "confirmatory" studies by the same group, however, reported that there were no significant histopathological changes in the liver nor the brain. The "cyst-like spaces" reported in the brain in the earlier studies were thus not confirmed and may have arisen from inadequate tissue fixation procedures (Molck et al. 1998). In this study the clinical biochemical examinations revealed

increased plasma glucose, alkaline phosphatase and ALAT and a decreased creatinine in the dosed group.
In later studies the liver toxicity of pulegone has been confirmed and a mechanism of action has been proposed based on its metabolism to menthofuran and other reactive metabolites, which are the ultimate hepatotoxins (see SCF report).

Pharmacovigilance information

A literature review of cases of human intoxication with pennyroyal oil (pulegone content 62-97%) indicate that ingestion of 10 ml (corresponding to ca 5.4-9 g pulegone, ca 90-150 mg/kg bw for a 60 kg person; calculated with a relative density of 0.9 as for peppermint oil) resulted in moderate to severe toxicity and ingestion of greater than 15 ml (corresponding to ca 8-13 g pulegone, ca 130-215 mg/kg bw for a 60 kg person) resulted in death. The clinical pathology was characterised by massive centrilobular necrosis of the liver, pulmonary edema and internal haemorrhage (SCF, 2002).
A non-urgent information request was sent out to the member states concerning use and association of licensed herbal medicinal products containing pennyroyal oil, peppermint oil and mint oil with reports of liver damage.
The highest recommended daily dose in EU is 1.2 ml peppermint oil i.e. 1080 mg peppermint oil, which contains maximum 140 mg pulegone + menthofuran (Ph Eur). For a 60 kg person this would correspond to a daily intake of 2.3 mg/kg bw. Clearly, this recommended daily dose of peppermint oil in herbal medicinal products results in an intake of pulegone/menthofuran that exceeds the TDI (0.1 mg/kg) set for food by CEFS.
No certain cases of liver damage caused by peppermint oil or mint oil were reported. (EMEA, 2005)

7.1. SAFETY

Interactions with other drugs: none known for peppermint leaves

Internal Use of Peppermint leaves:

Dosages
recommended daily allowance:

Cattle	25,0 - 50,0 g
Horse	20,0 - 40,0 g
Sheep, Goat	5,0 - 10,0 g
Pig	2,0 - 5,0 g
Dog	1,0 - 3,0 g
Cat	0,5 - 1,0 g
Fowl	0,2 - 0,5 g

(by Gachnian and Assenov 1986)

Peppermint tincture:

Cattle, Equids	5,0 - 15,0 ml
Sheep, Goat, Pig	2,0 - 5,0 ml
Dog	0,3 - 0,8 ml
Cat	0,1- 0,3 ml

(by Gachnian and Assenov 1986)

Prescription:

Rp.		Rp.	
Menthae pip. fol.	10,0	Peppermint leaves	10,0
Aqu. comm.	190,0	Drinking water	190,0
M.f.infus.		make an infusion.	

D.S. Internal use for a horse with intestinal spasm [modified by Rabinovich 1987].

Presently it is not allowed to use Peppermint leaves (Menthae piperitae folium) as an active agent for foodstuff delievering animals in the EU, but it is allowed to use Peppermintoil (Menthae piperitae aetheroleum) as an active agent for foodstuff delievering animals in the EU. [VO (EWG) Nr. 2377/90]. [Reichling et al., 2005]

Dosages and Administrations for humans:

Unless otherwise prescribed: 6–12 drops per day essential oil and galenical preparations for internal and external application.

Internal:

Essential oil: Average single dose 0.2 ml. Essential oil enterically coated form: Average daily dose 0.6 ml (for IBS). Inhalant: Add 3–4 drops of essential oil to hot water; deeply inhale the steam vapor.

External:

Essential oil: Some drops rubbed in the affected skin areas (may be diluted with lukewarm water or vegetable oil). Liniment: Oily preparation containing 5–20% essential oil in base of paraffin or vegetable oil applied locally by friction method. Ointment or unguent: Semi-solid preparation containing 5–20% essential oil in base of petroleum jelly or lanolin spread on linen for local application. Nasal ointment: Semi-solid preparation containing 1–5% essential oil. Tincture: Aqueous-alcoholic preparation containing 5–10% essential oil for local application.

Peppermint oil: People with esophageal reflux should avoid use of menthol-containing herbs, such as peppermint; the volatile oils in these plants may decrease the pressure in the lower esophageal sphincter and make the reflux worse (Sigmund CJ, McNally EF., 1969).

Contraindications: Obstruction of bile ducts, gallbladder inflammation, severe liver damage. In case of gallstones, to be used only after consultation with a physician. Preparations containing peppermint oil should not be used on the face, particularly the nose, of infants and small children.

[Ed. Note: Peppermint oil is contraindicated in infants and small children due to the potential risk of glottic spasms or respiratory arrest (Schulz et al., 1998).]

KBB

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ORIGANUM SP.

	
Origanum sp	

1. INTRODUCTION

Oregano is the world´s comercially most valued spice. It´s name, Oregano, is the common name for a general aroma and flavor primarily derived from more than 60 plant species used all over the world as a spice. The majority of them belong to the Lamiaceae and Verbenaceae families, while a large distinction is made between the European (*Origanum* sp.) and Mexican (*Lippia* sp.) oregano. European oregano is used as a flavoring in meat and sausage products, salads, stews, sauces and soups. Prior to the introduction of hops, oregano was used to flavor ale and beer. The essential oil and oleoresin, used extensively in place of the plant material, are found in food products, cosmetics, and alcoholic liqueurs. Oregano is also a good salt replacement in tomato-containing recipes. Mexican oregano is used predominantly in flavoring Mexican foods, pizza and barbecue sauces. Mexican oregano has a sharper and more pungent flavor than European oregano.

The genus *Origanum* (Lamiaceae) includes 39 species widely distributed in the Mediterranean region (Vokou et al., 1993). The species Origanum vulgare predominates in occurence, while *Origanum dictamnus* is endemic of the Island of Crete in southern Greece. The plants are perennial herbs spontaneously growing in calcareous substrates. One of the striking morphological characteristics of the Origanum plants is the presence of glandular and nonglandular hairs covering the aerial organs. Both types of hair originate from epidermal (protodermal) cells. Every epidermal cell, according to Netolitzky (1932), practically possesses the potentiality to develop into a hair. The glandular hairs are numerous on the vegetative organs (stems, leaves, bracts), while their density becomes reduced on the reproductive organs (calyces, corollas). On the stamens and the gynoecium of the flowers, no glandular hairs have been reported to occur (Werker *et al*., 1985b). The glandular hairs secrete an essential oil with a characteristic odour, mainly due to the major components of the oil, the monoterpenes carvacrol and thymol. The essential oils of *Origanum* have been found to develop strong antioxidant, antimicrobial, insecticidal and genotoxic activities (Scheffer *et al.,* 1986; Sivropoulou *et al*., 1996; Karpouhtsis *et al*., 1998).

Table 1 Different species called 'oregano'

Table 1. Species used commercially in the world as oregano

Family	Species	Commercial name/s found in literature
Labiatae	*Calamintha potosina* Schaf.	oregano de la sierra, oregano, origanum
	Coleus amboinicus Lour. (syn. *C. aromaticus* Benth)	oregano, oregano brujo, oregano de Cartagena, oregano de Espana, oregano Frances
	Coleus aromaticus Benth.	oregano de Espana, oregano, origanum
	Hedeoma floribunda Standl.	oregano, origanum
	Hedeoma incona Torr.	oregano
	Hedeoma patens Jones	oregano, origanum
	Hyptis albida H.B.K.	oregano, origanum
	Hyptis americana (Aubl.) Urb. (*H. gonocephala* Gris.)	oregano
	Hyptis capitata Jacq.	oregano, origanum
	Hyptis pectinata Poit.	oregano, origanum
	Hyptis suaveolens (L.) Poit.	oregano, oregano cimarron, origanum
	Monarda austromontana Epling	oregano, origanum
	Ocimum basilicum L.	oregano, origanum
	Origanum compactum Benth. (syn. *O. glandulosum* Salzm, ex Benth.)	oregano, origanum
	Origanum dictamnus L. (*Majorana dictamnus* L.)	oregano, origanum
	Origanum elongatum (Bonent) Emberger et Maire	oregano, origanum
	Origanum floribundum Munby (*O. cinereum* Noe)	oregano, origanum
	Origanum grosii Pau et Font Quer ex letswaart	oregano, origanum
	Origanum majorana L.	oregano
	Origanum microphyllum (Benth) Vogel	oregano, origanum
	Origanum onites L. (syn. *O. smyrneum* L.)	* Turkish oregano, oregano, origanum
	Origanum scabrum Boiss et Heldr. (syn. *O. pulchrum* Boiss et Heldr.)	oregano, origanum
	Origanum syriacum L. var. syriacum (syn. *O. maru* L.)	oregano, origanum
	Origanum vulgare L. subsp. *gracile* (Koch) letswaart (syn. *O. gracile* Koch, *O. tyttanthum* Gontscharov)	oregano, origanum
	Origanum vulgare subsp. *hirtum* (Link) letswaart (syn. *O. hirtum* Link)	oregano, origanum
	Origanum vulgare subsp. *virens* (Hoffmanns et Link) letswaart (syn. *O. virens* Hoffmanns et Link)	oregano, origanum, oregano verde
	Origanum vulgare subsp. *viride* (Boiss.) Hayek (syn. *O. viride*) Halacsy (syn. *O. heracleoticum* L.)	* Greek oregano, oregano, origanum
	Origanum vulgare L. subsp. *vulgare* (syn. *Thymus origanum* (L.) Kuntze)	oregano, origanum
	Origanum vulgare L.	oregano, orenga, Oregano de Espana
	Poliomintha longiflora Gray	oregano

	Salvia sp.	oregano
	Satureja thymbra L.	oregano cabruno, oregano, origanum
	Thymus capitatus (L.) Hoffmanns et Link (syn. *Coridothymus capitatus* (L.) Rchb.f.)	* Spanish oregano, oregano, origanum
Verbenaceae	*Lantana citrosa* (Small) Modenke	oregano xiu, oregano, origanum
	Lantana glandulosissima Hayek	oregano xiu, oregano silvestre, oregano, origanum
	Lantana hirsuta Mart. et Gall.	oreganillo del monte, oregano, origanum
	Lantana involucrata L.	oregano, origanum
	Lantana purpurea (Jacq.) Benth.& Hook. (syn. *Lippia purpurea* Jacq.)	oregano, origanum
	Lantana trifolia L.	oregano, origanum
	Lantana velutina Mart.&Gal.	oregano xiu, oregano, origanum
	Lippia myriocephala Schlecht.&Cham.	oreganillo
	Lippia affinis Schau.	oregano
	Lippia alba (Mill) N.E. Br. (syn. L. involucrata L.)	oregano, origanum
	Lippia Berlandieri Schau.	oregano
	Lippia cordiostegia Benth.	oreganillo, oregano montes, oregano, origanum
	Lippia formosa T.S.Brandeg.	oregano, origanum
	Lippia geisseana (R.A.Phil.) Soler.	oregano, origanum
	Lippia graveolens H.B.K.	* Mexican oregano, oregano cimarron, oregano, origanum
	Lippia helleri Britton	oregano del pais, oregano, origanum
	Lippia micromera Schau.	oregano del pais, oregano, origanum
	Lippia micromera var. *helleri* (Britton) Moldenke	oregano
	Lippia origanoides H.B.K.	oregano, origano del pais
	Lippia palmeri var. *spicata* Rose	oregano
	Lippia palmeri Wats.	oregano, origanum
	Lippia umbellata Cav.	oreganillo, oregano montes, oregano, origanum
	Lippia velutina Mart. et Galeotti	oregano, origanum
Rubiaceae	*Borreria* sp.	oreganos, oregano, origanum
Scrophulariaceae	*Limnophila stolonifera* (Blanco) Merr.	oregano, origanum
Apiaceae	*Eryngium foetidum* L.	oregano de Cartagena, oregano, origanum
Asteraceae	*Coleosanthus veronicaefolius* H.B.K. del campo	oregano del cerro, oregano del monte, oregano
	Eupatorium macrophyllum L. (syn. *Hebeclinium macrophyllum* DC.)	oregano, origanum

* Species of main economic importance according to Lawrence and Reynolds (1984)

Definition: Oregano are the dried leaves and flowers of *Origanum onites* L. or *Origanum vulgare* L. subsp. *hirtum,* or a mixture of them both (Pharmeuropa, April 2006).

2. DESCRIPTION OF THE PLANT

Origanum is one of over 200 genera in the Lamiaceae (mint family), and the genus includes culinary, fragrant, medicinal and ornamental plants. Herbaceous perennials or subshrubs, origanums are native to the Mediterranean and Eurasia, and grow in mountainous areas with rocky, calcareous soil. Some species grow in mounds that are only 2-3 inches high while others grow erect up to 39 inches tall.

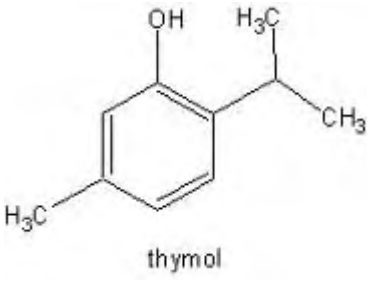
All members of the genus have flowers that occur in spikes; for most species these form a panicle with multiple branched stems growing from a central stalk. In *O. onites*, the spikes grow in a "false corymb", forming a convex or flat-topped open inflorescence. Corollas may be purple, pink or white depending on the species. In some species flowers are arranged in whorls. The calyx, or small vase-like receptacle that supports and protects the corolla and reproductive organs of the flower, can be bell-shaped or tubular with one or two lips. The shape of the calyx is the principal plant character used to distinguish between *Origanum* species. Flower stems can be erect or trailing/cascading. Trailing types like the ornamental *O. rotundifolium* and the cultivar 'Kent Beauty,' a hybrid of *O. rotundifolium* and another *Origanum* species, have a graceful, drooping appearance. The leaves and flowering parts of the plant contain essential oil glands that secrete volatile oils responsible for the plant's fragrance.

Plants in the genus Origanum have bracts, or non-typical leaves, that surround the calyx and corolla. In some instances the bracts are so beautiful and colourful that the casual observer might mistake them for the flower. In these cases, the flower is actually hidden within the bracts. In some species, like *O. rotundifolium* and *O. dictamnus* (dittany of Crete), bracts overlap and resemble hops.

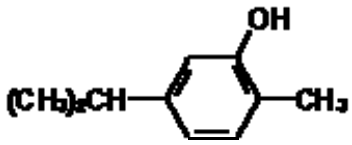
Both the stems and leaves of origanums are often covered with fine hairs. Leaves can be of various shapes including round, heart-shaped and oval and may be shiny/waxy or hairy-fuzzy in appearance. Stems may be woody or non-woody. All species also bear tiny brown fruits called nutlets.

3. INGREDIENTS / CONSTITUENTS

Origanum is known widely in the world of herbs and spices for its volatile oils. Oregano is the commercial name of those species zhat are rich in the phenolic monoterpenoids, mainly carvacrol, occasionally thymol, while marjoram is the commersial name of those that are rich in bicyclic monoterpenoids *cis*- and *trans*- sabinene hydrate. A number of chemically related compounds i.e. *γ*-terpinene, *p*-cymene, thymol and carvacrol methyl ethers, thymol and carvacrol acetates; also compounds such as *p*-cymenene, *p*-cymen-8-ol, *p*-cymen-7-ol, thymoquinone, and thymohydroquinone are also present. Furthermore, when *cis*- and/or *trans*-sabinene hydrate is the main compound, α-thujene, sabinene, *cis*- and *trans*-sabinene hydrate acetates, cis- and trans-sabinol and sabina ketone can also be found. Two more biochemical groups, usually of less significance quantitatively, are present in *origanum*: that of the acyclic monoterpenoids such as, geraniol, geranyl acetate, linalool, linalyl acetate and β-myrcene and that of bornane type compounds such as camphene, camphor, borneol, and bornyl and isobornyl acetate. To the above groups of compounds some sesquiterpenoids, such as β-caryophyllene, β-bisabolene, β-bourbonene, germacrene-D, bicyclogermacrene, α-humulene, α-muurolene, γ-muurolene, γ-cadinene, allo-aromadendrene, α-cubebene, α-copaene, α-cadinol, β-caryophyllene oxide and germacrene-D-4-ol should also be added.



thymol



carvacrol

3.1 CHEMICAL ANALYSIS

Volatile oil is quantitatively determined by water/steam distillation, and the percentage content of phenols expressed as thymol is determined by spectrophotometric analysis. Thin-layer chromatographic analysis is used for thymol and carvacrol.

3.2 STANDARDISATION

The content of essential oil should be at least 25 ml/kg related to the dry (aquatic-free) drug. Carvacrol and Thymol should be at least 60% of the essential oil-content (Zeitschrift Pharmeuropa, April 2006).

4. PHARMACOLOGY

4.1 PIG

Effects on meat stability

In a study the influence of feeding sage and oregano on the oxidative stability of raw belly bacon was demonstrated. Raw belly bacon produced from animals fed oregano demonstrated improved stability ($P < 0.05$) compared to that produced by the control group, after 34 weeks of storage (Bauer *et al.*, 2001).

Oreganum and its extract have an antioxidant capacity, which could be used as stabilisers of fat, in order to improve quality and shelf-life of meat and fat-containing food. On the one hand it seems possible to keep perishable fat-containing food longer by an addition of an extract of sage or oregano due to their antioxidative properties, on the other hand administration of feed additives of dried herbs (*Origanum vulgare*) to pigs had no effect on quality and shelf-life of fat obtained from these animals (Vichi *et al*., 2001).

Effect on the weight gain and feed conversion in pigs

A study showed the effect of high vitamin E and oregano phytogenic feed additives on the performance of "slow growing" fattening pigs. It was observed a significant ($P < 0.01$) positive effect of this oregano phytogenic feed additive in combination with high vitamin E regarding average daily gain when compared to a non-treated control group of animals, but for feed conversion there was no significant difference (Bilkei and Gertenbach, 2001).

A study evaluated the effect of dietary oregano etheric oils as non-specific immunostimulating agents in growth-retarded, low-weight growing-finishing pigs. A group of pigs were fed with commercial fattening diet supplemented with 3000 ppm oregano additive (Oregpig®, Pecs, Hungary), composed of dried leaf and flower of *Origanum vulgare*, enriched with 500 g/kg cold-pressed essential oils of the leaf and flower of *O. vulgare*, and containing 60 g carvacrol and 55 g thymol/kg. Dietary oregano improved growth in growth-retarded growing-finishing pigs and had non-specific immunostimulatory effects on porcine immune cells (Walter and Bilkei, 2004).

A study evaluated the effects of vaccination and of a phytogenic feed additive (1000 ppm dried oregano [Origanum vulgare] powder) on postweaning mortality due to *Escherichia coli* and on piglet performance, that the vaccination with purified VT2e toxoid and/or oregano feed supplementation can prevent losses due to postweaning coli complex in weaned pigs (Ken and Bilkei, 2003).

Effects of vaccination against *Haemophilus parasuis* serovar 5 (HPS 5) and *E. coli* and a phytogenic feed additive (a diet supplemented with 1000 ppm oregano) improved the performance of pigs in a Swiss nursery. The average daily nursery liveweight gain, feed efficiency and weaner mortality were recorded, observing that, from day 35, the groups of weaners fed the oregano supplement were significantly ($P < 0.05$) heavier than the groups fed without the supplement (Sads and Bilkei, 2003).

A study revealed that the inclusion of essential oil of oregano in a pigs' diet significantly improved the average daily weight gain and feed conversion ratio of the pigs. Pigs fed the essential oils had higher carcass weight, dressing percentage and carcass length than those fed the basal and antibiotic-supplemented diet. In the pigs, which received the essential oil supplementation had a significantly lower fat thickness. Also lean meat and ham portions from these pigs were significantly higher. Therefore, the use of *Origanum* essential oil? ~~essential oils~~ as feed additive~~s~~ improves the growth of pigs and has greater positive effects on carcass composition than antibiotics (Onibala *et al*., 2001).

Ropadiar®, an essential oil of the oregano plant, was supplemented in the diet of weaning pigs as alternative for anti microbial growth promoters (AMGP), observing its efficacy on the performance of the piglets. Ropadiar liquid contains 10% Oregano oil and has been designed to be added to water. Compared to the negative control (without AMGP), Ropadiar® ~~i~~mproved performance only during the first fourteen days after weaning. Based on the results of this trial it can not be argued the usefulness of Ropadiar® as an alternative for AMGP in diets of weanling pigs. However its addition in prestarter diets could improve performance of these animals (Krimpen and Binnendijk, 2001).

The objective of another trial was to ascertain, if by adding an oregano oil to the feed, there will be an effect on nutrient digestibilities, N-balance as well as on parameters of microbial activity in the gastrointestinal tract of weaned pigs. The apparent digestibility of crude nutrients (except fibre) and the N-balance of the weaned piglets in this study was not influenced by feeding piglets restrictively with this feed additive (which additive? essential oil?, at which percentage?). By direct microbiological methods, no influence of the additive on the gut flora could be found (Moller, 2001).

Antibiotic-Resistance

A study compared the resistance patterns of *Escherichia coli* isolated from pig herds receiving antibiotics or oregano (Oregpig®, Pecs, Hungary) as preventive feed additives. Oregano-fed pig-herds demonstrated significantly ($P < 0.001$) lower MICs (minimum inhibitory concentrations; µg/ml) for ampicillin, doxycyclin, enrofloxacin, gentamycin, oxytetracyclin and sulfamethacin against *Escherichia coli* compared to herds with prophylactic use of antibiotics. Resistance to ceftiofur revealed significant ($P < 0.05$) differences between the antibiotic- or oregano-treated units (Docic and Bilkei, 2003).

Effect of <u>Origanum vulgare</u> on weaning-to-estrus interval and farrowing rate in sows

Sows during lactation or until the first standing oestrus were fed with a diet supplemented either with oregano (*Origanum vulgare),* chlortetracycline or no supplement. Results showed that weaning-to-oestrus interval was shorter in sows supplemented with oregano compared to other

treatments. Farrowing rate was greater in sows supplemented with either chlortetracycline or oregano compared to untreated sows. (Kis and Bilkei, 2003).

Also another study reported that dietary Oregano supplementation improved the sows´reproductive performance. Farrowing sows receiving 1000 ppm Oregpig® (Pecs, Hungary) in their lactation diet showed a lower mortality rate, a lower culling rate during lactation, an increased farrowing rate, more liveborn piglets and less stillbirth piglets per litter compared with untreated females (Kovac and Bilkei, 2003).

Alternate diets containing 1000 ppm of oregano feed supplementation (Oregpig®) were provided in farrowing groups of sows during lactation, from parturition throughout lactation and from weaning to mating. Compared to untreated sows, oregano treated sows showed lower annual mortality rate, lower culling rate during lactation, increased farrowing rate, more liveborn piglets per litter and less stillbirths. Results showed that oregano as a feed additive of natural origin may be useful in pig breeding (Mauch and Bilkei, 2004).

4.2 FOWL

Effect of oregano essential oil on performance of broilers

A dietary supplementation of oregano essential oil (300 mg/kg) showed a positive effect on performance of broiler chickens experimentally infected with *Eimeria tenella*. Throughout the experimental period of 42 days, oregano essential oil exerted an anticoccidial effect against *E. tenella*, which was, however, lower than that exhibited by lasalocid. Supplementation with dietary oregano oil to *E. tenella* infected chickens resulted in body weight gains and feed conversion ratios not differing from the non-infected group, but higher than those of the infected control group and lower than those of chicken treated with the anticoccidial lasalocid (Giannenas *et al*., 2003).

In another study effects of the addition of herbal mixture (which is not specified by the authors) or essential oil to chickens feed-ratio did not alter the final liveweight after 35 days, but the feed to gain ratio significantly decreased in the chicks supplemented with herbal mixture and essential oil. The chickens recieved 1 g essential oil (oregano/clove, 1:1?) or 1 g essential oil (oregano/cinnamon, 1:1?) per kg body weight. (Halle *et al*., 2001).

4.3 RUMINANTS

Effects of natural plant extracts on protein degradation and fermentation profile

Eight dual-flow continuous culture fermenters were used in four consecutive periods of 10 days to study the effects of six natural plant extracts on ruminal protein degradation and fermentation profile. Fermenters were fed a 60:40 (52:48?), lucerne hay:concentrate diet (DM). One of the treatments was 7.5 mg/kg DM of extracts of *Origanum vulgare*, which increased the acetate and decreased the propionate proportion during the first 6 days of fermentation, in comparison to controls untreated with the plant extract. However, these differences disappeared after day 7, suggesting that rumen microbes were adapted to the additives within 7 days and that data from short-term *in vitro* fermentation studies may lead to erroneous conclusions, and should be interpreted with caution (Cardozo *et al*., 2004).

4.4 HUMANS AND ANIMALS

Treatment of mastitis

Origanum vulgare is used in a pharmaceutical preparation for treatment of mastitis in animals and humans, which consists of a mixture of a decoction and infusion, in ammonia spirit, of 16 medicinal herbs (Deryabin AM; PCT International Patent Application 1990; -13305).

5. MICROBIOLOGY

5.1 ANTIFUNGAL ACTIVITY

The antifungal activity is strongly correlated with the composition of the essential oil, its concentration and pH of the testing medium *in vitro*. Phenols are believed to be the most potent antimicrobials followed by alcohols, ketones, ethers and hydrocarbons. These presumptions are in accordance with the findings of Biondi *et al.* (1993), who reported that carvacrol - rich *O. onites* L. essential oil showed more potent antifungal activity against *A. niger, Aspergillus terreus, C. albicans* and against *Fusarium* spp. than the oregano oil, composed prevently of terpinene-4-ol and of γ-terpinene. A potent antifungal effect of *O. vulgare* essential oil (rich in carvacrol and thymol) at concentration of 1 µl/ml against the common spoilage fungus *A. niger* was observed also by Baratta *et al*. (1998a).

Other authors documented the inhibitory effect of *Origanum* essential oil against *Candida albicans in vitro* as well as after oral administration to mice *in vivo* (Hammer *et al*., 1999; Manohar *et al*., 2001).

However, there are many differences among genera of fungi with regard to sensitivity to the antifungal effects of oregano and of its essential oils. Also, the concentration of an essential oil and its origin may significantly influence their antifungal activity (Kintzios et al., 2002).

5.2 ANTIBACTERIAL ACTIVITY

Based on a broad spectra of antibacterial activity, oregano seems to be one of the most inhibitory spices tested. Essential oils of *O.vulgare* and *O. majorana* have been investigated for their activity against *Acinetobacter baumanii, Aeromonas veronii biogroup sobria, Streptococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* subsp. *enterica* serotype *typhimurium, Serratia marcescens* and *Staphylococcus aureus*. It was found that *O. vulgare* (Australian origin) yielded one of the most potent antibacterial agents, which considerably inhibited the growth of all tested microorganisms. The lowest minimum inhibitory concentration of *O. vulgare* essential oil was 0,12 per cent (v/v) and 0,25 per cent (v/v) of *O. majorana*. Among the tested bacteria, the most resistant was *Pseudomonas aeruginosa*, that was inhibited by *O. vulgare* essential oil at 2 per cent (v/v), but not by *O. majorana* oil (Hammer *et al*., 1999).

An evaluation of the antibacterial properties of *Origanum* essential oil against *S. aureus*, *Bacillus anthracis*, *E. coli*, *K. pneumoniae*, *Helicobacter pylori* and *Mycobacterium terrae*, revealed a bactericidal activity to all tested organisms. The essential oil was bacteriostatic *only against B. anthracis* (Presuss *et al*., 2005).

5.3 ANTIOXIDANT ACTIVITY

The antioxidative effects of *O. vulgare* drug (Origani herba) have been studied in the light of both direct use of *O. vulgare* as stabilisers of fat and, indirectly, as feed additives in order to improve the shelf-life of meat and fat-containing food obtained from animals treated with *O. vulgare* (Vichi *et al*., 2001). In contrast with the significant antioxidative and stabilising effects of oregano extracts in lard (measured by photochemiluminescence), no effect on the quality or shelf life of the fat obtained from animals fed with oregano additives was observed (Vichi *et al*., 2001). Antioxidant properties were demonstrated also for a hydroalcoholic extract of *Origanum vulgare* leaves by its iron-reducing activity *in vitro* (Yoshino *et al*., 2006).

5.4 ANALGESIC, ANTIAGGREGANT, ANTIINFLAMMATORY AND ANTISPASMODIC ACTIVITY

Carvacrol-rich (67 per cent) essential oil of *Origanum onites*, collected at the Izmir locality (Turkey), showed a marked dose-dependent analgesic activity as assessed by the tail-flick method in male albino mice. After administration, at 0,33 ml/kg, the activity of *O. onites* oil was comparable to that of morphine (1 mg/kg), but at 0,03 ml/kg the essential oil was more potent than fenoprofen (~~at~~ 8 mg/kg) (Aydin *et al.*, 1996). Findings obtained in the study strongly suggested that the analgesic activity of the essential oil is related to its carvacrol content (Aydin *et al*., 1996). The antiaggregant effect of a methanol extract of *O. majorana* on human platelets

has been studied by Okazaki et al. (1998). *O. majorana* extract concentration-dependently inhibited platelet aggregation induced by collagen (2,0 µg/ml) or ADP (2,0 µg/ml). Successive fractionation of the methanol extract led to isolation of the hydroquinon β-D-glucopyranoside, arbutin, which strongly inhibited platelet aggregation induced by (collagen, ADP, arachidonic acid, and thrombin (Okazaki *et al*., 1998).
Only a few reports on the topical anti-inflammatory effects of *O. vulgare* refer to oregano-herbal mixtures or their decoctions, used in the treatment of inflammation as supporting therapies (Deryabin, 1990 and 1991). A hydroalcoholic extract of *Origanum vulgare* leaves, orally administered to mice, significantly prevented gastritis induced by cold-resistant stress, while its cutaneous application prevented contact hypersensitivity induced by oxazolone (Yoshino *et al*., 2006).
While analysing the antispasmodic effects of *Origanum*, it was found that water macerates of *O. compactum* significantly inhibited smooth muscle response induced by any of the tested spasmogens (acetylcholine, histamine, serotonine, $BaCl_2$, nicotine) in the guinea-pig ileum. Moreover, in the pharmacological inhibition of smooth muscular activity, non-specific and non-competitive mechanism of action was attributed to thymol and carvacrol: they caused both direct musculotropic (muscle relaxant activity) and indirect neurotropic (inhibition of the nerve action potential) actions on the smooth muscle (van den Brouke and Lemli, 1980). The same results were obtained by testing the antispasmodic effects of pure active components, i.e. thymol and carvacrol. It was concluded that both phenols act as non-competitive Ca^{2+} antagonists, which block nerve fibre conduction and induce musculotropic and neurotropic spasmolyse (van den Brouke and Lemli, 1982).

5.5 Immunostimulating, Antimutagenic and protective effects

Some studies have been shown that oregano extracts or herbal mixtures with *Origanum* spp. possess *in vitro* antiviral activity or have immunostimulating effects both *in vitro* and *in vivo*. However, little knowledge has been attained so far on mechanism of immunomodulating activity or underlying active compounds (Kintzios et al., 2002).
A strong and dose-dependent capacity of inactivating dietary mutagen Trp-P-1 in the *Salmonella typhimurium* TA 98 assay was observed for *O. vulgare* water extracts, that exhibited significant antimutagenic effects *in vitro* (Ueda *et al*., 1991).
A study evaluated the effect of oral administration of *O. majorana* (volatile oil, alcoholic and aqueous extracts) on the toxicity induced by oral administration of lead acetate in the diet of mice for one month. Mice treated with the 3 different preparations of *O. majorana*, one month before and maintained with lead acetate administration, showed a significant decrease in serum transaminases, alkaline phosphatase, urea and creatinine, and improved the liver and kidney histology in comparison with lead acetate treated group. Alcoholic extract significantly reduced the rate of micronucleus, number of aberrant cells and different kinds of chromosomal aberrations. Volatile oil extract significantly reduced the rate of micronucleus and chromosomal fragments. Aqueous extract and volatile oil significantly reduced also the number of gaps, ring chromosome and stickiness. The study demonstrated that *O. majorana* plays an important role in ameliorating liver and kidney functions and genotoxicity induced by lead acetate (El-Ashmawy, 2005).

6. Efficacy

The claimed efficacy for this herb has not been documented.

7. TOXICOLOGY

Single components of *Origanum* essential oils - such as d-limonene from *O. majorana* - showed a carcinogenic effect in male rats (de Vincenzi and Mancini 1997) This carcinogenic effect as well as other renal damages observed in male rats are species and gender specific: consequently they are not predictable for human toxic effects (Meek et al., 2003)!
and several studies confirmed the allergenic potential of *Origanum* spp. *Origanum vulgare* showed cross-sensitivity with other plants of the Lamiaceae family, confirmed in *in vitro* and *in vivo* studies (references). The potential allergic response, that could be evoked in sensitive patients after the ingestion of food seasened with *O. vulgare*, comprises an increased serum level of specific IgE and induced systemic allergic reactions (Benito *et al*., 1996). Perioral dermatitis has been reported to be induced by *O. majorana* food flavouring (Farkas, 1981). *O. vulgare* has been shown also to induce allergic contact dermatitis, as clinically evaluated by patch test (Futrell and Rietschel, 1993). Due to empirically proven emmenagogue and abortifacient effects, excessive use of *O. vulgare* or *O. majorana* should be avoided during pregnancy (Brinker, 1998).

7.1 SAFETY

Dosages for a dog:
Internal use:
1,5 - 2g origanum-wort, 3 times a day as an infusion.
The dosage depends on the age and the weight of the dog (Reichling *et al*., 2005).

Presently it is not allowed to use *Origani herba* as an active agent for foodstuff delievering animals in the EU [VO (EWG) Nr. 2377/90] (Reichling et al., 2005).
Presently there are no experiences with Origani herba and pregnant or lactating animals (Reichling *et al*., 2005).

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PETROSELINUM CRISPUM

Petroselinum crispum

1. INTRODUCTION

Parsley is known in the Mediterranean region since about 2000 years, and it was initially predominantly used for medical purposes. Nowadays, it is used as a spice in nearly all temperate regions of the world (Teuscher, 2003). It is also extensively used in food industry for flavouring meat, sauces and for the production of spice concentrates (Frank, 1994).
As far as its medical use is concerned, the stimulation of gastric and bile secretion and a digestive action is attributed to the plant because of its essential oil content (Teuscher, 2003). It is also stated to possess spasmolytic, diuretic, emmenagogue, expectorant, antirheumatic and antimicrobial properties. Traditionally, it has been used for dyspeptic disorders, colic, cystitis, dysuria, bronchitic cough, dismenorrhoea, functional amenorrhoea and myalgia (Barnes *et al.*, 2002). The volatile oil and its main compound apiole have been misused in high doses as an abortefacient (Frank, 1994).
Most of these indications are not yet scientifically documented, and so far only the use of parsley root and herb as a diuretic in disorders of the urinary tract and for the prevention and therapy of renal gravel is recommended. The use of parsley fruits, however, has been negatively judged by the Commission E, due to their higher content of the volatile oil compounds apiole and myristicin which are associated with negative side effects (Blumenthal, 1998; Wichtl, 2002). In herbal veterinary medicine, parsley roots are occasionally used as an ingredient of diuretic herbal teas (Bentz *et al.*, 1989). However, also for this application the efficacy remains to be documented.

2. DESCRIPTION OF THE PLANT

The genus Petroselinum HILL belongs to the Apiaceae (Umbelliferae) family, subfamily Apioideae, tribus Ammineae. It only consists of 2 species, namely *P. segetum* (L.) KOCH and *P. crispum* MILL. (Synonyma: *P. sativum* HOFFM., *P. hortense* Auct. non HOFFM.), the latter one being

used for medical and culinary purposes. The species *P. crispum* MILL. is divided in two subspecies: *P. crispum* ssp. *crispum* is available with a unruffled and a ruffled leaf form, and from this subspecies mainly the leaves are used, whereas *P. crispum* ssp. *tuberosum* BERNH. ex RCHB. is the main source for parsley roots. Furthermore, in *P. crispum* three chemical races can be differentiated depending on the composition of the volatile oil (Frank, 1994).

2.1 SYSTEMATICS

The genus *Petroselinum* HILL belongs to the Apiaceae (Umbelliferae) family, subfamily Apioideae, tribus Ammineae. It only consists of 2 species, namely *P. segetum* (L.) KOCH and *P. crispum* MILL. (Synonyma: *P. sativum* HOFFM., *P. hortense* Auct. non HOFFM.), the latter one being used for medical and culinary purposes. The species *P. crispum* MILL. is divided in two subspecies: *P. crispum* ssp. *crispum* is available with a unruffled and a ruffled leaf form, and from this subspecies mainly the leaves are used, whereas *P. crispum* ssp. *tuberosum* BERNH. ex RCHB. is the main source for parsley roots. Furthermore, in *P. crispum* three chemical races can be differentiated depending on the composition of the volatile oil (Frank, 1994).
The plant parts used for flavouring and medical purposes are the fruits and the volatile oil obtained from them, the roots and the herbs. For medical applications, normally tea preparations are used (Frank, 1994).

2.3 PLANT PARTS USED

Confusions of the leaves with the leaves from *Aethusa cynapium* L. or *Conium maculatum* L. have been described. The discrimination is possible by macroscopic criteria, and also by chemical analysis. Parsley root is sometimes mistaken with the roots of *Pastinaca sativa* L. In contrary to parsnip, parsley root bark becomes red when $FeSO_4$ is added (Teuscher, 2003; Frank, 1994).

3. CONSTITUENTS

The whole plant contains volatile oil with the phenylpropanes myristicin and *p*-apiole as well as mono- and sesquiterpenes. Furthermore flavones and small amounts of furanocoumarins (bergapten, imperatorin, isopimponellin, xanthotoxin, psoralen) are present.
The fruits have the highest volatile oil content (2-6%). Depending on the composition of the volatile oil in the ripe fruits, 3 chemical races can be distinguished: the myristicin-type (49-77% myristicin, 0-23% apiole and 1-23% allyltetramethoxybenzene, ATMOB); the apiole-type (58-80% apiole, 9-30% myristicin, up to 6% ATMOB); and the ATMOB-type (50-60% ATMOB, 26-37% myristicin and apiole in traces). The main flavone is apiin, and furanocoumarins are present at a concentration of about 0.003%. Additionally, the fruits are very rich in fatty oil with petroselinic acid as the main compound (60- 80%).
The volatile oil content of the herb is much lower (0.016-0.3% in the fresh herb; up to 1.2% in the dried herb). The flavonoid content of the dried herb is 1.9-5.6%. Furthermore small amounts of coumarins are present. The herb is very rich in chlorophyll (about 1%), why it has been used for chlorophyll extraction in the past.
The dried roots contain 0.05-0.12% essential oil, and the fresh ones up to 0.73%. The flavonoid content is 0.2-1.3% of the dry weight, calculated as apiin. Furthermore, small amounts of furanocoumarins and phthalides (ligustilide, senkyunolide) were found, and also polyacetylenes (falcarinol, falcarinon, falcarinonol) are present.
The volatile oil can be qualitatively and quantitiatively analyzed by GC and/or TLC after steam distillation. The flavonoid spectrum can be analyzed by TLC and HPLC. Adulteration with *Aethusa cynapium* L. can be detected by the presence of volatile oil compounds like aethusanole and aethusin, which must not be present in the GC chromatograms of *P. crispum* (Frank, 1994).

4. PHARMACOLOGY

Scientific studies to document the claimed effects of parsley are rather rare, and clinical trials in humans or productive livestock do not exist at all.

4.1: IN VITRO AND ANIMAL TESTING

According to older investigations in rats and mice, the volatile oil of parsley shows a diuretic effect. A diuretic and spasmolytic activity is also postulated for the flavonoids (Teuscher, 2003).
A parsley extract (0.25-1 ml/kg, i.v.) has been reported to lower the blood pressure of cats by more than 40%, and to decrease both respiratory movements and blood pressure in anaestzesized dogs. Parsley exhibits a tonic effect on both intestinal and uterine muscle. The uterine effect has been attributed to the apiole content, but has also been observed with apiole-free aqueous extracts (Barnes *et al*., 2002).
A methanolic parsley herb extract exhibited estrogenic activities in an estrogen sensitive breast cancer cell line. Flavonoids were isolated as active compounds. Estrogenic activities were enhanced by removal of the glycoside moiety. Apigenin, diosmetin and kaempferol were the most active compounds, with estrogenic activities nearly equal to those of the isoflavones daidzein and genistein. Furthermore, the crude extract, apiin and apigenin restored the uterus weight of ovariectomized mice after p.o. administration for 7 days (Yoshikawa *et al*., 2000).
Polyacetylenes like falcarindiol, which had also been detected in parsley, were shown to possess medium level cytotoxicity *in vitro* against myeloma, leukaemia and lymphoma cell lines, with IC_{50} values around 30 µM. Colorectal cancer cell lines were less susceptible, with IC_{50} values around 100 µM (Zidorn *et al*., 2005). Myristicin from parsley leaf oil inhibited benzo[a]pyrene induced tumorgenesis in female A/J mice. This could be due to its high activity as an inducer of the detoxifying enzyme glutathione-S-transferase, which was observed in mouse liver and small intestinal mucosa (Zheng *et al.* 1992).
The antioxidant activity of parsley leaf and stem aqueous and methanolic extracts has been shown in several *in vitro* test systems (for example Hinneburg *et al*., 2006; Wong and Kitts, 2006).
Recently, some evidence for a potential antidiabetic effect of parsley was found:
Yanardag *et al.* (2003) investigated the effect of an aqueous parsley leaf extract, which was administered at a dose of 2 g/kg every day for 28 days, to streptozotocin-induced diabetic rats. They found a significant reduction of blood glucose levels, and the activity of the extract was not insulin-like. The authors suggest inhibition of gluconeogenesis and direct stimulation of glycolysis to be ivnolved in the mechanism of action.
Using the same model, Bolkent and coauthors (2004) investigated the hepatoprotective effect of this extract. The degenerative changes observed in the hepatocytes of diabetic rats were significantly reduced or absent in the hepatocytes of diabetic rats treated with parsley. Furthermore, treated rats demonstrated significantly lower levels of alanine transaminase and alkaline phosphatase than untreated diabetic rats. Oszoy-Sacan *et al*. (2006) compared the hepatoprotective effect of this extract to the effect of the antidiabetic agent glibornuride using the same animal model. They found a better protective effect against liver damage for the parsley extract and suggested that it was caused by the extract´s antioxidant potential. Göksel *et a*l. (2003) showed, that the same extract reversed the effects of diabetes on tissue lipid peroxidation and glutathione levels in the aorta and heart tissues in this animal model. Aorta lipid peroxidation was significantly decreased, and the decrease of glutathione levels in the heart was prevented. According to the authors, these effects might be caused by the hypoglycaemic effect as well as by the antioxidant activity of parsley. Despite the promising results of these studies, the observed effects could not yet be attributed to a certain compound or class of compounds. Additionally, the high dose administered in these studies (2 g/kg b.w.!) should be considered.
Mekhfi *et al.* (2004) found an inhibitory effect on platelet aggregation of an aqueous parsley herb extract *in vitro* using rat platelets. Also in this assay high doses were used (10 mg/ml).

Finally, an ethanolic parsley herb extract (1 or 2 g/kg b.w.) was shown to possess gastroprotective properties as evidenced by its significant inhibition of basal gastric secretion and of the formation of gastric lesions induced by pylorus ligation, hypothermic restraint stress, indomethacin, ethanol and strong alkali (Al-Howiriny *et al*. 2003).

4.2 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

The only available data concern the metabolism of myristicin, a constiutent of the volatile oil. Lee *et al.* (1998) investigated the *in vitro* and *in vivo* metabolism of myristicin in rats. Metabolites were determined by GC-MS analysis. Myristicin was metabolized to 1,2-dihydroxy-4-methoxy-5-allylbenzene and to a lower extent to 1´-hydroxymyristicin by rat liver microsomes *in vitro*. After p.o. administration of myristicin (100 mg/kg b.w.) to rats, the same metabolites were found in the urine, and enzymatic hydrolysis suggested that these metabolites were also conjugated to different extent. The presence of amphetamine derivatives, which had been found as myristicin metabolites in earlier studies, was not confirmed in this trial. Yun *et al*. (2003) investigated the importance of P450 enzymes for the formation of 5-allyl-1-methoxy-2,3-dihydroxybenzene. The results of this study provide evidence that CYP 3A4 and CYP 1A3 play a role in the formation of the major myristicin metabolite, thus the authors conclude that administration of myristicin would alter the metabolism of drugs metabolized by those enzymes and cause interactions.

5. MICROBIOLOGY

Lyophilized parsley leaf powder showed antibacterial activity in concentrations from 0.12 to 8% in the agar, depending on the microorganism. For example, the growth of *Escherichia coli*, *Listeria monocytogenes*, *Listeria innocua* and *Erwinia carotovora* was inhibited (Manderfeld *et al.*, 1997). The inhibitory potential of a methanolic parsley leaf extract and of pure coumarins was tested against a variety of gram positive and gram negative bacteria, yeasts, mold and plant-pathogenic fungi. The extract showed modest antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Saccharomyces cerevisiae*, and bacteriostatic activity against *Bacillus subtilis* at an amount of 500 µg. Concerning the plant pathogenic fungi, the extract clearly inhibited *Heterobasidion annosum*, but promoted the growth of *Phytophtora*. The tested coumarins were effective against some plant pathogens, but only showed very weak effects on bacteria, yeasts and mold (Ojala *et al*., 2000).

El Astal *et al*. (2005) tested aqueous, ethanolic, methanolic and phenolic leaf extracts and a commercial volatile oil of parsley against 10 pathogenic microorganisms using the hole plate diffusion technique. The methanolic extract inhibited *Salmonella typh*i and *Staphylococcus aureus* at a concentration of 5 mg/ml, and the essential oil (100 µl) additionally inhibited *Pseudomonas aeruginosa*. The other extracts were even less active.

Wong and Kitts (2006) assessed the antimicrobial activity of aqueous and methanolic parsley leaf and stem extracts against *E. coli* and *B. subtilis* by determination of cell damage and growth inhibition. The methanolic extracts showed the higher activity than the aqueous ones.

6. EFFICACY

The efficacy in most of the indications parsley is traditionally used for is not documented by scientific studies so far. At the moment, only the use of parsley herb and root as a diuretic is recommended. Also this indication is not documented by clinical trials. The use of parsley fruits is not recommended because of the negative risk-benefit ratio and the lack in documentation of efficacy (Blumenthal, 1998; Wichtl, 2002). Concerning the medical use in productive livestock, no scientific data are available so far.

The plant is extensively used in food for flavouring purposes, and it is listed by the Council of Europe as natural source of food flavouring in category N2. This category indicates that parsley can be added to foodstuffs in small quantities, with a possible limitation of an active principle

(as yet unspecified) in the final product. In the USA, the plant is listed as GRAS (Generally Recognized As Safe) (Barnes *et al*., 2002).

7. TOXICOLOGY

7.1 SAFETY

Potentially toxic constituents of parsley are the volatile oil compounds apiole and myristicin and the furanocoumarins like psoralen, bergapten and xanthotoxin.

Apiole and myristicin are documented to be hepatotoxic. The compounds are documented to have a similar chemical structure and acute toxicity to safrole, which is known to be carcinogenic and hepatotoxic. The carcinogenic potenitial of apiole and myristicin has not been evaluated. LD_{50} values for apiole and myristicin have been documented as 50 mg/kg and 200 mg/kg b.w. respectively (mouse, i.v. injection) (Barnes *et al*., 2002). In comparison, the LD_{50} for an ethanolic extract of the aerial plant parts was determined as 13 g/kg b.w. in rats (i.p. injection) (Al-Howiriny *et al*., 2003).

High doses of parsley oil or apiole have been misused as abortefacient (up to 10 g apiole or 1 g daily for 1-2 weeks) due to the tonic effect of apiole in the uterine smooth muscle (Frank, 1994). Also parsley tea is sometimes misused for this purpose (Ciganda and Laborde, 2003). Apiole intoxications are characterized by gastroenteristis, headache, elevated pulse rate, shock, coma, kidney irritation and liver damage (Frank, 1994). The ingestion of approximately 10 g apiole has been reported to cause acute haemolytic anaemia, thrombocytopenia purpura, nephrosis and hepatic dysfunction. However, 10 g apiole are equivalent to a dose of more than 200 g parsley, thus the amount of apiole ingested by normal dietary consumption of parsley is stated to be non hazardous (Barnes *et al*., 2002).

Myristicin has been documented to cause vertigo, deafness, hypotension, a lowered pulse rate and paralysis followed by fatty degeneration of the liver and kidney. Additionally, the compound is known to possess hallucinogenic properties. However, when compared to nutmeg, parsley contains a relatively low amount of myristicin (less than 0.05% in parsley leaf, about 0.4-0.89% in nutmeg). Parsley seed is potentially hazardous due to its higher volatile oil content (Barnes *et al*., 2002).

After external application, the undiluted volatile oil was not found to be irritant on mouse and pig skin, but under occlusion for 24 hours it led to severe irritations in rabbits. The external application of the essential oil did not elicit phototoxic reactions in mice and pigs. 2% solutions of the volatile oil did not provoke reactions over 24 hours in humans using the patch test (Frank, 1994).

Parsley contains phototoxic furanocoumarins. Photodermatitis resulting from oral ingestion of parsley is thought to be unlikely due to the rather low content. The estimated daily ingestion of furanocoumarins from parsley is 20 µg in Switzerland; for comparison, the minimum erythema producing dose of xanthotoxin is 240 µg/kg b.w. per day (about 14 mg per capita per day). However, a phototoxic reaction from topical contact is possible (Barnes *et al*., 2002; Frank, 1994; Teuscher, 2003). Allergic reactions are not very common, but are more likely, if already an allergy against other Apiaceae or against mugwort (Asteraceae) exists (Teuscher, 2003).

The use of parsley should be avoided during pregnancy and lactation, and when suffering from inflammatory renal diseases (Wichtl, 2002).

To summarize, the plant should not be ingested in excessive amounts by humans in view of the documented toxicities of apiole and myristicin (Barnes *et al*., 2002). To what extent these safety and toxicity data are also valid for the application in productive livestock, remains to be clarified. So far, no data exist on this issue.

7.2 DOSAGE

Leaf/ root: 2-4 g daily (infusion 3 times daily)
(Seed: 1-2 g daily)
Liquid extract (1:1 in 25% alcohol) 2-4 ml three times daily (Barnes *et al*., 2002).

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PIMPINELLA ANISUM

Pimpinella anisum

1. INTRODUCTION

The use of anise (*Pimpinella anisum* L.) for flavouring and medical purposes has a long tradition. The fruits are extensively used as a spice for flavouring bread and bakery products, and the essential oil is often added to foodstuff and liqueurs as a sensory and flavoring agent. Aniseed is listed by the Council of Europe as a natural source of feed flavouring, and in the USA it is listed as GRAS (Generally Recognized as Safe) (Staesche *et al*., 1994, Barnes *et al.,* 2002).
As far as its medical use is concerned, aniseed is stated to possess expectorant, antispasmodic, carminative and parasiticide properties. In traditional medicine, the drug has been used internally for bronchial catarrh, pertussis, spasmodic cough, flatulent colic, and externally for pediculosis and scabies. Furthermore it has been used as an estrogenic agent. It has been recommended to increase milk secretion, promote menstruation, facilitate birth, alleviate symptoms of the male climacteric and increase libido (Barnes *et al*., 2002).
Nowadays, it is stated to be effective in the treatment of dyspeptic complaints such as mild spasmodic gastrointestinal complaints, bloating, flatulence (internally) and against catarrh of the upper respiratory tract (internally and externally) (Wichtl, 2002; ESCOP, 2003). The documented pharmacological effects support some of the medical uses (Barnes *et al*., 2002).
Additionally, the essential oil is used as a fragrance additive in pharmaceutical and cosmetic products (toothpastes, perfumes, soaps, detergents, creams, lotions), and as a repellent against flying insects and lice. The residue from essential oil distillation is often used as fodder in husbandry because of its high protein and fatty oil content (Staesche *et al*., 1994).

2. DESCRIPTION OF THE PLANT

Anise *(Pimpinella anisum)* is a herbaceous, annual plant becoming 30-50 cm high. The stem is terete, channelled and branched in the upper part. The leaves are not partite in the lower part of the plant and becoming more and more pinnatisect towards the upper part. The flowers are arranged to double umbels consisting of about 7 to 15 rays. The fruits consist of 2 greyish-brown, about 2 mm long, inversely pyriform, slightly corrugated schizocarps (cremocarp), which normally do not fall apart. Each fruitlet has 5 straight ribs. The smell is anethole-like, the taste is sweetish-aromatic (Staesche *et al.*, 1994; Wichtl, 2002).

2.1 SYSTEMATICS

The genus *Pimpinella* belongs to the Apiaceae family, subfamily Apioideae, Tribus Apieae, subtribus Apiinae, and comprises about 90 species. The genus is divided into 2 sections, according to macroscopic criteria. *P. anisum* belongs to section Anisum. The original provenience of the plant is unknown. Nowadays it is cultivated in many parts of the world, mainly in southern Europe, India, China, Japan, Chile, Argentina and Mexico.

2.2 PLANT PARTS USED

The plant parts used are the fruits (Fructus Anisi) and the essential oil (Aetheroleum Anisi). Fructus Anisi consists of the whole dry cremocarp of *P. Anisum*. Aetheroleum Anisi is the essential oil obtained by steam distillation of the cremocarp of *P. anisum*. Both materials comply to the respective monographs of the European Pharmacopoea. In the past, the EAB also had permitted the use of the essential oil of *Illicium verum L.* (star anise) as Aetheroleum Anisi. This has changed, and according to the latest monograph version, only *P. anisum* oil is allowed (Ph. Eur. 5, 2005a, b).
The squeezed or powdered fruits are prepared as tea, and the essential oil can be used internally or externally (for inhalation). The essential oil can also be processed to spirit of anise or distilled anise water (Staesche *et al*., 1994).
As the drug is nowadays mainly obtained from cultivation, adulterations do not occur so often any more. The most dangerous adulteration were the toxic fruits of *Conium maculatum* L. (poison hemlock). They can be distinguished from *P. anisum* fruits by macroscopic criteria and by chemical detection of coniin. The essential oil of *I. verum* can be identified by the detection of foeniculin, which is not present in *P. anisum*. Admixture of synthetic anethole can be demonstrated by GLC-MS, as synthetic anethoel contains up to 15% *cis*-anethole which is normally only present in very small amounts in natural Aetheroleum Anisi (Staesche *et al*., 1994).

3. CONSTITUENTS

P. anisum fruits contain 2-6% essential oil, 30% fat, 20% proteins, 6-7% gum, 6-7% minerals, 5-7% pentosane, 4% sugars, 3% furfurol; furthermore phenolic acids and flavonol and flavone glycosides. The predominant constituent of the essential oil is *trans*-anethole, which makes up 80-95% of the total oil. The *cis*-anethole content is normally only 0.3-0.4%, but under the influence of direct sunlight, *trans*-anethole can partly isomerize to the more toxic *cis*-anethole; also small amounts of 4,4´-dimethoxystilben are produced due to UV-light; Oxygen leads to polymerization and oxidation to anise aldehyde and anisic acid. For this reason, the storage in closely sealed, lightproof flasks is obligatory (Staesche *et al*., 1994, Barnes *et al.,* 2002).
Furthermore, small amounts of estragol, pseudoisoeugenyl-2-methylbutrate, epoxypseudo-isoeugenyl-2-butyrate and anisaldehyde are present in the volatile oil (Staesche *et al.*, 1994). The European Pharmacopoea specifies a minimum essential oil content of 20 ml/kg or Fructus Anisi. The qualitative and quantitative analysis of the essential oil compounds can be performed by TLC and/or GC-MS. The *trans*-anethole content of the volatile oil has to be between 87 and 94% (Staesche *et al*. 1994, EAB, 2005).

4. PHARMACOLOGY

The pharmacological effects of aniseed are largely due to the presence of anethole, which is structurally related to the catecholamines adrenaline, noradrenaline and dopamine. Anethole dimers closely resemble the estrogenic agents stilbene and stilbestrol (Barnes *et al*., 2002).

4.1 IN VITRO AND ANIMAL TESTING

The antispasmodic activities of the essential oil have been shown in several *in vitro* models using isolated smooth muscles. Also an aqueous and an ethanolic aniseed extract showed significant spasmolytic acitivity in such a model (ESCOP, 2003). The relevance of these *in vitro* results for the therapeutic use against disorders of the gastrointestinal and the upper respiratory tract is questionable, because plasma levels are normally much lower than the concentrations used in the *in vitro* tests. However, in case of p.o. and inhalative application, effective concentrations might be reached locally (Staesche *et al*., 1994).

The expectorant and secretolytic activity of an aniseed infusion (200 µl, 4.6%) was shown *in vitro* using isolated ciliated epithelium of frog oesophagus. Dilutions of the essential oil increased respiratory tract fluid *in vivo* in anaesthesized guinea-pigs, rats and cats. A similar action was observed in anaesthesized rabbits inhaling anise oil (Barnes *et al.,* 2002).

Aniseed aqueous infusions and essential oil exhibited rather weak anticonvulsant effects i*n vivo*, and also a sedative effect could be shown for the essential oil and for its main compound *trans*-anethole respectively (Barnes *et al*., 2002, ESCOP, 2003).

For *trans*-anethole, also local anaesthetic, estrogenic, anti-tumor and anti-genotoxic effects were found *in vitro* and *in vivo* (ESCOP, 2003). The reputed lactagogic action of anise has been attributed to anethole, which exerts a competitive antagonism at dopamine receptor sites, and to the action of polymerized anethole due to its structural similarity to the estrogenic compounds stilbene and stilbestrol (Barnes *et al.*, 2002). Kassi *et al.* (2004) report that aqueous aniseed extracts show SERM (selective estrogen receptor modulator)-like effects on bone, breast and uterine cells in *vitro*. The observed effects were not attributed to a certain compound of the extract. Tabanca *et al.* (2004) tested 11 Pimpinella species for estrogenic activity *in vitro* using the yeast estrogen screen assay. Generally, the authors only found weak estrogenic activity, and there was no significant correlation between anethole content and estrogenic activity of the essential oils. This indicates that other constituents may contribute to the estrogenic acitivity of the essential oil.

Antioxidative activity was reported for aqueous and ethanolic anise fruit extracts in several *in vitro* models (Gülçın *et al*., 2003; Al-Ismail and Aburjai, 2004).

Additionally, the reputed insecticidal activities of aniseed have been assessed in several studies: Prajapati *et al.* (2005) found strong larvicidal and ovicidal activity of *P. anisum* essential oil against three mosquito species. Lee (2004) performed a bioassay guided fractionation of Anise essential oil and found, that p-anisaldehyde is mainly responsible for the acaricidal activity of the oil against two house dust mite species. The compound was even more effective then the synthetic acaricides benzoyl benzoate and N,N-diethyl-*m*-toluamide. It also showed very high activity against the stored food mite (*Tyrophagus putrescentiae*) (Lee, 2005). Also anethole, anisaldehyde and myristicin have exhibited mild insecticidal properties (Barnes *et al.*, 2002).

4.2 VETERINARY STUDIES

Ruminal microbial activity is essential for the decomposition of structural carbohydrates and the synthesis of high-quality protein in ruminants. However, microbial fermentation may lead to considerable energy and protein losses due to methane and ammonia production. For this reason, many feed additives have been developed to improve the efficacy of nutrient use by decreasing methane or ammonia production. Ionophore antibiotics have been used successfully, however the use of antibiotics in animal feed has been prohibited in the European Union in 2006. So, the search for alternatives, particularly from plants, is under progress (Cardozo *et al*., 2004).

Several *in vitro* studies have included anise oil and anethole, yielding mixed results:

Cardozo *et al.* (2004) evaluated the effects of several plant extracts on ruminal microbial fermentation *in vitro*. Volatile fatty acid (VFA) concentration and protein degradation were determined in a dual-flow continuous culture system inoculated with ruminal fluid from dairy cows. Anise oil and some other extracts affected the molar proportions of acetate, propionate and butyrate during the adaption period, however these differences disappeared after day 6, indicating that the ruminal microbes built up a tolerance to the additive after 6 days. The addition of 7.5 mg/kg dry matter (equivalent to 0.22 mg/l rumen fluid) of anise essential oil

(86% anethole) increased the peptide N concentration by 79% 2 h after feeding, but did not affect the average peptide concentration throughout the 8 hours feeding interval. The higher average amino acid concentration, the higher ammonia concentration at 0,2,4, and 6 hours after feeding and the higher average ammonia concentration indicate that the anise extract stimulated peptidolysis and deamination. According to the authors, the accumulation of peptides and amino acids in ruminal fluid may stimulate microbial protein synthesis or improve the flow of amino acids to the small intestine.
In a second study, Cardozo and coauthors (2005) tested among others anise essential oil and anethole at various doses (0.3-300 mg/l) for their influence on the ruminal microbial fermentation profile in an *in vitro* batch culture fermentation system at 2 different pH (7.0 and 5.5). The authors found out that the effects of plant extracts on ruminal microbial fermentation are pH-dependent. At high pH, the plant extracts exhibited no beneficial effects for beef production. At pH 5.5 however, beneficial effects were observed for some preparations. In case of anise essential oil and anethole the VFA concentration did not change, and ammonia nitrogen and branched-chain volatile fatty acid concentrations were decreased, suggesting that deamination was inhibited or that bacteria used peptides and amino acids as a nitrogen source, thus decreasing ammonia concentration. High doses of all tested extracts resulted in lower VFA concentrations, confirming their antimicrobial effect. Considering the pH-dependency of the effects of the tested plant extracts, the authors suggest that *in vivo* studies should be conducted due to the daily fluctuation of pH generally observed *in vivo*.
Busquet *et al*. (2006) also used *in vitro* batch culture of rumen fluid to test several essential oils and pure compounds at different doses. In accordance to Cardozo and coauthors (2005), they reported detrimental effects on rumen microbial fermentation of many of the tested preparations at high doses (3000 mg/l) due to their antimicrobial activity.
Anise oil and anethole both decreased total VFA and the proportion of acetate and propionate, and increased the proportion of butyrate, anethole exhibiting the stronger effects than anise oil. Ammonia concentration was not affected. As anethole reduced the proportion of acetate and propionate, which are the main precursors for ruminant fat and glucose synthesis, the authors suggest that they may not be nutritionally beneficial to dairy cattle. However, in this study, a pH of 7.0 was used, which had been reported to be unfavourable by Cardozo *et al*. (2005), and as a batch culture fermentation system was used, long-term effects such as adaption of the microorganisms could not be considered in this study.

4.3 HUMAN STUDIES

There is a lack of documented clinical studies on aniseed (Barnes *et al*., 2002).

4.4 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

Few pharmacokinetic data exist about *trans*-anethole with regard to toxicity:
In mice and rats, *trans*-anethole is reported to be metabolized by O-demethylation and by oxidative transformation of the C_3-side chain. After ingestion of low doses (0.05 and 5 mg/kg b.w.) predominantly *O*-demethylation occurs, whereas at higher doses (up to 1500 mg/kg b.w.) there is a shift towards oxygenated metabolites (ESCOP, 2003). Especially in female rats, the hepatotoxic metabolite anetholepoxide is predominantly produced at higher concentrations (Newberne *et al.,* 1999).
In humans, already after administration of 1 mg *trans*-anethole the oxidative metabolism is predominant, with the 4-methoxyhippuric acid as the main metabolite. After p.o. administration of 1, 50 and 250 mg *trans* -anethole to healthy volunteers respectively, the substance was readily absorbed, and 54 to 69% of the dose (detected as ^{14}C) were eliminated in the urine and at least 13-17% in exhaled carbon dioxide, irrespective of the dose level. The substance was nearly completely eliminated within the first 8 hours (Staesche *et al.,* 1994, ESCOP, 2003).
No data are available concerning the pharmacokinetics of other anise constituents, and no pharmacokinetic studies have been performed in productive livestock.

5. MICROBIOLOGY

As many other essential oil containing plants, *P. anisum* shows antimicrobial activity:
An acetone extract of aniseed inhibited the growth of a range of bacteria including *Escherichia coli* and *Staphylococcus aureus*. The essential oil also inhibited the growth of *E. coli* (MIC 0.5% V/V), *Staphylococcus aureus* (MIC: 0.25% V/V) and *Salmonella typhimurium* (MIC 2.0%), when tested using the agar dilution method (ESCOP, 2003). Singh *et al.* (2002) found a quite high activity of *P. anisum* essential oil against several gram positive and gram negative organisms using the filter paper disc agar method, but no MIC was determined. Also aqueous and ethanolic extracts were reported to possess inhibitory activity against a several gram positive and gram negative bacteria, but for testing high concentrations were used, and no MIC was determined (Gülçın *et al.,* 2003). Recently, only weak activity against *Helicobacter pylori* was reported for an aniseed methanolic extract (MIC >100 µg/ml) (Mahady *et al*., 2005).
The acetone extract and the essential oil exhibit antifungal effects against *Candida albicans* and other organisms (MIC of the essential oil against *C. albicans* 0.5% using the agar dilution method) (ESCOP, 2003).
Trans-anethole was assayed for its fungicidal activity in *Saccharomyces cerevisiae*, grown under hypoxic and aerobic conditions. The results indicate that the antifungal activity of the compound is affected by the concentration of dissolved oxygen in the culture broth. Furthermore, the authors demonstrated that anethole does not exhibit fungicidal activity against non-growing cells, and they suggest that the compound might only be active against fermentatively growing cells (Fujita and Kubo, 2004).

6. EFFICACY

As far as their medical use in humans is concerned, *P. anisum* fruits and essential oil are stated to be effective in the treatment of dyspeptic complaints such as mild spasmodic gastrointestinal disorders, bloating, flatulence (internally) and against catarrh of the upper respiratory tract (internally and externally) (Wichtl, 2002; ESCOP, 2003). Up to now, no clinical data exist on the efficacy in these applications, so these indications are based on experience and on the available *in vitro* and *in vivo* data.

Studies on the efficacy of *P. anisum* in productive livestock are very limited. The outcomes of *in vitro* studies investigating the potential of *P. anisum* essential oil as a feed additive to improve nutrient use in ruminants are inconclusive, and more and larger studies, especially *in vivo* studies, will be necessary for evaluation of efficacy.
El-Deek and coauthors (2003) investigated the potential of aniseed as alternative growth promoting substance in broilers. When admixed to the feed at a concentration of 0.05% at day 12 to 47 of age, aniseed improved weight gains significantly by 19.1% and feed conversion ratio by 10.85% with respect to the control group. Admixture of 0.1% only enhanced feed conversion ratio by 1.92%, related to a 2.97% decrease in feed intake; also fat content of the meat was significantly reduced with this diet. Use of a two or three way mixture of anise, ginger and fennel had no additive effect on growth as compared to the control group.
Grela *et al.* (2003) investigated the influence of dietary supplementation with two different herbal mixtures, one of them containing *P. anisum* fruits, on the general performance and blood parameters of piglets. After 40 days, they compared the results to a control group receiving standard feed and a positive control receiving 20 mg avilamycin/kg diet. The group fed the mixture containing *Urtica dioica* leaves, *Plantago lanceolata* herb, lyophilized *Allium sativum* bulbs, *P. anisum* fruits and *Echinacea purpurea* fruits had the best daily gains, feed utilization and blood indices; the production gains were even better than with the antibiotic additive. Due to the complexity of the mixture, the contribution of aniseed to this effect can not be estimated.
Aniseed is extensively used as a sensory and flavouring agent in humans. To what extent it is also suitable for this purpose in productive livestock, remains to be investigated.

By the Council of Europe, it is listed as a natural source of food flavouring in category N2. This category allows small quantities of aniseed to be added to foodstuffs, with a possible limitation of an active principle (as yet unspecified) in the final product. In the USA, aniseed is listed as GRAS (Generally Recognized As Safe). Anethole was reaffirmed as GRAS in 1997 (Barnes *et al.,* 2002.)

7. TOXICOLOGY

7.1. SAFETY

Contact dermatitis reactions to aniseed and aniseed oil have been attributed to anethole. Reactions have been reported with products, such as creams and toothpastes flavoured with aniseed oil. The volatile oil and anethole have been stated to be irritant and sensitizing (Barnes *et al.,* 2002).

Most toxicity studies relevant to aniseed have been conducted on *trans*-anethole, the main constituent of the essential oil:

As far as the acute toxicity is concerned, the LD_{50} in rats is 2.7g/kg b.w. for the essential oil and 2.1-3.2 g for *trans*-anethole respectively. Intraperitoneal LD_{50} values for *trans*-anethole have been determined as 0.65-1.41 g/kg b.w. in mice and 0.9-2.67 g/kg b.w. in rats (ESCOP, 2003).

Concerning the repeated-dose toxicity, hepatic changes have been described in rats fed *trans*-anethole in their daily diet (1%) for 15 weeks, although at a level of 0.25% no changes were observed after 1 year. Rats fed with 0.1% *trans*-anethole in their diet for 90 days showed no signs of toxicity, but at higher doses (0.3-3%) liver edema was observed. In therapeutic doses *trans*-anethole is reported to cause minimal hepatotoxicity.

Animal studies on the reproductive toxicity of *trans*-anethole (50-80 mg/kg) demonstrated that the compound has antifertility activity and might also have an early abortifacient activitiy. Significant estrogenic activity was observed, but no anti-estrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity (Barnes *et al*., 2002, ESCOP, 2003).

In the Ames test, a dry ethanolic aniseed extract was only mutagenic at high concentrations (5 mg/plate) to streptomycin dependent strains of *Salmonella typhimurium* TA 98. The essential oil and *trans*-anethole were mutagenic at 2 mg/plate, and mutagenicity was potentiated by S13 activiation. Other investigations with metabolic activation have confirmed that *trans*-anethole is weakly mutagenic. Estragol, a minor constituent of the essential oil, has also shown mutagenic potential in various Ames tests, demonstrating the need for carcinogenicity studies.

Trans-anethole did not show carcinogenic potential in a 1-year experiment with mice receiving the compound in their diet. In another long term study of *trans*-anethole in mice, there were no histological differences between treated and control animals.

In a study in rats, the highest dose feeding group (1% *trans*-anethole for 117 weeks) showed hyperplastic and partially neoplastic changes, only in the livers of female rats. This led to the review of safety data on *trans*-anethole as a flavouring substance by the Expert Panel of the Flavour and Extract Manufacturer Association (FEMA). The Expert Panel stated that the observed neoplastic changes in the liver of female rats were not due to a direct genotoxic effect, but were secondary to hepatotoxicity caused by continuous exprosures to high hepatocellular concentrations of anethole epoxide (AE). At low levels, *trans*-anethole is efficiently detoxicated in rodents and humans primarily by *O*-demethylation and ω-oxidation. At high dose levels, particularly in female rats, a metabolic shift occurs resulting in increased epoxidation and formation of AE. The continuous intake of high levels of *trans*-anethole has been shown to induce a continuum of cytotoxicity, cell necrosis and cell proliferation due to AE production. However, the intake of *trans*-anethole as a flavouring substance was stated to be very low (estimated 54 µg/kg b.w./day), and at this level it is efficiently detoxicated in humans. For this reason the Expert Panel concludes that the neoplastic effects in female rats associated with dose dependent hepatotoxicity are not indicators of any significant risk to human health from the use of *trans*-anethole as a flavouring substance, and the compound was reaffirmed as GRAS (generally regarded as safe) (Newberne *et al*., 1999). However, clinical safety data do not exist.

The safety of aniseed taken during pregnancy and lactation has not been established. Aniseed may be used at the recommended dosage as an aqueous infusion only. Preparations containing the essential oil or ethanolic extracts should not be used (ESCOP, 2003).
As aniseed may cause allergic reactions, the use of the volatile oil should be avoided in dermatitis and inflammatory or allergic skin conditions. Aniseed should not be used by persons with known sensitivity to anethole (Barnes *et al*., 2002).
Herb-drug interactions are not known, and experimental data on weak enzyme induction in rodents can not directly be extrapolated to humans (ESCOP, 2003).

7.2. DOSAGE

Dried fruit:
Adult average daily dose: 3 g of crushed fruits as an infusion or similar preparations
Children average daily dose: 0-1 year 0.5 g crushed fruits as an infusion; 1-4 years 1g; 4-10 years 2g; then adult dose (ESCOP, 2003).
*Oil:*0.05-0.2 ml 3 times daily.
Spirit of anise: 0.3-1 ml 3 times daily.
Distilled anise water: 15-30 ml 3 times daily (Barnes *et al.* 2002).

ANIMALS:
For internal use either powdered aniseed or infusions (1:10) out of it will be prepared and added to the fodder (Reichling et al. 2005).

Internal Use of Aniseed:
Dosages
recommended daily allowance:

Cattle	25,0 - 50,0 g
Horse	10,0 - 25,0 g
Sheep	5,0 - 10,0 g
Pig	3,0 - 10,0 g
Chicken	0,2 - 0,5g

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RIBES NIGRUM

Ribes nigrum

1. INTRODUCTION

The use of *Ribes nigrum* L. (Grossulariaceae) has a long tradition in Europe. The leaves are mainly used for medical purposes. The have been used in traditional medicine internally as diuretics, for the treatment of gout, rheumatic diseases, diarrhea, cramps and cough and externally for the treatment of wounds and insect bites. The drug is nowadays mainly used in the form of tea as a diuretic and as an adjuvant in the therapy of rheumatic disorders (Wichtl, 2002; ESCOP, 2003). The efficacy in these indications is not yet fully documented (Seitz, 1994).

The fruits are used in traditional medicine internally as syrup, juice, jelly or lozenges for the treatment of common cold, sore throat and cough and as a vitamin C source, against diarrhea and stomach pain. The dried berries are sometimes chewed to treat disorders of the urinary tract. In France, they are traditionally used in the treatment of venous disorders such as heavy legs, hemorrhoids and for the symptomatic treatment of hematoma. Also in this case the efficacy in most indications is not yet documented (Seitz, 1994).

Furthermore blackcurrant fruits are used in industry for the production of fruit juices and concentrates, marmelades and candies. The seed containing pomace is often used as animal feed in husbandry because of its high nutrient value. The seed oil can also be used for the extraction of pure γ-linolenic acid and concentrates rich in γ-linolenic acid which are for example used as food supplements and in cosmetic industry (Seitz, 1994; Wichtl, 2002).

2. DESCRIPTION OF THE PLANT

Ribes L. is the only genus the Grossulariaceae family consists of. It comprises 140-150 species, which are split up into 6 subgenera. *R. nigrum* belongs to the subgenus Coreosma (Seitz, 1994). *R. nigrum* is a deciduous shrub with a height of up to 2 meters. The leaves are divided in 3-5 lobes, are more or less cordiform at the leaf base, and the leaf edges are doubly serrate. The flowers are greenish-white and arranged in racemous inflorescences. The fruits are brownish-black spheroidal berries with a peculiar smell. The plant occurs in the European-asian woodland, in Canada and Australia. In central and eastern Europe, *R. nigrum* sometimes still can be found as a wild plant, but in most regions it is cultivated (Seitz, 1994; Wichtl, 2002).

The plant parts used in medicine are the leaves and the fruits. Blackcurrant leaves consist of the dried leaves of *Ribes nigrum* L., harvested during or breviously after the anthesis. The material complies with the monograph of the Pharmacopée francaise (Seitz, 1994, ESCOP, 2003). Blackcurrant fruits are harvested at full ripeness and are immediately processed to various preparations or frozen (Seitz, 1994).

3. CHEMICAL CONSTITUENTS

Blackcurrant leaves

The most important constituents are flavonol glycosides, mainly mono- and diglycosides of quercetin, kaempferol, isorhamnetin and myricetin (main glycosides: isoquercitrin, rutoside) (Seitz, 1994, ESCOP, 2003). According to the monograph of the Pharmacopée francaise, the drug must contain a minimum of 1.5% flavonoids, expressed as rutin (ESCOP, 2003). Determination of flavonoid content can be performed photometrically after addition of aluminium chloride solution to a methanolic extract of the material. The identity can be controlled by TLC examination of the methanolic extract, using isoquercitrin and rutoside as standard compounds.

Furthermore the drug contains about 0.4% oligomeric proanthocyanidines, vitamin C and traces of essential oil (Seitz, 1994).

Blackcurrant fruits

The main constituents are anthocyanins (up to 2% of fresh weight), with cyanidin-3-glucoside (17% of anthocyanin fraction), cyanidin-3-rutinoside (35%), delphinidin-3-glucoside (13%) and delphinidin-3-rutinoside (30%) as the main compounds (Seitz, 1994). The anthocyanins can be analyzed and quantified by HPLC with PDA and/or MS detection (for example Rubinskiene *et al*., 2005). Using this method, the authors did not only quantify the main anthocyanins present in various *R. nigrum* cultivars, they also investigated the effect of temperature, light and storage time on the stability of these compounds. They found that all anthocyanins are to a certain extent susceptible to UV light and to a variable degree also to temperature. The anthocyanin showing the best thermal stability was cyanidin-3-rutinoside. After storage at 8°C for 12 months it could be shown that rutinosides were more stable than glucosides.

Additionally, flavonol glycosides are present in blackcurrant fruits, with isoquercitrin, myricetin-D-glucopyranoside and rutoside as the main compounds. Organic acids and hydroxycinnamic acid derivatives like caffeoylquinic acids, p-coumaroylquinic acids and feruloylquinic acids are also present. The specifications concerning the vitamin C content vary tremendously from 10 to 300 mg/100 g, depending on the used determination method.

The seeds contain up to 30% fatty oil which is a rich source of γ-linolenic acid. Besides, 6-8% invert sugar and pectins are present (Seitz, 1994).

4. PHARMACOLOGY

4.1 IN VITRO AND ANIMAL TESTING

4.1.1 BLACKCURRANT LEAVES

A 14%-ethanolic extract from blackcurrant leaves produced dose-dependent anti-inflammatory effects in carrageenan induced rat paw edema after oral administration (ESCOP, 2003). Tits *et al.* (1991) isolated prodelphinidin oligomers from *R. nigrum* leaves that also showed inhibitory activity in this model.

In another study, a lyophilized 15%-ethanolic extract showed dose dependent anti-inflammatory activity in several animal models of inflammation. The same lyophilizate exhibited potent analgesic activity in the acetic acid-induced writhing test after single dose intraperitoneal administration to mice (ESCOP, 2003).

Garbacki *et al.* (2002) tested proanthocyanidins isolated from *R. nigrum* leaves to evaluate their chondroprotective potential and their COX inhibition properties using human cartilage, purified COX-1 and COX-2 enzymes and a COX whole blood assay. The compounds stimulated type II collagen production and inhibited purified COX with a certain selectivity against COX-2, but they had no effect on COX in the whole blood assay.

In 2004, Garbacki *et al.* found, that the anti-inflammatory activity of proanthocyanidins isolated from blackcurrant leaves is related to an interference with the migration of leukocytes, associated with the reduction of pro-inflammatory factors such as TNF-α, IL-1β and CINC-1, a decrease of NOx level and a decrease of plasma exsudation.

The transvasation of the leukocytes from blood to the inflammatory exudates requires activation of leukocytes and the expression of adhesion molecules on the surface of endothelial cells. Garbacki *et al.* (2005) showed that the proanthocyanidins did not significantly reduce the expression of adhesion factors on the surface of the leukocytes, but greatly reduced the production of adhesion molecules on the surface of endothelial cells *in vivo* during carrageenin-induced pleurisy. This indicates that the anti-inflammatory activity of *R. nigrum* procyanidins is related to the down-regulation of endothelial adhesion molecules.

Furthermore, diuretic and antihypertensive effects have been shown in several animal studies, and a reduction of capillary permeability has been shown in a rat model (Seitz, 1994; ESCOP, 2003).

4.1.2 BLACKCURRANT FRUITS AND SEEDS

4.1.2.1 ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY

The antioxidant activity of blackcurrant fruit extracts and polyphenolics has been tested *in vitro* in many different assays using various reference antioxidants such as trolox, L-ascorbic acid or α-tocopherol. This makes the comparison of results very difficult.

For example, Ehala *et al.* (2005) used an ABTS radical cation decolorization assay for determination of the antioxidant activity of various berry extracts. The radical was produced with potassium persulfate. Bermúdez-Soto *et al.* (2004) determined the antioxidant activity of fruit juice concentrates in the ABTS-assay using MnO_2 for radical production and in the DPPH-assay. Wu and coauthors (2004) used hydrophilic and lipophilic $ORAC_{FL}$ assays for the determination of the antioxidant activity of berry extracts. Blackcurrant showed very high hydrophilic antioxidant capacities, whereas the lipophilic antioxidant capacity was rather low.

In all of these studies, the antioxidant activity of the extracts showed quite good correlation with their total phenolic content.

The relevance of these *in vitro* results for *in vivo* effects is difficult to estimate and the antioxidant activity of blackcurrant could not yet be verified by clinical studies.

In foods, lipid oxidation can cause protein oxidation due to close interaction between lipids and proteins. Viljanen *et al.* (2004) investigated the effect of black currants and other berries on the oxidative stability of proteins and lipids in a liposome system. They found an antioxidant effect in both assays. The effect was more pronounced toward lipid than protein oxidation. According to the authors, this antioxidant protection may result in an improved quality of foods, especially of dairy origin.

Večeřa and coauthors (2003) compared the effects of blackcurrant seed oil rich in (ω-3) and (ω-

6) polyunsaturated fatty acids in rats fed a diet rich in cholesterol to the effects of lard fat rich in saturated and monounsaturated fatty acids on antioxidant parameters, the lipoprotein profile and liver lipids. The results of the study indicate that currant oil, when substituted to lard fat in a high cholesterol diet, partly exerts a positive effect on lipid metabolism in the liver, but has partly adverse effects on the antioxidative status, especially in the blood.

4.1.2.2 ANTI-INFLAMMATORY, ANTITUMOR AND IMMUNOSTIMULATORY ACTIVITY

A polysaccharide rich fraction from blackcurrant fruit juice showed macrophage stimulating activity *in vitro* and antitumor activity in Ehrlich carcinoma-bearing mice with a certain cytotoxicity directly against tumor cells (Takata *et al*., 2005).

Because the eicosanoid cascade is regulated in part by the distribution of arachidonic acid among phospholipid subclasses, the effects of feeding blackcurrant seed oil, which is rich in γ- and α- linolenic acid, were studied in mouse peritoneal macrophages after 4 weeks of dietary treatment. The authors could show that feeding with γ- and α- linolenic acid can increase potential anti-inflammatory precursor levels of 20:3 (ω-6) and (ω-3) polyunsaturated fatty acids in the macrophages (Chapkin *et al*., 1990).

4.1.2.3 OTHER ACTIVITES

In vivo antihypertensive (Seitz*,* 1994) activity and *in vitro* vasorelaxant effects (Nakamura *et al.,* 2002) were found.

4.2 VETERINARY STUDIES

The effect of a herbal mixture containing extracts from *R. nigrum*, *Echinacea angustifolia, Agrimonia eupatoria* and *Cinchona succirubra* (plant parts not given) was tested in broiler chickens challenged with *Eimeria tenella* using lasolacid as a positive control. The herbal feed supplement led to a certain coccidiostatic effect, which was however significantly lower than that of lasolacid (Christaki *et al*., 2004). The contribution *of R. nigrum* to the observed effect can not be estimated.

4.3 HUMAN STUDIES

The effect of dietary supplementation with blackcurrant seed oil (BCO) was investigated in patients suffering from rheumatoid arthritis (RA) as well as in healthy volunteers, using sunflower oil as a placebo. A significant improvement in morning stiffness was noted in the RA patients receiving BCO. Cytokine and PGE_2 production of the cultured macrophages was markedly altered in subjects given BCO. The results suggest that the beneficial effects of polyunsaturated fatty acids in inflammatory diseases may be due to a reduction of the secretion of proinflammatory cytokines via redirection of eicosanoid metabolism (Watson *et al*., 1993).

Also Levental *et al*. (1994) found certain positive effects of BSO administered to patients with RA and active synovitis in a double blind, placebo controlled, 24 week trial. BSO treatment resulted in in a reduction in signs and symptoms of disease activity in patients with RA, but overall clinical responses were not significantly better than in the placebo group.

Møller *et al.* (2004) performed a three- week, controlled, parallel intervention study investigating the antioxidative effect of blackcurrant juice and of an anthocyanin-rich drink in humans. They determined the steady state level of oxidative DNA damage in mononuclear blood cells using the alkaline comet assay. Daily doses ranged from 475 to 1000 ml juice according to body weight (mean anthocyanin intake for blackcurrant juice and anthocyanin drink was 397 and 365 mg respectively). The study shows that even large amounts of dietary antioxidants did not decrease the (already low) steady state levels of oxidative DNA damage in healthy, adequately nourished humans.

4.4 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

There are some data available concerning the pharmacokinetics of blackcurrant anthocyanins in humans and animals:
Netzel *et al.* (2000) analyzed urinary samples from 3 men before and after ingestion of 200 ml blackcurrant juice for delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside. The cumulative amounts of excreted unchanged anthocyanins in the urine were only 0.02-0.05% of the ingested doses. Degradation during the digestion process, losses in feces and intermediary metabolism may contribute to the low urinary output.
Wu and coauthors (2005) investigated the metabolism of anthoycanins in weanling pigs fed with freeze dried powder of chokeberry, black currant or elderberry in a single meal. Blood and urine samples were collected at particular times after ingestion and analyzed by HPLC-ESIMS^2.The authors found that the aglycone and sugar moieties can alter absorption and metabolism of the anthocyanins: Delphinidin was not metabolized to any measurable extent. Cyanidin was metabolized via methylation and/or glucuronidation, the same happened to monoglycosides other than the glucoside. Di-or triglycosides were excreted in the urine primarly in the intact form (over 80% of the anthocyanins containing rutinose or sambubiose).
According to the authors, also the total anthocyanin composition of the respective berry influences uptake and metabolism, thus the results must be interpreted in the context of the composition of the complete berry.
Nielsen *et al.* (2003) performed a study to compare the pharmacokinetics of blackcurrant anthocyanins in humans and Watanabe heritable hyperlipidemic (WHHL) rabbits, a model for human homozygous familial hypercholesterolemia due to LDL receptor deficiency. In a single dose study, the rabbits were intubated with black currant juice concentrate, blackcurrant anthocyanins dissolved in aqueous citric acid or aqueous citric acid only. The normolipidemic volunteers participating in the study received either a blackcurrant concentrate alone, blackcurrant concentrate in combination with a rice cake, or control solution in a partial crossover study. Blood and urine samples were taken at particular times after ingestion, and the four major blackcurrant anthocyanins were quantified in plasma and urine by HPLC (metabolites were not analyzed). In the rabbit study a food matrix effect was observed resulting in a higher absorption of anthocyanins from juice than from anthocyanin solution. In the human study the plasma concentration and urinary excretion of anthocyanins were found to be dose dependent and not influenced by intake of the rice cake. A higher plasma concentration and urinary excretion of anthocyanin rutinosides than of glucosides was found, whereas the nature of the aglycone did not influence the bioavailability. This might be due to the cleaveage of the anthocyanin glucosides but not rutinosides by intestinal β-glucosidases. The anthocyanins did not affect the antioxidant capacity of plasma measured as trolox equivalent antioxidant capacity and ferric acid reducing ability in rabbits. This indicates that a possible protective effect on the development of atherosclerosis is unlikely to be due to an increased redox capacity of the plasma.

5. MICROBIOLOGY

Puupponen-Pimiä *et al*. (2001) measured the antimicrobial activity of several berry extracts against selected gram positive and gram negative bacteria, including probiotic bacteria and intestinal pathogens. Among the 8 extracts tested, blackcurrant showed the least antimicrobial activity. In liquid culture, only *Escherichia coli* CM 871 (a DNA repair-deficient strain) and *Salmonella enterica* were strongly inhibitied by the extract, the growth of several *Lactobacillus rhamnosus* and *paracasei* strains was slightly increased.

Cavanagh and coauthors (2003) tested several commercial berry drinks (100% fruit) as well as fresh berries for their inhibition of the growth of various bacteria and the yeast *Candida albicans*. The blackcurrant drink inhibited all 12 bacteria and *C. albicans* when diluted 1:5.

Crude and purified polysaccharides from blackcurrant seeds were investigated on their effect against *Helicobacter pylori* in *in situ* adhesion studies on sections of human gastric mucosa. After pre-treatment of *H. pylori* with the polysaccharide solutions, the epithelial binding of the bacteria was considerably reduced in a concentration dependent manner. As none of the active fractions showed inhibitory effects on bacterial growth *in vitro*, the authors concluded that the

polysaccharides are able to block *H. pylori* surface receptors, thus inhibiting their interaction with specific binding factors located on human gastric epithelia (Lengsfeld *et al*., 2004).

There are also two reports concerning antiviral activity of Kurokarin®, a crude extract of wild *R. nigrum* fruits commercially available in Japan:

Knox *et al.* (2003) investigated the antiviral activity of Kurokarin against influenza virus IVA and IVB *in vitro*. The extract inhibited IVA and IVB with an IC_{50} of 3.2 µg/ml. Moreover, heat treatment at 121°C for 20 minutes did not affect antiviral activity of the extract. This is an important finding for the potential of *R. nigrum* in industrial processing applications. Suzutani *et al*. (2003) tested the same extract for *in vitro* anti-herpesvirus activity. The extract inhibited HSV 1 attachment on the cell membrane completely at a 100fold dilution, as well as the plaque formation of HSV 1 and HSV 2, and varicella-zoster virus by 50% at a 400 fold dilution. The latter activity is due to the inhibition of protein synthesis in infected cells from the early stage of infection.

6. EFFICACY

Blackcurrant berries are rich in vitamin C. The seeds contain high amounts of fatty oil; because of this high nutrient value blackcurrant pomace is often used in husbandry as a mast feed (Hänsel, 1994). No scientific data exist about the efficacy of blackcurrant fruits of leaves as a sensory and flavouring agent for animal feed production.

In humans, the leaves are nowadays considered to be effective as an adjuvant in the treatment of rheumatic conditions (ESCOP, 2003). Because clinical studies are very rare, the efficacy for most traditional indications of blackcurrant fruits and berries is not yet documented.

7. TOXICOLOGY

7.1 SAFETY

A lyophilized 14% ethanolic blackcurrant leaf extract, administered orally to rats at 2 g/kg/day for 21 days or 1.34 g/kg/day for 28 days, revealed no signs of toxicity and no gastric ulceration was observed.

Rats treated orally for 28 days with a lyophilized blackcurrant leaf 15% ethanolic extracts had no gastric ulceration. No changes were apparent in food and fluid consumption or body weight, nor in results from blood analysis and histopathological evaluation of 14 different organs. In an acute toxicity study of the same lyophilizate in mice, the intraperitoneal LD_{50} was 1.90 g/kg. Oral doses of up to 3g/kg showed no overt toxicity. In another study, the LD_0 and the LD_{50} of a blackcurrant leaf fluid extract in mice were equivalent to 22 and 49 g blackcurrant leaf/kg respectively (ESCOP, 2003).

After feeding of dried blackcurrant fruit peel and pulp to mice (1000 and 2000 mg/kg orally for 7 days), no toxic effects were observed (Hänsel, 1994).

7.2 DOSAGE

Adults: dried leaves as an infusion (20-50 g/litre), 250-500 ml daily; fluid extract (1:1), 5 ml twice daily, taken before meals (ESCOP, 2003).

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ROSMARINUS OFFICINALIS




Rosmarinus officinalis

1. INTRODUCTION

The genus and common name are derived from the Latin "ros marinus", meaning "dew of the sea" as the plant grows profusely near the Mediterranean sea coast and sea foam sprays upon it. Rosemary has long been considered a symbol of friendship and loyalty. The Ancients were well acquainted with the rosemary, which had a reputation for strengthening the memory. On this account, it became the emblem of fidelity for lovers. Not only was it used at weddings, but also for decking churches and banqueting halls at festivals, and incense in religious ceremonies and in magical spells. The Spaniards revere it as one of the bushes that gave shelter to the Virgin Mary in the flight into Egypt, and call it Romero, the Pilgrim's Flower. Both in Spain and Italy, it has been considered a safeguard from witches and evil influences generally (Grieve, 1996).
In early times Rosemary was freely cultivated in kitchen gardens. In addition to the well known culinary use, various medicinal properties are also associated with the herb. Documented antibacterial, antinflammatory and spasmolytic actions, which support the traditional use, are attributable to the essential oil (Barnes, 2002).
The rosemary oil as reported by the European Pharmacopeia (Ph.Eur. 5, 2005) is obtained by steam distillation from the flowering tops. Nevertheless almost all the commercial oil is distilled from the stem and leaves of the plant before it is in flower.

2. DESCRIPTION OF THE PLANT

Rosmarinus officinalis L. is a small evergeen branched shrub, 50 to 150 cm high, with erect , climbing or occasionally decumbent brown branches. The leaves are sessile, thick aromatic, linear, coriaceous and entire-margined. The upper surface is dark green, glabrous and grainy, the lower surface is greyish-green and densely tomentose, 15 to 40 mm by 1.2 to 3.5 mm, with recurved edges. The flowers grow on tomentose inflorescences in the leaf axils of the upper part of the branches. The calyx is 3 to 4 mm, green or reddish, initially tomentose, later 5 to 7 mm and glabrous. The corolla is 10 to 12 mm long, bluish, occasionally pinck or with. The nutlet is brown.

2.1 SYSTEMATICS

Rosmarinus officinalis belongs to the Family Lamiaceae being the only species of this genus.
It has various vernacular synonyms: Folia Anthos, Folia Roris marini (Lat.), Sea Dew, Our Lady's Rose, Polar Plant, Compass Weed, Romero, and Rosemarine.
Not to be confused with Ledum palustre, Andromeda polifolia, Teucrium montanum, Taxus baccata, Santolina rosmarifolia and S. chamaecyparissus.

2.2 OCCURRENCE

It's native in the Mediterranean region and Portugal and is cultivated there as well as on the Central Asia, India, Southeast Asia, South Africa, Australia and the U.S.

2.3 PLANT PARTS AND PRODUCTS

Rosemary is available as whole crude drug of the flowering dried twig tips, the dried leaves, the fresh leaves, the fresh aerial parts collected during flowering and the flowering branches. The second main product is rosemary essential oil. But also powder, infusion, dry extracts or other galenic preparations for internal (oral administration) and external (topical application) use are on the market. It is also available in composed preparations.

The essential oil of rosemary distilled from the flowering tops, is superior to that obtained from the stems and leaves but nearly all the commercial oil is distilled from stems and leaves of the plant before it is in flower.

The essential oil is colourless or pale yellow with the characteristics of rosemary and a warm camphoraceous taste. It is used in the perfume industry and as flavour agent. It is, however, known that variants of the same species can differ in their constituents essential oils.

3. INGREDIENTS/CONSTITUENTS (CHEM. ANALYSIS, STANDARDIZATION)

Rosmarini aetheroleum is the volatile oil obtained by steam distillation from the aerial parts of *Rosmarinus officinalis*. Its composition may vary according to chemotype. Characteristic components of the oil are: 1,8-cineole (20-50%), alfa-pinene (15-26%), camphor (10-25%), bornyl acetate (1-5%), borneol (1-6%), camphene (5-10%) and alfa-terpineol (12-24%). Limonene, beta-pinene, beta-caryophyllene and myrcene are also present in the oil (ESCOP, 2003).

Flavonoids

Include diosmetin, diosmin, genkwanin and derivatives, luteolin and derivatives, hispidulin, neptin, nepitrin and apigenin (ESCOP, 2003; PDR, 2004).

Other charateristic constituents

Phenols: caffeic, chlorogenic, labiatic, neochlorogenic.

phenolic diterpenes suchs as carnosol (up to 4.6%), carnosolic acid, rosmanol, isorosmanol, epirosmanol, rosmaridiphenol, rosmariquinone, hydroxycinnamic derivatives, e.g. rosmarinic acid (2-3%) (diterpenes); triterpenoids such us oleanolic and ursolic acids, α- and β-amyrin, and rofficerone (ESCOP 2003).

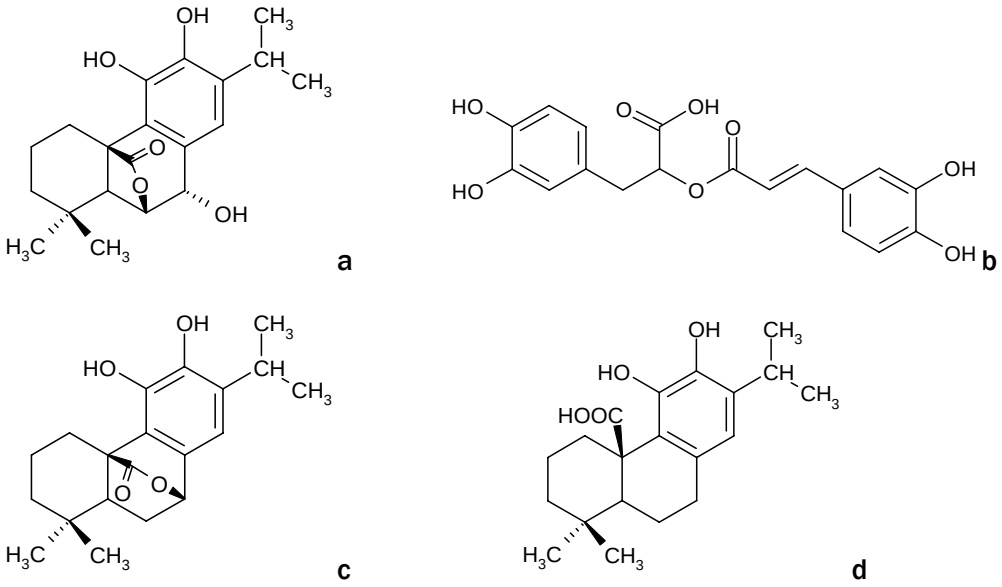

Figure 1. Chem. structure of rosmanol (a), rosmarinic acid (b), carnosol (c) and carnosic acid (d).

3.1 CHEMICAL ANALYSIS: METHODS TO DETERMINE THE COMPOUNDS

Rosemary oil

The essential oil of rosemary is analysed by gas-chromatography / mass spectroscopy (GLC-MS) and gas- liquid chromatography / flame ionization detector (GLC-FID).

The chemical identification test is performed by Thin-Layer Chromatography (Ph.Eur. 5, 2005). The chromatograms obtained in the test are examined for their fingerprint profile (Wagner and Bladt 1996). A yellowish-brown zone corresponds to bornyl acetate; a violet zone corresponds to cineole and a violet brown zone corresponds to borneol in the reference solution.

Another chemical identification test refers to the percentage content of total hydroxycinnamic derivatives expressed as rosmarinic acid by measuring the absorbance of the test solution. The specific absorbance of rosmarinic acid is 400 (Ph.Eur. 5, 2005).

Peng et al. (2005) used a capillary electrophoresis with electrochemical detection (CE-ED), method for the determination of rosemary active components The method was successfully used with relatively simple extraction procedures, and the assay results were satisfactory. The detection electrode was a 300 micro m carbon disc electrode at a working potential of +0.90 V (versus SCE). The analytes can be well separated with 25 min in a 75 cm length fused-silica capillary at a separation voltage of 16 kV in an 80 mmol/l borate buffer (pH 9.0). The current response was linear over about three orders of magnitude with detection limits (S/N=3) ranging from 2x10-7 to 1x10-6 g/ml for all the analytes.

3.2 STANDARDIZATION, STABILITY

According to Ph.Eur 5 (2005) *Rosmarini folium* (dried leaves of *Rosmarinus officinalis*) contain minimum 12 ml/kg of essential oil (anhydrous drug) and minimum 3% of total hydroxycinnamic derivatives expressed as rosmarinic acid. The powder is greyish-green to yellowish –green and is examined under a microscope using chloral hydrate solution R. A measurable decrease of essential oil of rosemary leaves could be detected only after 18 months of storage.

Rosmarini aetheroleum Ph.Eur.5 is tested on its identity by TLC, on its quantitative composition by GLC. The composition (main compounds: pinene, camphene, cineol, camphor and borneol) depends on the origin (Spain, North Africa).The most important adulterants of Rosemary essential oils are turpentine and petroleum.

The essential oil has to be stored in a well-filled, airtight container, protected from light, at a temperature not exceeding 25°C (Ph.Eur. 5, 2005).

4. PHARMACOLOGY

Rosemary has a long list of claims pertaining to its medicinal uses including antibacterial (Del Campo *et al*., 2000) and antioxidant properties (Ozcan, 2003). It is known to be an effective chemopreventive agent (Plouzek *et al*., 1999), an antimutagenic (Minnunni *et al*., 1992) and has been shown to be non-toxic in animal models (Lemonica *et al*., 1996).

Rosmarinus officinalis is stated to act as a carminative, spasmolytic, anticonvulsant, sedative, diuretic, cholagogic, choleretic, antihepatotoxic, antioxidant, antimicrobial, antiviral, anti-inflammatory, anti-ulcerogenic effects. Topically, rubefacient, mild analgesic, mild antiseptic, wound healing and parasiticide properties are documented (Grainger - Bisset, 1994).

The German commission E approved internal use for: blood pressure problems, dyspeptic complaints, loss of appetite and rheumatism and external use as supporting therapy for rheumatic disease and circulatory problems.

4.1 ANTIOXIDANT ACTIVITY

Rosmarinus officinalis was found to contain several antioxidant compounds such as rosmarinic acid (Lamaison *et al*., 1990), diterpenoids like carnosic acid, carnosol, rosmanol and epirosmanol (Haraguchi *et al*., 1995), carotenoids and a-tocopherol (Munne-Bosch *et al*., 1999). A good correlation between the antioxidant activities and total phenol content in the extracts

was found, although all rosemary extracts showed a high radical scavenging activity (Moreno *et al*., 2006). Furthermore, rosmanol is more effective than both R-tocopherol and BHT. The volatile oil of rosemary is an antioxidant with a thermal resistance that is stronger than that of BHA and BHT (Nakatani, 1994); the latter compounds volatize easily at high temperatures (Yaakob et al., 2000).
Carnosol, rosmanol, and epirosmanol had an inhibitory activity to lipid peroxidation and oxidized apo B formation in human bloods LDL. The IC50 were 7-10 micromol/L. The antioxidant mechanism was related to the scavenging activities to lipid free radical (Zeng *et al*., 2001).

4.2 ANTICARCINOGENIC ACTIVITY

Carnosol, a constant constituent of *Rosmarinus officinalis* extracts, is a phenolic diterpene shown to have antioxidant and anticarcinogenic properties. Infact, carnosol inhibited the invasion of highly metastatic mouse melanoma B16/F10 cells in vitro (Huang et al., 2005).

4.3 ANIMAL STUDIES

4.3.1 HEPATOPROTECTIVE EFFECTS

Rosemary extracts have been shown to have potent hepatoprotective effects against a variety of hepatotoxic agents including CCl4, t-BHP and cyclophosphamide. including CCl4 (Fahim *et al*., 1999), tert-Butylhydroperoxide (t-BHP) (Joyeux *et al*., 1990) and cyclophosph-amide (Fahim *et al*., 1999).
A water extract from this herb has a hepatoprotective effect against azathioprine-induced hepatoxicity. Animals pretreated with water extracts from rosemary extract under investigation not only failed to show necrosis of the liver after azathioprine administration, but also retained livers that, for the most part, were histologically normal. In addition, these herbs blocked the induced elevated levels of alanine aminotransferase and aspartate aminotranferase in serum. The extract of Carnosol, one of the main constituents of *Rosmarinus*, has been shown to have antioxidant and scavenging activities. The effectiveness of carnosol to normalize biochemical and histological parameters of CCl(4)-induced acute liver injury, was evaluated (Sotelo *et al*., 2002). Carnosol normalized bilirubin plasma levels, reduced malondialdehyde (MDA) content in the liver by 69%, reduced alanine aminotransferase (ALT) activity in plasma by 50%, and partially prevented the fall of liver glycogen content and distortion of the liver parenchyma. Carnosol prevents acute liver damage, possibly by improving the structural integrity of the hepatocytes. To achieve this, carnosol could scavenge free radicals induced by CCl(4), consequently avoiding the propagation of lipid peroxides.
Aqueous extracts of *Rosmarinus officinalis* young sprouts show a significant hepatoprotective effect on plasma GPT levels when given as pretreatment before carbon tetrachloride intoxication while the whole plant extract was inactive (Hoefler *et al*., 1987).
The ability of rosemary to modulate cytochrome P450 (CYP) and detoxication enzymes in rat liver was evaluated by comparing the effects of rosmarinus extracts with different chemical compositions: essential oil (EO) containing monoterpenes, a dichloromethane extract (DCME) containing phenolic diterpenes and a water-soluble extract (WSE) containing phenolic compounds such as rosmarinic acid and flavonoids. Male Wistar rats received rosemary in their diet at 0.5% (w/w) for 2 weeks. The study reports that essential oil selectively induced CYP, particularly CYP2B. Water-soluble estract enhanced both CYP and detoxication enzymes (Debersac *et al*., 2001).

4.3.2 CHOLERETIC ACTIVITY

Rosmarinus officinalis ethanol extracts prepared from young sprouts show a significant dose-related choleretic activity and are more active than the total plant extract (Hoefler *et al*., 1987).

4.3.3 ANTIULCEROGENIC ACTIVITY

Rosmarinus officinalis L. crude hydroalcoholic (70%) extract was evaluated for antiulcerogenic activity employing different experimental models. The crude hydroalcoholic extract (CHE) decreased the ulcerative lesion index produced by indomethacin, ethanol and reserpine in rats.

The pharmacological mechanism has no relationship with nitric oxide (NO) and has no relationship with prostaglandins. The crude hydroalcoholic extract of R. officinalis L. has active substances that increase the mucosal nonprotein sulfhydryl groups content (Dias *et al*., 2000).

4.3.4 ANTISPASMODIC AND ANTICONVULSANT ACTIVITIES

The volatile oil of *Rosmarinus officinalis* leaves inhibited the contractions of rabbit tracheal smooth muscle induced by acetylcholine stimulation and the contractions of guinea pig tracheal smooth muscle induced by histamine stimulation. Also, the volatile oil inhibited the contractions of rabbit and guinea pig tracheal smooth muscle induced by high potassium (K+) solution and the contraction induced by acetylcholine and histamine stimulation, respectively, in Ca(2+)-free solution. These data suggest that the volatile oil of *Rosmarinus officinalis* leaves has a calcium antagonistic property (Aqel, 1991).

Rosemary oil , 1-8 cineole and bornyl acetate have exerted a spasmolytic action in both smooth muscle (guinea pig ileum) and cardiac muscle (guinea-pig atria prepration). In sooth muscle this spasmolytic effect has been attributed to antagonism of acetylcholine.

4.3.5 ANTICARCINOGEN ACTIVITY

A methanol extract of the leaves of the plant *Rosmarinus officinalis* L. was evaluated for its effects on tumor initiation and promotion in mouse skin. Application of rosemary to mouse skin inhibited the covalent binding of benzo(a)pyrene [B(a)P] to epidermal DNA and inhibited tumor initiation by B(a)P and 7,12-dimethylbenz[a]anthracene (DMBA). Application of rosemary to mouse skin also inhibited TPA-induced ornithine decarboxylase activity, TPA-induced inflammation, arachidonic acid-induced inflammation, TPA-induced hyperplasia, and TPA-induced tumor promotion. Topical application of carnosol or ursolic acid isolated from rosemary inhibited TPA-induced ear inflammation, ornithine decarboxylase activity, and tumor promotion (Huang Shiu-Chen *et al*., 2005).

4.3.6 EFFECT ON METABOLISM OF ESTROGENS

The effects of a methanol extract from the leaves of the plant *Rosmarinus officinalis* L. (rosemary) on the metabolism and action of estradiol and estrone were evaluated in female CD-1 mice. Feeding female mice with a 2% rosemary diet for 3 weeks increased the liver microsomal oxidation and glucuronidation of estradiol and estrone and inhibited their uterotropic action (Zhu *et al*., 1998).

4.3.7 OTHER ACTIVITIES

The volatile oil of *Rosmarinus officinalis* has hyperglycemic and insulin release inhibitory effects in the rabbit. In normal rabbits, the intramuscular administration of the volatile oil (25 mg/kg) increases in plasma glucose levels following the administration of the intraperitoneal glucose test. The same treatment also resulted in a 30% decrease in serum insulin level (Al-Hader *et al*., 1994).

In alloxan diabetic rabbits, *Rosmarinus officinalis* volatile oil increased fasting plasma glucose levels.

The aqueous and ethanol extracts of *Rosmarinus officinalis* aerial parts diminish morphine withdrawal syndrome in mice. Dependence was induced using subcutaneous injections of morphine daily for 3 days. On day 4, morphine was injected 2 h prior to the intraperitoneal injection of naloxone. The number of jumps during the 30 min period after naloxone injection was considered as a measure of the withdrawal syndrome. The results indicated that the aqueous (1.68 g/kg and 2.4 g/kg, i.p.) and ethanol (0.96 g/kg, i.p.) extracts reduced the number of jumps (Hosseinzadeh *et al*., 2003).

Rosemary showed significant antithrombotic activity in vitro and in vivo, in experimental models of thrombosis. The mechanism of the antithrombotic effect of rosemary may involve a direct inhibitory effect on platelets (Yamamoto *et al*., 2005).

Essential oils of *Zingiber officinale* and *Rosmarinus officinalis* were found to be ovicidal and repellent, respectively, towards three mosquito species (Prajapati *et al*., 2005).

4.4 HUMAN STUDIES

Rosemary (*Rosmarinus officinalis* L.) extract enhanced the production of Nerve Growth Factor, a factor vital for the growth and functional maintenance of nerve tissue, in T98G human glioblastoma cells. Furthermore, the results indicated that carnosic acid and carnosol, which are major components of the rosemary extract, were able to promote markedly enhanced synthesis of NGF (Kosaka et al., 2003).

4.4.1 ANTI-INFLAMMATORY EFFECTS

Rosmarinic acid is well absorbed from gastrointestinal tract and from the skin. It increases the production of prostaglandin E2 and reduces the production of leukotriene B4 in human polymorphonuclear leukocytes, and inhibits the complement system. It is concluded that rosemary and its constituents especially caffeic acid such as rosmarinic acid have a therapeutic potential in treatment or prevention of inflammatory diseases (Al-Sereiti *et al*., 1999).

4.5 ADME

The *in vitro* organic-matter digestibility of the *Rosmarinus officinalis* varies between 36-52%, the effective degradability of the crude proteins (CP) varies between 45-68%, and the concentration of undegraded dietary protein varies from 32-55 g/kg dry matter. The availability of protein is 61% (RO), calculated protein digestibility of the undegraded dietary protein leaving the rumen at the degradability estimated was negative for *Rosmarinus officinalis*. The digestibility of the total carbohydrates and the efficiency of volatile fatty acid production were not affected by the supplementation.
Rosmarinic acid is rapidly eliminated from the circulation t1/2=9 minutes following intravenous administration.
Rosmarinic acid is well absorbed from gastrointestinal tract and from the skin.

5. MICROBIOLOGY

5.1 ANTIBACTERIAL ACTIVITY

Several compounds found in *Rosmarini aetheroleum* have been reported to be inhibitory to micro-organism (Guenther, 1974). Although the essential oil of *Rosmarinus officinalis* from the Mediterranean region appears to be well studied, its composition, as well as its antimicrobial properties, could differ from those of the variants growing in South Africa because of the differences in the two environments (Janssen *et al*., 1987).
Several components of essential oils have been reported to possess biological activities. Camphene, for instance was found to be effective against *Staphylococcus aureus* and *Escherichia Coli*. α-Pinene significantly reduced growth of *Erwinia amylovora* while β-Pinene was inhibitory to bacteria, especially at higher bacterial population (Scortichini *et al*., 1991).
The major components were assessed for their antibacterial activities against strains of *Staphylococcus aureus* possessing efflux mechanisms of resistance. Minimum inhibitory concentrations ranged from 16 to 64 µg/ml. Incorporation of carnosic acid and carnosol into the growth medium at 10 l µg/ml caused a 32- and 16-fold potentiation of the activity of erythromycin against an erythromycin effluxing strain, respectively (Oluwatuyi et al., 2004).
Methanol extract containing 30% of carnosic acid, 16% of carnosol and 5% of rosmarinic acid was the most effective antimicrobial against Gram positive bacteria (minimal inhibition concentration, MIC, between 2 and 15 mug/ml), Gram negative bacteria (MIC between 2 and 60 mug/ml) and yeast (MIC of 4 mug/ml). By contrast, water extract containing only 15% of rosmarinic acid showed a narrow activity. The antimicrobial rosemary extracts efficacy was associated with their specific phenolic composition. Carnosic acid and rosmarinic acid may be the main bioactive antimicrobial compounds present in rosemary extracts.

6. EFFICACY

6.1 QUALITY OF THE ANIMAL PRODUCT

The influence of rosemary (*Rosmarinus officinalis*) and vitamin E during 21 day of feeding were evaluated in broilers on the growth and qualitative parameters of poultry meat. Broilers fed with rosemary achieved higher body weights, better feed conversion and lower feed consumption compared with the other groups. Sensory evaluation showed better sensory properties of poultry meat from chickens fed with rosemary. The most significant differences were in the taste and flavour of the meat. The meat from broilers fed rosemary extract had lower concentration of total cholesterol oxidation products and the value of TBARS of refrigerated meats were significantly lower (Marcincak *et al*., 2004).

One experiment was conducted to evaluate the effect of supplementation of aromatic plants (dried rosemary leaves and stems, as well as oregano essential oil) in layers' diets, on the performance and egg traits. The results of this study showed that the addition of dried rosemary leaves and stems oil in layers diet did not significantly affect either the performance of laying hens or the egg traits. No distortion of the oviduct no any significant effects on the kidneys' condition were found (Mitsopoulos et al., 2005).

A study was conducted to evaluate the effect of rosemary herbal supplementation in broiler diets on some prevalent species of gut microbes. There was no effect of treatment on the concentration of gastrointestinal bacteria compared to the control. (Lopez-Bote et al., 1998).

Periparturient Saanen goats were fed a standard diet supplemented with 800 or 1600 mg/day *Rosmarinus officinalis*. Rosemary extract does not affect milk yield and milk and colostrum characteristics when given at 800 mg/day.

Different antioxidants, either α-tocopheryl acetate, BHT, or a rosemary extract were tested in fish diet. The results showed that the use of antioxidants altered the fatty acids composition of fish fillet (Sant'Ana *et al*., 2000).

In an other study Rosemary extract was administered to New Zealand White male rabbits in order to evaluate its effect on lipid content and fatty acid composition of liver, *Longissimus lumborum* muscle and abdominal fat. More than 60% of the fatty acid composition was quantitatively different in liver; 40% in adipose tissue while only few differed in *Longissimus lumborum* muscle (Tava *et al*., 2002).

7. TOXICOLOGY

Rosmarinic acid exhibits low toxicity (LD50 in mice is stated as 561 mg/kg for intravenous administration) and is rapidly eliminated from the circulation. Acute LD50 values quoted include % ml/kg (rat, oral) and >10 ml/kg (rabbit, dermal). Some botanical compounds might be activated by P450 to short-lived electrophilic intermediates capable of alkylating cellular biomolecules or participating in redox cycling reactions and causing cellular damage.

Pulsed ultrafiltration-mass spectrometry is a drug discovery technique invented for the affinity selection and mass spectrometric identification of small molecules that interact with a macromolecular receptor. This new assay provides a rapid in vitro toxicity screen to determine whether botanical dietary supplements should be tested for safety in animals and humans. *Rosmarinus officinalis* (rosemary) contains rosmarinic acid, carnosic acid, carnosol, and eugenol. Eugenol can be metabolized by P450 to a quinine methide and has been shown to be toxic to rat liver hepatocytes with an LC50 of 200-300 μM. As a food product, the leaves rosemary should pose no threat to human health if consumed in moderation, since small amounts of electrophilic quinones, if formed, could be deactivated by reaction with endogenous GSH. However, certain extraction methods might concentrate compounds such as rosmarinic acid or eugenol and increase the risk of toxicity of dietary supplements containing rosemary. Since this data indicate that a methanol extract of rosemary contains compounds that may be activated metabolically to redox active compounds or electrophilic species (Johnson *et al*., 2001).

7.1 SAFETY

7.1.1 ADMINISTRATION - DOSAGE

Rosemary is available as whole, crude and powdered drug forms. The daily dosage is 4 to 6 g drug. *Rosmarini aetheroleum* is commonly used as flavouring agent in foods and was not considered necessary limited use in veterinary medicine. No information on possible residues or residue depletion was considered necessary.
Considering that *Rosmarinus officinalis* is a normal component of human food and a normal component of the feed of food producing animals, the EMEA Committee on Veterinary Medicinals included it in Annex II of Council Regulation (EEC) No 2377/90 as a substance that does not need a MRL level (EMEA/MRL/290/97).

7.1.2 SIDE EFFECTS/ ADVERSE REACTION /CONTRAINDICATIONS/ PRECAUTIONS

Topical preparations containing rosemary oil should be used with caution by hypersensitive individuals.
Rosemary preparations should not be to used during pregnancy.
RO is a normal constituent of the animal diet in some Member State, and widely used a spice in the human diet. No information on possible residues or residue depletion was considered necessary.

7.1.3 INTERACTIONS WITH OTHER DRUGS

In a laboratory study, rosemary extract increased the effectiveness of doxorubicin in treating human breast cancer cells. Human studies will be necessary to determine whether this is true in people. Meanwhile, those taking doxorubicin should consult with a healthcare practitioner before taking rosemary.
The effects of rosemary was studied on the permeability of some commonly used drugs.
The permeabilities of verapamil, metoprolol, ketoprofen,, paracetamol, and furosemide were studied across Caco-2 cell monolayers. Mechanisms of interactions between drugs and herbs are similar to well known mechanisms of interactions between drugs and minerals (chelation, adsorption). Many herbal food supplements contain large amounts of flavonoids. These can interact with efflux proteins present in absorptive enterocytes, and there may be severe interactions with drugs administered contemporaneously (8–10) (Laitinen *et al*., 2004).
Rosemary extracts, has been reported to enhance the oral and systemic availabilities of a variety of drugs. Effects on systemic availability were explained by acute inhibition of the MDR1 efflux pump system that exists in various parts of the small intestine. Methanolic extracts of rosemary have been reported to inhibit P-gp efflux activity in breast-cancer cell cultures that over-expressed P-gp. The mechanism underlying the phenomenon is believed to be the ability of the rosemary extract to alter the activity of P-gp but not its expression (Castro *et al*., 1997). Furosemide permeability was strongly increased by the presence of 1 mg/ml rosemary extract, by opening of tight junctions between Caco-2 cells.

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SALVIA OFFICINALIS

		
Salvia officinalis L.		*Salvia sclarea* L.

1. INTRODUCTION

The name of the genus, *Salvia*, is derived from the Latin *salvere,* to save, in reference to the curative properties of the plant, which was in olden times celebrated as a medicinal herb.
This species was used in ancient Egyptian, Greek, and Roman medicines. Ancient Egyptians used it as a fertility drug (Bown, 1995). In the first century C.E. Greek physician Dioscorides reported that the aqueous decoction of sage stopped bleeding of wounds and cleaned ulcers and sores. He also recommended sage juice in warm water for hoarseness and cough. Pliny the Elder (ca. 23–79 C.E.) reported that sage enhanced memory functions. He prescribed decoctions, boiled in water or wine, of sage, rosemary, honeysuckle, plantain, and honey for gargles to treat sore mouths and throats (Grieve, 1979). Its uses in traditional Greek medicine spread to India, where the dried leaf (*Salbia-sefakuss* in Hindi) and fluid extract are used in traditional Indian Ayurvedic, Siddha, and Unani medicines. Eight other related Indian species of *Salvia* (e.g., *S. plebeia*) are also listed in the *Ayurvedic Pharmacopoeia* and used interchangeably with the Mediterranean species. In India, its use is indicated for flatulent dyspepsia, pharyngitis, uvulitis, stomatitis, gingivitis, and glossitis (Karnick, 1994; Nadkarni, 1976).
Hippokrates (5th c BC), Theophrastus (4th c BC) and Dioscorides (1st c AD) referred to them with the names "elelisfakon" and "sfakon".
In modern Greece, three *Salvia* species, named "faskomilo" and "alisfakia", are used as spices or as ingredients of folk remedies: S. fruticosa, which is known in the international trade as "Greek sage", S. officinalis, the commercially known "Dalmatian or garden sage", and S. pomifera, the "Cretan sage".
The essential oil of *S. fruticosa* is a folk remedy for toothaches and intestinal complaints (Fragaki 1969; Vokou *et al*., 1993; Rivera *et al.,* 1994). Recent studies have revealed that a leaf infusion of S. fructicosa has a hypoglycemic effect (Perfumi *et al*., 1991); furthermore it has been shown that its essential oil exhibits antibacterial, cytotoxic and antiviral activities (Sivropoulou *et al.,* 1997).
Sage is an evergreen perennial subshrub, native to the Mediterranean rim, especially around the Adriatic Sea, now cultivated in Albania, Turkey, Greece, Italy, France, the United Kingdom, and the United States (Bruneton, 1995; Budavari, 1996; Leung and Foster, 1996; Wichtl and Bisset, 1994). The material of commerce comes mainly from southeastern European countries, such as Albania and the former Yugoslavia (BHP, 1996; Wichtl and Bisset, 1994). Its cultivation in northern Europe dates back to medieval times, and it was introduced to North America during the seventeenth century (Bown, 1995).
In Germany, sage is licensed as a standard medicinal tea to treat gastrointestinal catarrh and night sweats. The tea is also applied topically as a rinse or gargle for inflammations. Sage fluidextract, tincture, and essential oil are all used in prepared medicines for mouth and throat

and as gastrointestinal remedies in fluid (e.g., juice) and solid dosage forms (e.g., capsules, drageès) (Leung and Foster, 1996; Wichtl and Bisset, 1994). In the United States, sage is used as a component of dietary supplement products for similar conditions, usually in aqueous infusion or alcoholic tincture dosage forms. Sometimes the dry herb or dry extract is used in capsules and tablets. Sage was formerly official in the *United States Pharmacopoeia* from 1840 to 1900 as a gargle in inflamed sore throat (Boyle, 1991).

2. DESCRIPTION OF THE PLANT

The genus *Salvia*, one of the largest Labiatae genera, includes c. 900 species and has almost cosmopolitan distribution. Southwest and central Asia is considered its major centre of origin (Hedge 1992).
The sage plants of greece, the three sage species (*S. fruticosa, S. officinalis,* S. *pomifera*) are strongly aromatic, many branched shrubs and reach heights up to 1.60 m. *S. fruticosa* and *S. officinalis*, members of the Section *Salvia*, have actinomorphic calyces with 5 equal or subequal teeth or bilabiate calyces with a 3-toothed upper lip and a 2-toothed lower lip. S. *pomifera* belongs to the Section *Hymenosphace* Bentham and is clearly distinguished from the other two species by its membranous-reticulate, bilabiate calyces. The upper lip of these calyces is entire, sinuate or shortly three-mucronate, whereas the lobes of the lower lip are obtuse, emarginate or mucronate (cf. Hedge 1972, 1982). Glandular trichomes, where the essential oils are secreted and accumulated, appear in all aerial parts of the three *Salvia* species. Two types of glandular trichomes are found: (a) capitate glandular hairs consisting of a unicellular, bicellular or multicellular stalk and a unicellular or bicellular secretory head, and (b) peltate glandular hairs consisting of a unicellular stalk and a multicellular (up to 12-celled) head (Verzar -Petri and Then 1975; Gupta and Bambie 1980; Bini Maleci *et al*., 1983; Venkatachalam *et al*., 1984; Werker *et al.,* 1985; Corsi and Corsi 1988; Werker 1993; Länger 1997). Galls are frequently formed on the stems of *S. fruticosa* and *S. pomifera*. The three *Salvia* species have the same chromosome number, 2n=14 (Fedorov 1969; Bothmer 1970; Kuštrak *et al*., 1986).
Salvia species of Africa, particularly southern Africa, have not been very well studied. With regard to taxonomy, a revision of *Salvia* in Africa was carried out by Hedge (1974). The 59 species recognized were devided into 18 species-groups, of which the seven occur in southern Africa. Most of the species are restricted to Africa.
Southern Africa is home to 30 species of the genus *Salvia*, for example *S. sclarea* (Clary sage), *S. africana-lutea, S. chamelaeagnea, S. coccinea, S. reflexa S. tiliifolia* and *S. verbenaca*.
The ESCOP sage leaf monograph requires that the material comply with the *European Pharmacopoeia* (ESCOP, 1997).

3. INGREDIENTS / CONSTITUENTS

Sage leaf consists of the whole or cut dried leaves of *Salvia officinalis* L. The whole drug contains not less than 15 ml/kg of essential oil and the cut drug not less than 10 ml/kg of essential oil, both calculated with reference to the anhydrous drug.
The ingredients of sage leaf (*Salviae officinalis folium*) are essential Oil, up to 2,5%, containing monoterpenes such as α- and β- thujone (up to 63% and 13% respectively), camphor and 1,8 - cineole. Other constituents are monoterpene glycosides and diterpenoids such as carnosic acid and its derivatives, e.g. carnosol and a quinone methide. Also triterpenoids including ursolic acid, oleanolic acid and their derivatives. Flavonoids, e.g. 5-methoxysalvigenin. Phenolic compounds, e.g. rosmarinic acid and derivatives, a caffeic acid trimer and flavonoid and phenolic glycosides are ingredients of sage leaf. [ESCOP]
Sage leaf contains 3–8% condensed catechin-type tannins (salviatannin); phenolic acids (rosmarinic, caffeic, chlorogenic, ferulic, and gallic acids); 1–3% flavonoids (apigenin and luteolin derivatives); 1.5–2.8% volatile oil, mostly monoterpenoids of which 18–60% is *a*-thujone, 3–10% *b*-thujone, 4.5–24.5% camphor, 5.5–13% cineole, 0–12% humulene, 1–6.5%

a-pinene, 1.5–7% camphene, 0.5–3% limonene, max. 1% linalool, and max. 2.5% bornyl acetate; diterpenoid bitter principles carnosol, carnosic acid, and rosmanol; triterpenoids oleanolic acid and ursolic acid; and resin (Bruneton, 1995; Budavari, 1996; ESCOP, 1997; Leung and Foster, 1996; Newall et al., 1996; Wichtl and Bisset, 1994).
Pharmacopeial grade sage leaf must contain not less than 1.5% (v/m) thujone-rich volatile oil calculated with reference to the anhydrous drug as determined by DAB (*German Pharacopoeia*) method V.4.5.8 (DAB, 1997; ESCOP, 1997; AB, 1981; Ph.Helv.VII, 1987; Wichtl and Bisset, 1994). It must contain not less than 16% water-soluble extractive, among other quantitative standards. Botanical identity requirements are carried out by thin-layer chromatography (TLC) as well as by examination of macroscopic and microscopic characteristics (BHP, 1996). Adulteration is determined with a TLC method that can be used to examine the volatile oil composition and flavonoids profile (DAB 1997; Wichtl and Bisset, 1994). The *French Pharmacopoeia* requires between 2–3% volatile oil (Bruneton, 1995; Ph.Fr.X., 1990).
Pharmacopeial grade sage tincture (*Salviae Tinctura*) must contain not less than 0.1% thujone-rich volatile oil. Its drug-to-extract ratio is 1:10 in ethanol 70% (v/v), manufactured by percolation according to the *German Pharmacopoeia* Tincture monograph. Botanical identity of the finished tincture is determined by DAB TLC method V.6.20.2 (DAB, 1997).

3.1. S. OFFICINALIS AND S. FRUTICOSA

S. officinalis is considered to have the highest essential oil yield among *Salvia* species, along with a higher total ketone content and a lower total alcohol content (Ivanic and Savin 1976, Newall *et al.,* 1996). The major components of the essential oil of *S. officinalis* are α- and β-thujones (35-50%, mainly α). At least two chemotypes of S. officinalis exist, one with a low β-thujone content (4-8%) and another with a relatively high content (16-32%) (Boelens and Boelens 1997).

3.2 S. SCLAREA

The volatile fraction of the Greek *S. sclarea* differs from many *Salvia* species growing in the Mediterranean region, in regard to compounds, in the variety of its components and their relative quantity. While the oil of *S. sclarea* contains mainly monoterpenoid alcohols such as linalool, geraniol, terpineol, nerol, and linalyl acetate, geranyl acetate, neryl acetate, the essential oils from many *Salvia* species contain α- and β-pinenes, camphor, phelladrene, cineol and bornyl acetate as major constituents.

4. PHARMACOLOGY

The Commission E approved the internal use of sage leaf for dyspeptic symptoms and excessive perspiration, and external use for inflammations of the mucous membranes of nose and throat.
ESCOP indicates its use for inflammations such as stomatitis, gingivitis and pharyngitis, and hyperhidrosis (ESCOP, 1997). The German Standard License for sage infusion indicates its use for inflammation of the gums and the mucous membranes of the mouth and throat; pressure spots caused by prostheses and in supportive treatment of gastrointestinal catarrh (Wichtl and Bisset, 1984).
Sage leaves act appetising and digestive because of the bitter flavour (Reichling *et al*., 2005).

4.1 ANIMAL AND *IN VITRO* TESTING

When applied topically (5% in vehicle) to rhesus monkeys, rosmarinic acid significantly lowered both gingival and plaque indices in comparison with placebo. [ESCOP]

4.2 HUMAN STUDIES

A dialysate of an aequeous extract from fresh sage leaf showed antihyperhidrotic activity in humans. Excessive sweat production induced by pilocarpine was inhibited.

Reduction of Sweat secretion:

One open study at an outpatient clinic investigated the equivalence of efficacy and compared tolerance between an aqueous dry extract product "Sweatosan" and sage tea. Eighty patients suffering from idiopathic hyperhidrosis were treated for four weeks. Forty patients were treated with 440 mg aqueous dry extract, corresponding to 2.6 g of dry leaf, and the other 40 with an aqueous infusion, 4.5 g leaf daily. The reduction of sweat secretion reported (less than 50%) was comparable for both treatment groups, though somewhat stronger in the aqueous dry extract group (ESCOP, 1997; Rˆsing, 1989).
In an open study with 80 patients both an aqueous infusion and a dried aqueous extract comparably reduced sweat secretion (ESCOP, 1997).

4.3 VETERINARY STUDIES

4.3.1 RUMINANTS

Positive effects on the rearing performance of calves with herbal mixtures including sage are reported (Kraszewski J, Wawrzynczak S, Wawrzynski M, 2002), but there is also a study wich did not show this effect (Stenzel R, Widenski K, Saba L.; 1998).
The efficacy of feeding high-yielding cows with concentrates containing 2% herb supplements (sage included) on cow performance, modification of milk chemical composition, technological value of milk for processing and nutritive value for humans was determined (Kraszewski J, Grega T, Wawrzynski M.; 2004) Positive immunological effects (increasing IgG-levels) of mineral-herbal mixtures as diet-supplements (containing sage) in growing calves were reported (Krukowski H, Nowakowicz DB, Saba L, Stenzel R.; 1998)

4.3.2 PIGS

It is reported that sage extracts improve the body weight gains and meat quality of pigs (Hanczakowska E, Wolski T, Urbanczyk J, Dobrowolska D, Lach H, Pilawski J; 2003; Wagner F., 2003)
Sage and its extract have an antioxidant capacity, which could be used as stabilisers of fat, in order to improve quality and shelf-life of meat and fat-containing food. On the one hand it seems possible to keep perishable fat-containing food longer by an addition of an extract of sage or oregano due to their antioxidative properties, on the other hand administration of feed additives of dried herbs (sage) to pigs had no effect on quality and shelf-life of fat obtained from these animals (Vichi S, Zitterl EK, Jugl M, Franz C.; 2001) (Bauer F, Siller D, Kleineisen S, Luf W, Pfannhauser W, Fenwick GR, Khokhar S.; 2001)

5. MICROBIOLOGY

The Commission E reported antibacterial, fungistatic, virustatic, astringent, secretion-promoting, and perspiration-inhibiting properties.

Drug preparations act antimicrobial (against *Staphylococcus aureus, Escherichia coli and others),* antiviral (amongst others Influenza A2, Vacciniaviruses and Herpes-simplex-Virus), antihypertensive (a.o. in cats, rats), choleretic (for ex. aqueous infusion for rats), antisecretory (sweat), spasmolytic (for example at rats duodenum, guineapig-ileum) and antioxidative (Lipidperoxidation is limited because of rosmarinic acid). (Reichling *et al.,* 2005, Heilpflanzenkunde für Tierärzte).
Antisecretory and antimicrobial acticity:
The Merck Index reported its therapeutic category as antisecretory agent (Budavari, 1996). Its volatile oil has antimicrobial activity attributed to its thujone content. Its traditional uses may be explained by its volatile oil and tannin content (Jalsenjak et al., 1987; Newall et al., 1996).
Bactericidal and fungicidal effects:
In in vitro experiments sage oil exhibited bactericidal and fungicidal properties when tested on Gram-positive and Gram-negative bacteria, filamentous fungi and yeasts, e.g. *Candida albicans.*

An aequeous and a 50%- ethanolic extract of sage leaf exhibited strong inhibitory effects on the collagenolytic activity of Porphyromonas gingivalis.
Aerial parts of sage contain diterpenes with antiviral activity against vesicular stomatitis virus. [ESCOP].

5.1 Antioxidant and antiinflammatory activity

Two phenolic glycosides isolated from sage leaf, 6-O-caffeoyl-β-D-fructofuranosyl-α-D-glucopyranoside and 1-O-caffeoyl-β-D-apiofuranosyl-β-D-glucopyranoside, were found to have moderate antioxidant activity in the DPPH and metmyoglobin tests.
Rosmarinic acid has been shown to have antiinflammatory activity.
Urosolic acid , the main component of the chloroform dry extract from sage leaf was found to be the major contributor to its anti-inflammatory activity in in vivo experiments.
Oleanolic acid also showed anti-inflammatory activity in in vivo experiments but was less effective.

6. Efficacy

6.1. Stability

Sage -infusions shouldn´t be cooked, because of the volatility of the essential oil (Wagenauer and Wiesenauer, 2003)

7. Toxicology

In the case of overdose, there are no toxic effects reported. [ESCOP].
Epilepic cramps were reported because of the use of the essential oil in a high dosage (BRAUN, 1994).

7.1 Safety

For internal use an infusion (1:10) out of sage leaves will be prepared.

Internal Use of Sage leaves:
Dosages
recommended daily allowance:

Cattle	30,0 - 80,0 g
Horse	25,0 - 60,0 g
Sheep	10,0 - 15,0 g
Pig	5,0 - 10,0 g
Dog	2,0 - 5,0 g
Cat, Fowl	1,0 - 2,0 g

(by Rabinovich 1981; Gachnian and Assenov 1986;; Lipnizkiji et al. 1987)

Prescription:

Rp.		Rp.	
Salviae fol.	2,5	Sage leaves	2,5
Aqu. comm.	47,5	Drinking water	47,5
M.f.infus.		make an infusion.	

D.S. Internal use 3 times daily a tablespoon of this infusion for a dog with diarroea [Gachnian and Assenov 1990]

It is allowed to use Sage leaves (Salviae officinalis folium) as an active agent for foodstuff delievering animals in the EU. [VO (EWG) Nr. 2377/90]. [Reichling et al., 2005]

Calves suffering from diarrhoea get an infusion of 300 - 400ml over 3 - 5 days. This therapy normalises the function of the alimentary system, activates the appetite and enhance the general condition.

Presently, there is no experience with sage leaves and pregnant or lactating animals. [Reichling et al., 2005]

Caution is required with the use of alcoholic preparations because of the presence of thujone. Given the potential toxicity of some constituents of the essential oil, the use of sage leaf is not recommended during pregnancy or lactation. [ESCOP]
Interactions with other drugs or undesirable effects are none known for sage leaves. [ESCOP]
The pure essential oil and alcoholic extracts should not be used internally during pregnancy. It´s not recommended (McGuffin et al., 1997).
Interactions with other drugs:
None known
Dosages for humans:
Unless otherwise prescribed: 4–6 g per day of cut leaf for infusions, alcoholic extracts, and distillates for gargles, rinses and other topical applications, and for internal use. Also pressed juice of fresh plants.

Internal:
Dried leaf: 1–3 g, three times daily.
Infusion: 1–3 g in 150 ml water, three times daily.
Dry aqueous extract 5.5:1 (w/w): 0.18–0.36 g, three times daily.
Fluidextract 1:1 (g/ml): 1–3 ml, three times daily.
Fluidextract: 1.5–3 g (Erg.B.6).
Tincture 1:10 (g/ml): 2.5–7.5 g (Erg.B.6).
Essential oil: 0.1–0.3 ml.
Succus: Pressed juice of fresh plant in 25% alcoholic preservation.

External:
Gargle or rinse: Use warm infusion: 2.5 g cut leaf in 100 ml water; or 2 to 3 drops of essential oil in 100 ml water; or use 5 ml of fluidextract diluted in 1 glass water, several times daily.
Paint: Apply the undiluted alcoholic fluidextract to the affected area with a brush or swab.

KBB

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SILYBUM MARIANUM



Silybum marianum

1. INTRODUCTION

Silybum marianum (L.) Gaertn. Synonyms: *Carduus marianus* (L.), Lady's Thistle, Mediterranean Milk Thistle, St. Mary's Thistle. It's beloings to the Asteraceae family (Compositae). European colonists reportedly transported the herb to the New World from Britain and it has since become naturalized in the eastern United States, California and parts of Canada. Milk thistle also grows in India, China, South America, Mexico, Australia and Africa. In many regions, it's a widespread wayside herb, often found in wastelands, along roadsides and on cultivated ground. Historically, grown in Europe as an vegetable, for edible peeled stalks; leaves salad green or pot herb; roots and flower receptacle eaten like artichoke; the food use as herb tea ingredient is reported.
Traditionally, Milk Thistle fruits have been used for disorders of the liver, spleen and gallbladder; It is stated to posses hepatoprotective, antioxidant and choleretic properties. Current interest is focused on the hepatoprotective activity of Milk Thistle and its use in the prophylaxis and treatment of liver damage and disease (Herbal Medicines, 1996).
Milk thistle seed extract are marketed in the European Union as dry extract, yellow brown powder (particle size: at least 95% must be less than 500 micron), to form capsules and tablet, and as a liquid extract used in infusion and tinctures.

2. DESCRIPTION OF THE PLANT

Milk thistle or Mary's thistle (*Silybum marianum*) is a herbaceous annual or biennial plant, easily recognized by its stout thistle, reddish-purple flowers, large prickly leaves with milky white zones and tubular–shaped flowers that terminate in sharp pines. *Silybum marianum* has a stem 20-150 cm high, rarely shorther, glabrous or slightly downy, erect and branched in the upper part. The leaves are alternate, large, white-veined, glabrous with strongly spiny margin.The inflorescences are large and round capitula, solitary at the apex of the stem or its branches, surrounded by thorny bracts. The florets are hermaphrodite, tubular in shape with corolla red-purple.The fruits are hard-skinned achenes 6 to 8 mm long, shiny, generally brownish in colour with a white silk-like pappus at the apex (Botanical Medicines, 2002).

2.1 SYSTEMATICS

The *Silybum* genus belongs to the Asteraceae Family and contains two species: *Silybum marianum* (L.) Gaertner and *Silybum eburneum*. Both species are indigenous to the Mediterranean region, although Kashmir is considered the native home of *Silybum marianum*.

2.2 PLANT PARTS USED

The fruits are harvested in July-August after blooming.
The medicinal part of the plant are the fruits (often referred to as “seeds”).
Other parts frequently used with different value: milk thistle herb, milk thistle (fatty) oil.

3. INGREDIENTS / CONSTITUENTS: CHEMICAL STRUCTURE CONTENT OF VARIATION

Milk thistle fruits contain:
Silymarin, present only in the fruit (1.5-3%), is a mixture of flavonolignans, containing silybin, silychristin, silydianin, and isosilybin. Silymarin CAS number is 65666-07-1. Silymarin extract is standardized by silybin content, the major constituent.
Flavonoids: apigenin, chrysoeriol, eriodictyol, naringenin, quercetin, taxifolin
Lipids (20-30%): linoleic acid, oleic acid and palmitic acid.
Sterol: cholesterol, campesterol and stigmasterol
Other constituents: mucilages, sugars (arabinose, rhamnose, xylose, glucose) amine and saponins.
Silymarin is a standardised ecxtract from the milk thistle fruit.
Silymarin is the pharmacologically active component of Milk thistle fruit; silybin is the main component of silymarin.
Silymarin usually contain 50-70% of flavonolignans (silybin, isosilybin, silydianin and silychristin) taxifolin and other minority compounds (Fig. 1). A residual fraction (30-50%) of silymarin, which has not yet been defined chemically in detail, contains polymerised polyphenolics.

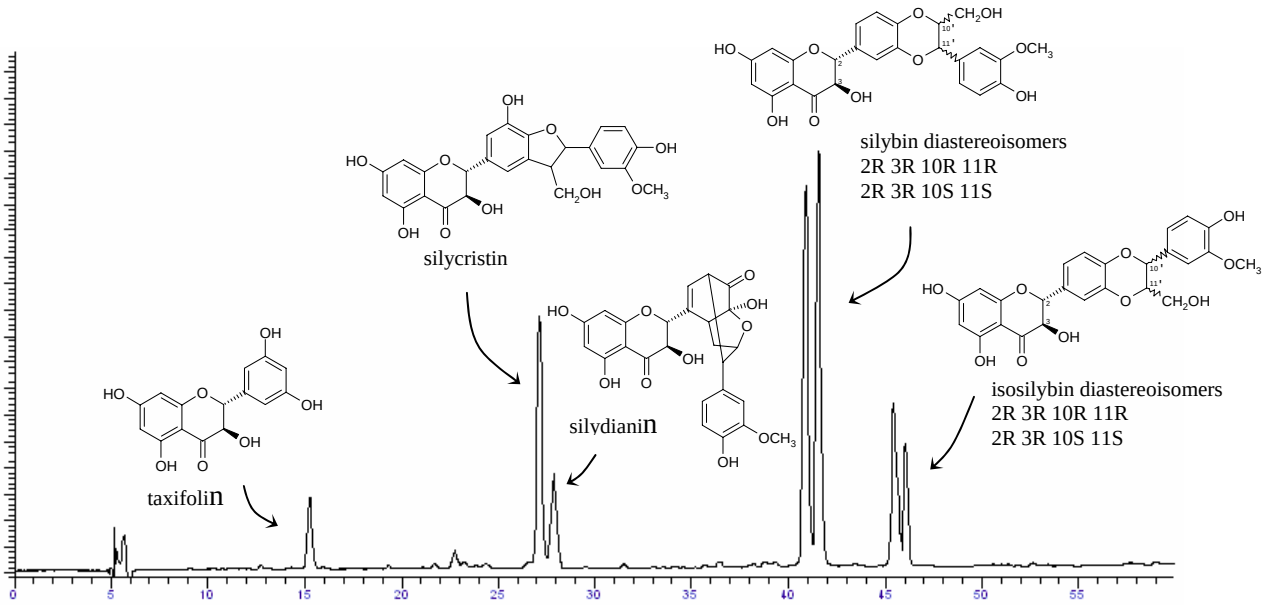

Figure 1. High performance liquid chromatogram of the silymarin mixture.

Milk thistle herb contain:
Flavonoids: apigenin, luteolin, kaempferol and their glycosides.
Steroids: sterols, including beta-sitosterol, beta-sitosterol glucoside
Organic acids: fumaric acid (3,3%)
Milk thistle oil is rich in unsaturated fatty acids.

3.1 CHEMICAL ANALYSIS: METHODS TO DETERMINATE THE COMPOUND

Separation and determination of *Silybum marianum* flavonoids (Silychristin, Silybin, Isosylibin and Silydianin) is achieved by HPLC as described in DAB 10 "Cardui mariae fructus extractum siccum" and in Pharmeuropa (2004), 1, 99 "Milk thistle dry extract, quantified and refined".
The method is suitable for the routine determination of the compounds of interest in *S. marianum* plant material and extracts in feed additive and in animal products.
A thin-layer chromatography method is reported in European Pharmacopoeia (01/2005:1860).

3.2 STANDARDIZATION, STABILITY

Silymarin is not soluble in water and is administered to the animals as a standard extract mixed in premixtures and feedingstuffs.
Stable under normal conditions, stored in a dry and cool place, away from direct light.

4. PHARMACOLOGY

Silybum marianum is a medicinal plant widely used in traditional European medicine.
In Europe, clinical use is widespread for toxic liver damage in supportive treatment of chronic inflammatory liver disorders and cirrhosis. In infusion therapy, silybin preparations is used for supportive treatment of Amanita mushroom poisoning and for acute liver toxicity induced by various toxic agents with different mechanism of action
In Germany, milk thistle is approved for the treatment of toxic liver disorders and as a supportive treatment in chronic inflammatory liver disease and hepatic cirrhosis. The seeds are approved by Commission E for dyspeptic, liver and gallbladder complaints.
In vitro studies using isolated hepatocytes have documented the protective activity of silymarin and several of its components against cell damage induced by various cytotoxic substances.
Silymarin stimulates RNA polymerase A, enhancing ribosome protein synthesis and resulting in activating the regenerative capacity of the liver through cell development. Silybin increases the rate of synthesis of ribosomal ribonucleic acid through stimulation of nucleolar polymerase. This reinforces protein synthesis and accelerates cell-regeneration process.

4.1 ANIMAL STUDIES

4.1.1 ANTIOXIDANT ACTIVITY

Due to its phenolic nature silymarin is considered to be an antioxidant able to react with numerous radicals in cell-free systems, including oxygen and hydroxylic radicals forming more stable and less reactive compounds (Mira et al., 1987).
Another reported property of silybin and silymarin is its role as regulators of the content of GSH in various organs. In rats treated with silybin intravenously or silymarin intraperitoneally, a significant increase in the amount of GSH contained in the liver, intestine and stomach was found, whereas there were no changes in the lungs, spleen and kidneys (Valenzuela et al., 1989).

4.1.2 HEPATOPROTECTIVE PROPERTIES

The hepatoprotective activity of the seed is from silymarin, in particular silychristin and silydianin. The compounds inhibits the entrance of toxins and block toxin binding sites through alteration of the liver cell's outer membrane and hindering the uptake of toxins as demonstrated in rabbit liver microsomes and mononuclear lipid layers.
It has been shown that silybin preserves the functional and structural integrity of hepatocytes membranes by preventing alterations of their phospholipid structure produced by carbon tetrachloride and by restoring alkaline phosphatase and GGT activities (Muriel and Mourelle, 1990). Other authors have shown that silymarin affords hepatoprotection against specific injury induced by microcystin, paracetamol, halothane, alloxane, ethanol, in several experimental models.

Furthermore silymarin significantly lowers the levels of serum γ-glutamyl transpeptidase, alanine transaminase, and aspartate transaminase in rats with ethanol-induced liver damage. Silybin, a major constituent of silymarin, stimulates phosphatidylcholine synthesis and increases the activity of cholinephosphate cytidyltransferase in rat liver both in normal condition and after intoxication by galactosamine (Wang et al., 1996).
The administration of silymarin reduces plasma level of cholesterol and low-density lipoprotein (LDL) cholesterol in hyperlipidaemic rats (Wagner et al., 1986). It has been reported that the polyphenolic fraction of silymarin positively modifies lipoprotein profile in plasma and reduces the development of fatty liver in rats (Skottova et al., 2003).
Fibrotic transformation plays a key role in the pathogenesis of hepatic cirrhosis. The animals treated with silymarin had a 50% decrease in hepatic collagen content after 6 weeks. The dose dependence of the antifibrotic effect was also tested. A highly significant reduction in hepatic collagen accumulation was obtained with a dose of 50mg silybin/kg/day but not with 25 mg/kg/day (Boigk et al., 1997).

4.1.2.1 ANTIHEPATOTOXIC

Some authors reported that silymarin through a competitive inibition of α-amanitin uptake has hepatoprotective properties in experimental intoxication with *Amanita phalloides*. The efficacy of silymarin against phalloidin, hepatotoxiic chemicals, and alcohol is based on its tendency to bind to proteins and receptors on cell membranes, displacing toxic substances and preventing their entry into the cell. The antihepatotoxic properties of silymarin have been tested in dogs, rabbits, rats, mice. Histochemical and histoenzymological studies have shown that silymarin, administered 60 minutes before or no longer than 10 minutes after induction of acute intoxication with phalloidin, is able to neutralise the effects of the toxin and to modulate hepatocyte function (Desplaces et al., 1975, Choppin et al., 1978). Other authors studied the ability of silymarin to inhibit the hepatic cytochrome P450 detoxification system (Baer-Dubowska et al., 1998). It has been reported that silymarin counteracted aflatoxin B_1 (AFB_1) induced changes in liver and serum in rats (Rastogi et al., 2000). In AFB_1 experimentally intoxicated broiler chickens silymarin administration reduced hepatic histopathological damage, prevented the changes in serum ALT activity, and completely counteracted the effects of AFB_1 intoxication on growth performances (Tedesco et al., 2004b).

4.1.3 ANTI-INFLAMMATORY EFFECTS

In vitro studies have shown that silybin at a concentration of 100 µmol/L reduces the proliferation of stellate cells isolated from fresh liver of rats by about 75%, reduces the conversion of such cells into myofibroblasts, and down regulates gene expression of extracellular matrix components indispensable for fibrosis. In rats silymarin administered at a dosage of 50 mg/kg/day for six weeks is able to reduce fibrosis by 30 to 45% when compared with control (Fuchs et al., 1997)

4.1.4 NEPHROPROTECTIVE PROPERTIES

Silybin injected into rats prior to administration of cisplatin afforded protection of glomerular and proximal tubular function.

4.1.5 ANTICANCER ACTIVITY

Silymarin when applied to the skin of SENCAR mice, gave protection against the effects of the tumor promoters 12-O-tetradecanoylphorbol (TPA) and okaidic acid (OA). Topical application of silymarin prior to that of TPA and OA completely inhibited induction of tumor necrosis factor α (TNFα) mRNA expression in the epidermis. Substantial protection from photocarcinogenesis in mice treated with phorbol ester or 7,12-dimethylbenze(a)anthracene has been demonstrade. The antitumour effects is primarily
At stage 1 tumour promotion and silymarin acts by inhibiting cyclooxygenase 2 (COX-2) and interleukin 1α (IL-1 α).

4.1.6 GASTRIC ULCER PROTECTIVE EFFECTS

Oral administration of silymarin to rats prevented gastric ulceration induced by cold-restraint stress, reducing histamine concentration. It was suggested that the anti-ulcrogenic effect of silymarin may be related to inhibition of enzymic perossidation by the lipoxygenase pathway.

4.2 HUMAN STUDIES

In human medicine the bioactive extract from *Silybum marianum* (milk thistle), silymarin, a mixture of flavanolignans composed of silycristin, silydianin, silybin and isosilybin, are responsible for the pharmacological effects and has clinical applications in the treatment of liver diseases.
Clinical trials with MilkThistle preparations have focused on their use in alcoholic liver disease, cirrhosis and acute viral and chronic hepatitis. However, several trials have included patients with liver disease of different aetiology, e.g. alcoholic and non alcoholic cirrhosis (Saller et al., 2001).

4.3 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

The pharmacokinetics and metabolism studies on silymarin and silybin are few. A plasma level of 500 µg/mL (as silybin) was found 90 min after oral administration in mice of 200 mg/kg of silybin. Silybin after oral administration showed peak time concentrations between 30 min and 1 h after administration and a bioavailability of approximately 30% (Bulles et al., 1975).
Approximately 20-50% of silymarin is absorbed following oral administration and approximately 80% of the dose whether administered orally or intravenously is excreted in the bile; about 10% enters the enterohepatic circulation. A low renal excretion (1-2% of Silybin) is registered.
Silymarin is excreted in bile as metabolites, which were identified, through hydrolysis with glucuronidase/arylsulfatase, as glucuronides and sulfates of silybin and dehydrosilybin (Bulles et al. , 1975). The ratio between dose and quantity excreted in bile was linear for oral doses up to 20 mg/kg and the minimum absorption percentage, evaluated on the basis of urinary and biliary excretion data, was 45%. The kinetics of the biliary excretion evidenced the maximum excretion 1 h after administration, which indicates a rapid absorption. After oral administration of 500-1000 mg/kg of silymarin in rats 75% was excreted in the faeces within three days (Meyer-Burg, 1972).
After 8 h the intraperitoneal administration of 3H-silybin in rats, the following percent values of the administered radioactivity were found in the various organs and tissues: liver 5.1%, spleen 0.3%, blood 2.1%, brain 0.1%. A similar trend was observed following the other way of administration. The 80% of the silybin found in the liver homogenate and in the cytosol fraction of the hepatic cells was the unconjugated form, while the remaining 20% was present as glucuronide or sulphate conjugated. In a pharmacokinetics study carried out in human healthy volunteers with oral administration of silymarin, the silybin metabolites other than glucuronides and sulphates were not found either in bile or urine (Flory et al., 1980). The biliary excretion of silybin after repeated oral administrations of silymarin reached the steady state at the latest on the second day of treatment and stopped within 72 h from the last administration (Sonnenbichler, 1980).
A pharmacokinetic profile of silymarin following oral administration of 200 mg/kg (as silybin) in rats has been reported (Morazzoni et al., 1993) . Silybin reached peak plasmatic level 2 h after silymarin administration, with a Cmax of 0.06 µg/mL and 6,7 µg/mL for unconjugated and total drug respectively. Maximum total biliary concentration of silymarin (106 µg/ml) was reached within 2 h.
After repeated oral administration of 10g/day of silymarin to dairy cows, no silybin residues were detected in milk samples (limit of detection 10 ppb) (Tedesco et al., 2004c).

5. MICROBIOLOGY

No data in literature suggest an antimicrobial activity of silymarin. Inhibitory activity has been tested in milk samples from animal receiving silymarin exctract, and results were negative (Tedesco et al., 2002).

6. EFFICACY

6.1 QUALITY OF THE ANIMAL PRODUCT

Data on the the safety and quality of food products after administration of silymarin have been provided in experimental studies on dairy ruminants. Concerning the residues in milk, no silybin residue was detected (detection limit =10 ppb) after 10 g/d administration for more than a week (Tedesco et al., 2004c). In dairy goats and cows treated with silymarin a higher milk production was found, without any variation in the milk composition, considering protein, fat and lactose content (Tedesco et al., 2004d).

7. TOXICOLOGY

The acute toxicity of silymarin and silybin were investigated after oral and intravenous administration in various animal species. Even in large doses silymarin is devoid of toxic effects. No mortality and signs of adverse effects were observed after oral administration of silymarin at the dose of 20 g/kg in mice and 1g/kg in dogs (Hahn et al., 1968). Long term oral administration of silymarin (100mg/Kg/day) to rats for 16 or 22 weeks did not reveal any adverse effect. The 50% lethal dose values (LD50) after intravenous infusion are 400 mg/kg in mice, 385 mg/kg in rats and 140 mg/kg in rabbits and dogs (Lecompte, 1975, Vogel et al., 1975). These data demonstrate a low acute toxicity of silymarin. Similarly, the subacute and chronic toxicity of silymarin are low. The compound is also devoid of embryotoxic potential and has no harmful action on the embryo (Wagner et al., 1986).
No adverse events were noted in a pharmacokinetic study involving healthy male volunteers following single oral doses of silymarin corresponding to up to 254 mg silybin. Some studies involving more than 3500 patients with various types of chronic liver disease treated with silymarin (560 mg/day) for eight weeks, have indicated that the frequency of adverse effects with silymarin is approximately 1%. Adverse effects are mainly transient, non serious, gastrointestinal complaints: in isolated cases, a mild laxative effect has been observed.

7.1 SAFETY (INFORMATION ON THE DOSAGE)

Silybum marianum seed extract is included in feed ration as a flavouring agent. The recommended usage range is 50 - 500 mg/kg.
Most liver remedies were introduced into therapeutic use because they were found to have protective properties in certain animal species. The doses of silymarin products under the heading of liver remedies used in clinical trials, have ranged from 280 to 800 mg/day (equivalent to milk thistle extract 400 – 1140mg/day standardised to contain 70% silybin).
No known contraindications and side effects are reported.
In human clinical trial Silymarin has a good safety profile and only rare case reports of gastrointestinal disturbances and allergic skin rashes have been reported.
Silymarin administration to dairy cows indicates a lack of transfer in milk, raising no concerns regarding the safety of the resulting milk and dairy products. Chemical and liver histological analysis did not provide any evidence for toxic effects in dairy cows and goats. The analysis of blood analytes did not show any differences between a group of animal treated with silymarin and a control group. Histological analysis carried out on hepatic biopsies is in agreement with a no harmful effect of silymarin extract (Tedesco et al., 2004a).
Further information made available and estensive search of published literature did not provide any further evidence for pharmacological or toxicological properties of *Silybum marianum*

alerting to specific health risks which may result from residues in food producing animals following the intended use.

Silybum marianum is included in the European Pharmacopoeia and is marketed in Europe under the trademark LEGALON®. *Silybum marianum* is included in Annex II of Council Regulation (EEC) N. 2377/90 for use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias.

Milk thistle is covered by a positive Commission E monograph and as the following applications:

- Crude drug: dyspeptic disorders;
- Preparations: for toxic liver damage; as supportive treatment in chronic inflammatory liver conditions and liver cirrhosis.

Milk thistle became official in mid 1999 in the United States Pharmacopeia-National Formulary (USP23-NF18,1995-June 1999). It does not have GRAS status. However, it is freely available as a dietary supplement in the USA under DSHEA legislation (1994 Dietary supplement Health and Education Act.).

Silymarin as other flavonoids has been shown to inhibit P-gp-mediated cellular efflux. The modulation of P-gp- activity may result in altered absorption and bioavailability of drugs that are P-gp- substrates.

It has been reported that silymarin inhibit cytochrome P450 enzymes and consequently an interaction with drugs primarily metabolized by P450s cannot be excluded.

Silymarin has an antagonistic effect with yohimbine and phentolamine when given simultaneously.

DT/SG

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THYMUS VULGARIS

Thymus vulgaris

1. INTRODUCTION

The genus *Thymus*, in the family *Lamiaceae*, consists of more than 315 species. Thyme is perennial, with wide natural distribution extending from the Mediterranean to the coasts of Greenland, Canary Islands, North Africa, highlands of Ethiopia, the southwestern Arabian highlands, Sinai Peninsula, arid regions of West and East Asia, Himalayas, and Northern Europe extending to Siberia. Thyme species – such as *T. serpyllum*, *T. vulgaris*, *T. pulegioides*, *T. cotroidora*, and *T. zygis* – are known to thrive well upon introduction to new growing areas, such as South and North America (including Canada), South and West Africa, Australia, and New Zealand.

Thyme is cultivated throughout the world for culinary, cosmetic, and medicinal purposes, the manufacture of perfume, and for red and white thyme oil. Common thyme (*Thymus vulgaris*) and Spanish thyme (*T. zygis*) are used interchangeably for medicinal purposes. Crude dried or fresh herb may be brewed as tea or extracted into an alcohol macerate. Common thyme contains 0.4–3.4% of the volatile oil; Spanish thyme contains 0.7–1.38%. The red or white thyme oil is manufactured commercially for use in cough drops, mouthwashes, liniment, toothpaste, detergent, and perfume. Because white thyme oil is a distilled red thyme oil product, red thyme oil is generally preferred (Leung and Foster, 1996).

Thyme has an integral place in Western culture and folklore, and has been used since ancient times not only as a traditional medicine, but also as a tonic and libido enhancer. The essential oil or the alcohol extract from its leaves are used as natural preservatives in conventional food, beverage, cosmetic, and personal care products. Thyme oil and extracts are among the top 10 natural products that have demonstrated antibacterial, antimycotic, antioxidative, and even some potentially anti-aging properties. Due to an increasing number of chemical studies, genetic improvement in the plant, and widening of the areas of its applications, thyme has been growing in international trade as a commodity of increased commercial importance.

It has been used in traditional medicine to treat heartburn, gastritis, asthma, laryngitis, pertussis, and bronchitis (Newall et al., 1996). Extracts demonstrate *in vitro* anti-inflammatory effects on guinea pig tracheal smooth muscle tissue (Leung and Foster, 1996), and the volatile oil in the herb most likely exerts spasmolytic effects on bronchial tissues in humans (Tyler, 1994). The herb is approved by ESCOP (2003) in the treatment of bronchitis, whooping cough, and upper respiratory inflammation.

Like the herb's infusions and extracts, thyme oil is also carminative, expectorant, and possesses antimicrobial and anthelmintic properties, due to concentrated thymol and carvacrol content (Leung and Foster, 1996), but it is extremely toxic. As an ingredient in toothpaste, thyme oil has been blamed for cases of inflamed lips and tongue reported in the toothpaste users. Signs of toxicity escalate from nausea to respiratory arrest (Newall et al., 1996). For these reasons, the herb is preferred to the oil.

Thyme was known to classic Rome; it was added to cheeses and alcoholic beverages. In the seventeenth century, herbalist Nicholas Culpepper wrote that thyme teas and infusions were useful for whooping cough, shortness of breath, gout, and mild stomach pains. He suggested that a thyme ointment be used to eliminate abscesses and warts. Thyme oil was used as a rubefacient and counterirritant, and was part of an herbal cigarette that was smoked to relieve stomach upset, headache, and fatigue. Thyme essence was used in perfumes and embalming oils (Grieve, 1979).
Thyme's common name may be derived from a Greek word meaning to fumigate, because the Greeks used thyme as an incense. It may also have come from the Greek word *thumus,* meaning courage. In medieval times, thyme was regarded as a plant that could impart courage and vigor, and women often embroidered a sprig of thyme on gifts for their favorite knight (Grieve, 1979).
Indigenous to southern Europe. It is a pan – European species that is cultivated in Europe, the United Sates of America and other parts of the world.

The aromatic plant of *Coridothymus capitatus* (L.) is a very common member of Labiatae family and it is distributed in the Mediterranean area, especially Turkey, Greece and Spain (Davis, 1982; Kokkini and Vokou, 1989) and it's known in trade as "Spanish Origanum". Oregano species, *Coridothymus, Satureja, Origanum, Thymus* etc., have commercial importance in Turkey as well as in the world (8000 ton plants/year). These species are collected, their leaves are used as thyme and also essential oil of species exported (Satıl *et al.*, 2002). Their essential oils are also important for the perfume, cosmetic, flavoring and pharmaceutical industries. They are used in folk medicine against cold, influenza and throat infection in Turkey (Baytop, 1962; Tabata *et al.*, 1988). Insecticidal (Karpouhtsis *et al.*, 1998), antioxidant (Demo *et al.*, 1998; Lagouri *et al.*, 1993), fungitoxic (Mullerriebau *et al.*, 1995), nematicidal (Oka *et al.*, 2000) activity studies and antibacterial effect of *C. capitatus* essential oil on potato storage (Vokou *et al.*, 1993), were reported along with their chemical composition (Karpouhtsis *et al.*, 1998; Fleisher and Fleisher, 2002; Ozek *et al.*, 1995). The essential oil of the Turkish*C. capitatus* was reported for three different localities which are Marmara island, Marmaris and Eceabat region of Turkey (Ozek *et al.*, 1995).

1.1 HISTORY (WHO)

Thyme extract has been used orally to treat dyspepsia and other gastrointestinal disturbances; coughs due to colds, bronchitis and pertussis; laryngitis and tonsillitis (as a gargle). Topical applications of thyme extract have been used in the treatment of minor wounds, the common cold, disorders of the oral cavity, and as an antibacterial agent in oral hygiene. Both the essential oil and thymol are ingredients of a number of proprietary drugs including antiseptic and healing ointments, syrups for the treatment of respiratory disorders, and preparations for inhalation. Another species in the genus, T. serpyllum L., is used for the same indications.
Uses described in folk medicine, not supported by experimental or clinical data:
As an emmenagogue, sedative, antiseptic, antipyretic, to control menstruation and cramps, and in the treatment of dermatitis.

1.2 OTHER SPECIES COMMERCIALLY USED AS THYME

Coridothymus capitatus, Origanum sp. and Satureja montana.

2. DESCRIPTION OF THE PLANT

An aromatic perennial sub-shrub, 20-30 cm in height, with ascending, quadrangular, greyish brown to purplish brown lignified and twisted stems bearing oblong – lanceolate to ovate – lanceolate greyish green leaves that are pubescent on the lower surface. The flowers have a pubescent calyx and a bilobate, pinkish or whitish, corolla and are borne in verticillasters. The fruit consists of 4 brown ovoid nutlets.
Herba Thymi is the dried leaves and flowering tops of *Thymus vulgaris* L. or of *Thymus zygis* L. (Lamiaceae).

General appearance:
-*Thymus vulgaris*: Leave 4-12 mm long and up to 3 mm wide; it is sessile or has a very short petiole. The lamina is tough, entire, lanceolate or ovate, covered on both surfaces by a grey to greenish grey indumentums; the edges are markedly rolled up towards the abaxial surface. The midrib is depressed on the adaxial surface and is very prominent on the abaxial surface. The calyx is green, often with violet spots, and is tubular; at the end are 2 lips of which the upper is bent back and has 3 lobes on it´s end; the lower is longer and has 2 hairy teeth. After flowering, the calyx tube is closed by a crown of long, stiff hairs. The corolla, about twice as long as the calyx, is usually brownish in the dry state and is slightly bilabiate.
-*Thymus zygis*: Leaf 1.7-6.5 mm long and 0.4-1.2 mm wide; it is acicular to linear-lanceolate and the edges are markedly rolled toward the abaxial surface. Both surfaces of the lamina are green to greenish grey and the midrib is sometimes violet; the edges, in particular at the base, have long, white hairs. The dried flowers are very similar to those of Thymus vulgaris.
Odour and taste aromatic.

2.1 Synonyms
Lamiaceae are also known as Labiatae.
Selected vernacular names: Common Thyme, farigola, garden thyme, herba timi, herba thymi, mother of thyme, red thyme, rubbed thyme, ten, thick leave thyme, thym, Thymian, thyme, time, timi, tomillo, za´ater.

2.2 Plant material of interest
Dried leaves and flowering tops.

3. Ingredients / Constituents

Constituents include essential oil containing the phenols thymol and carvacrol; terpenoids; glycosides of phenolic monoterpenoids; eugenol and aliphatic alcohols; the flavonoids thymonin, cirsilineol, and 8-methoxycirsilineol; biphenyl compounds of monoterpenoid origin; caffeic and rosmarinic acids; and saponins (ESCOP 2003). Other constituents include tannins, labiatic acid, ursolic acid, and oleanolic acid (Leung and Foster, 1996). Thyme also contains apigenin, luteolin, and 6-hydroxyluteolin glycosides, as well as di-, tri-, and tetramethoxylated flavones, which are all substituted in the 6-position (5,4'-dihydroxy-6,7,3'-trimethoxyflavone and its 8-methoxylated derivative, 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone, 5,4'-dihydroxy-6,7,8-trimethoxyflavone, 5,4'-dihydroxy-6,7-dimethoxyflavone) (Bruneton, 1995).

Herba Thymi contains about 2,5% but not less than 1% of volatile oil fluctuates depending on the chemotype under consideration. The principal components of Herba Thymi are thymol and carvacrol (up to 64% of oil), along with linalool, p-cymol, cymene, thymine, a-pinene, apigenin, luteolin, and 6-hydroxyluteolin glycosides, as well as di-, tri- and tetramethoxylated flavones, all substituent in the 6-position.
ESCOP: Essential oil containing phenols, predominantly thymol and/or carvacrol, and terpenoids; glycosides of phenolic monoterpenoids, eugenol, aliphatic alcohols and acetophenones; flavonoids, among with thymonin, cirsilineol and 8- methoxycirsilineol are characteristic; biphenyl compounds of monoterpenoid origin; caffeic acid and rosmarinic acid; saponins; long-chain saturated hydrocarbons and aliphatic aldehydes; and an arabinogalactan.

3.1. Chemical Analysis
Herba Thymi contains not less than 1.0% volatile oil, and not less than 0,5% phenols. Volatile oil is quantitatively determined by water/steam distillation, and the percentage content of phenols expressed as thymol is determined by spectrophotometric analysis. Thin-layer chromatographic analysis is used for thymol, carvacrol, and linalool.
3.2. Standardisation

Thyme consists of the whole leaves and flowers separated from the previously dried stems of Thymus vulgaris L. or Thymus zygis Loefl. ex L. or a mixture of both species. It contains not less than 1.2 per cent (V/m) of essential oil and not less than 0.5 per cent of volatile phenols, expressed as thymol ($C_{10}H_{14}O$; M_r 150.2), both calculated with reference to the anhydrous drug. The material complies with the European Pharmacopoeia. Fresh material may also be used, provided that when dried it complies with the European Pharmacopoeia.

Storage:

Store in a well-closed container, protected from light and moisture.

4. PHARMACOLOGY

The Commission E approved thyme for the treatment of symptoms of bronchitis and whooping cough and catarrhs of the upper respiratory tracts. It has also been used ~~as~~ to improve digestion (Stecher, 1968) and to treat pertussis, stomatitis, and halitosis (Wichtl and Bisset, 1994; ESCOP 2003).

4.1 SPASMOLYTIC AND ANTITUSSIVE ACTIVITIES

The spasmolytic and antitussive activity of thyme has been most often attributed to the phenolic constituents thymol and carvacrol, which make up a large percentage of the volatile oil (Reiter and Brandt, 1985). Although these compounds have been shown to prevent guinea pig ileum and trachea contractions induced by histamine, acetylcholine and other agents, the concentration of these phenolics in aqueous preparations of the drug is insufficient to account for this activity (Van den Broucke, 1980; Van den Broucke and Lemli, 1981). Experimental evidence suggests that the *in-vitro* spasmolytic activity of thyme preparations is due to the presence of polymethoxyflavones (Van den Broucke and Lemli, 1983). *In-vitro* studies have shown that flavones and thyme extracts inhibit responses to agonists of specific smooth muscle cells receptors, such as acetylcholine, histamine and L-norepinephrine, as well as to agents whose actions are not mediated by specific receptors, such as barium chloride (Van den Broucke and Lemli, 1983). The flavones of thyme were found to act as non-competetive and non-specific antagonists (Van den Broucke and Lemli, 1983); they were also shown to be Ca^{2+}-antagonists and musculotropic agents that act directly on smooth muscle (Van den Broucke and Lemli, 1983).

4.2 EXPECTORANT AND SECRETOMOTORIC ACTIVITIES

Experimental evidence suggests that thyme oil has secretomotoric activity (Gordonoff and Merz, 1931). This activity has been associated with a saponin extract from *T. vulgaris* (Vollmer, 1932). Stimulation of ciliary movements in tha pharynx mucosa of frogs treated with diluted solutions of thyme oil, thymol or carvacrol has also been reported (Freytag, 1933). Furthermore an increase in mucus secretion of the bronchi after treatment with thyme extracts has been observed (Schilf, 1932).

4.3 IN-VITRO/IN-VIVO STUDIES

- *In-vitro* tests (guinea pig trachea): flavonoids and ethanol extract of thyme revealed spasmolytic effect
- Antimicrobial effects: aqueous extract of thyme inhibited *Helicobacter pylori*
- Acetone extract of thyme inhibited *Mycobacterium tuberculosis*
- Fluid extract showed activity towards the pneumotropic bacteria *Moraxella catarrhalis*, *Klebsiella pneumoniae* and *Diplococcus pneumoniae*.
- Thyme essential oil: antibacterial, antifungal (thymol, carvacrol), prostaglandin biosynthesis inhibition
- Antiinflammatory effects: an ethanolic extract reduced the enzyme activity (rosmarinic acid)
- Antioxidant: dry acetone extract, supercritical carbon dioxide extract (phenolic constituents)
- *In vivo*-tests: fluid extract of thyme reduced carrageenan-induced rat paw oedema (comparable with phenylbutazone)

Intraperitoneal pretreatment of rats with essential oil protected against hepatotoxicity induced by carbon tetrachloride.
- Clinical studies: thyme syrup was as effective as medicinal expectorants such as bromhexine (humans)
- Acaricidal activity against *Dermanyssus gallinae*: thyme red and white oils (70 µg/cm²) provoked 100 % mortality of *D. gallinae* (Review of aromatic and medical plants 2004)

In vitro and in vivo tests
In vitro experiments found that the flavonoids thymonin, cirsilineol, and 8-methoxy-cirsilineol may be responsible for the bronchospasmolytic effect of thyme (ESCOP, 1997). *In vivo,* rosmarinic acid was shown to inhibit rat paw oedema induced by cobra venom factor but not the t-butyl hydroperoxide-induced paw oedema. Furthermore, it inhibited passive cutaneous anaphylaxis, and impaired activation by heat-killed *Corynebacterium parvum* of mouse macrophages. These observations demonstrate that its activity may be related to complement-related processes, according to its inhibitory action on complement C-3 convertase *in vitro* (Englberger et al., 1988; ESCOP, 1997).

4.4 TREATMENT OF MASTITIS

Thymus vulgaris is used in a pharmaceutical preparation for the treatment of mastitis in animals and humans, which consists of a mixture of a decoction of 16 medicinal herbs and an ammonia solution infusion of the same plants (Deryabin AM; PCT International Patent Application 1990; -13305).

4.5 VETERINARY STUDIES

4.5.1 HORSE

The effects of an oral preparation containing an extract of thyme and primula (Bronchipret; Bionorica) on the lung function of five horses suffering from heaves were determined in a longitudinal study (Austria). The horses accepted the product well. The plasma concentrations of the marker substance, thymol, indicated that at least one of the substances in the extract had been absorbed from the gastrointestinal tract. The compliance, pulmonary pressure and airway resistance of the horses' lungs were all significantly improved after one month of treatment. However, the severity of their clinical signs and their arterial oxygen partial pressure had not improved significantly (Hoven R. et al., 2003).

4.5.2 FOWL

The aim of the studies was to test performance, results of dissection, blood and selected meat and colour traits of chicken skin. The experiment involved 900 day-old cockerels allotted to 6 groups. Except that of the control group, diets contained 1.5% dried herbs (onion, caraway, camomile, thyme and dandelion) throughout the 51-day experiment. The addition of herbs had no effect on final body weight and feed conversion. Carcasses of chickens given herbal feeds contained less abdominal fat than control chickens. Organoleptic traits of meat were regarded as satisfactory in all the groups. The skin of chickens given herbs was redder, while the presence of yellow colour, its tone and intensity were most noticeable in controls ($P<0.05$) (Schleicher A. et al., 1998).

Diets were studied on gain and feed:gain ratio of broilers over 6 weeks. Studies were arranged in a factorial design with mashed feed compared with pellets with or without 10% virginiamycin Dried thyme (100 mg) improved feed:gain ratio by 3.2±1.5g/kg gain, but effect of virginiamycin was substantially greater. Spice supplements did not affect taste of broiler meat (Vogt H. et al., 1989).

On the other hand there is a study where groups of male, 1-day-old Lohmann broilers were given maize-soyabean meal diets, with oils extracted from thyme, mace and caraway or coriander, garlic and onion (0, 20, 40 and 80 mg/kg) for 6 weeks. Average daily gain and feed conversion

were not different between the broilers fed with the different oils; meat was not tainted with flavour or smell of the oils (Vogt H and Rauch HW., 1991).

4.5.3 PIG

4.5.3.1 PREVENTION AND TREATMENT OF DIARRHOEA

The Yugoslav product Fito-diaro-stop, prepared from thyme (Thymus vulgaris), was given orally as a 5 or 10% emulsion to 200 piglets at 3 and 10 days of age for prevention and treatment of diarrhoea and also used as a feed additive (1-2 kg/ton) for weaned pigs from 1 to about 3 months of age. In comparison with controls the treated animals showed higher daily weight gains, lower mortality, and lower drug expenses for their breeding (Teodorovic M. et al., 1990).

4.5.3.2 HERB MIXTURE AS AN ANTIBIOTIC SUBSTITUTE

In an experiment, 24 gilts and 24 crossbred barrows (Landrace x PLW x Duroc x Pietrain) were used to evaluate the efficiency of a multi-component herb mixture used as a substitute for fodder antibiotic in pig feeding. The herb mixture was composed of Melissa officinalis, Mentha x piperita, Urtica dioica, Thymus vulgaris, Agropyron repens [Elymus repens], Allium, Capsicum annuum, Origanum majorana, Coriandrum sativum, Taraxacum vulgare, and Silybum marianum. The gilts and barrows were assigned to 3 groups: 1. receiving feed mixture without any additives;2. receiving feed mixture with 0.5 % supplement of herbs; and 3. receiving feed mixture and 20 mg/kg of Flavomycine. Pigs fed the herb mixture supplement obtained the best average body weight gains (825 g) over the entire fattening period. The herbs added to the pig feed decreased backfat thickness by 8.4% and improved loin eye area (15.1%) and meat content in the carcass (6.7%). Animals receiving this mixture had the lightest thyroid glands, livers, and kidneys. There were no significant differences between the experimental groups in meat quality (colour, acidity, or water holding capacity). The cooked meat of the longissimus dorsi of pigs obtaining the herb supplement was more tender and juicier compared to the other fatteners. The obtained results confirmed the possibility of using the herb mixture as an antibiotic substitute in pig feed (Urbanczyk J. et al., 2002).

In another study, pigs were fed with a herbal mixture (30 g herb mixture per 1 kg diet), containing leaves of Urtica dioica and Plantago lanceolata, Rosmarinus officinalis, Thymus vulgaris and Thymus serpyllum and fruits of Juniperus communis. The experiment lasted 40 days. The herb supplements to the diets allowed for the attainment of even better production gains than in case of the antibiotic additive of the control group (Grela ER. et al., 2003).

In this study, 200 growing-finishing pigs were randomly divided into 5 feeding groups. Pigs in Group 1 (control group) were fed with a basal diet, whereas those in Group 2 were fed a basal diet plus an antibiotic (Tylosin, 25 mg/kg). Pigs in the remaining three groups were fed with a basal diet plus essential oils (at 25 mg/kg) of oregano (Group 3), thyme (Group 4) and garlic (Group 5). The inclusion of essential oil of spices in the pigs' diet significantly improved the average daily weight gain and feed conversion ratio of the pigs in Groups 3, 4 and 5, as compared to Groups 1 and 2 ($P < 0.01$). Furthermore, pigs fed the essential oils had higher carcass weight ($P < 0.01$), dressing percentage ($P < 0.01$), and carcass length ($P < 0.01$) than those fed the basal and antibiotic-supplemented diet. In Groups 3, 4 and 5, back fat thickness was significantly lower than those in Groups 1 and 2. Moreover, lean meat and ham portions from pigs in Groups 3, 4 and 5 were significantly higher than those from pigs in Groups 1 and 2. In conclusion, the use of essential oils as feed additives improves the growth of pigs and has greater positive effects on carcass composition than antibiotics (Onibala et al., 2001).

4.5.3.3 PERFORMANCE

Piglets fed rations containing sage, coriander, yarrow and thyme had significantly higher daily gain (by 7%) and feed conversion efficiency (by 3%) than controls (Wagner F., 2003).

Diet supplemented with 0.3% and 0.5% of a herbal mixture (couch grass rhizome, coriander seed, caraway seed, savory, camomile, thyme, mint, milk thistle endosperm) showed positive

effects on growing-finishing pigs. Pigs fed with 0.5% herbal supplementation showed best results in daily gain, the consumption of feed/kg gain, dressing percentage, right half-carcass weight, loin weight and ham weight. Backfat thickness was lower than in the control group (with no herbal supplementation) (Paschma J., 2000).

4.5.4 CATTLE

4.5.4.1 PERFORMANCE

Two groups of calves were fed with 0.5% or 1.0% herbs (*Mentha piperita, Thymus vulgaris, Salvia officinalis, Viola tricolor, Chamomilla recutita* and *Urtica dioica*) to their ration. Compared to a control group (fed with no herbal supplementation), they showed a tendency for higher intake of concentrates (which contained herbs) and forage. They had significantly higher final body weights at 120 days of age and higher mean daily weight gains. These groups also improved the conversion of ration nutrients per kg of body weight gain. The best rearing performance of calves was obtained in the group given 1% herbs in concentrate (Wawrzynczak S. et al., 2000).

The effect of feeding diets containing different proportions of herb mixtures (*Mentha piperita*, *Urtica dioica*, Matricaria chamomilla [Chamomilla recutita] *Thymus vulgaris, Salvia officinalis, Foeniculum vulgare, Viola tricolor*, and *Trigonella foenum-graceum*) on calves was investigated. Feeding concentrates with 1.0 and 2.0% herbs increased the intake of all solid feeds in the rations, ensured significantly higher body weights and daily gains in the experimental rearing periods, and improved the utilization of nutrients from the rations with respect to control animals fed without herb mixture. Best effects were obtained when heifer and bull calves were fed the 2.0% herb mixture concentrate (Kraszewski et al., 2002).

4.6 COMMERCIAL PRODUCTS FOR ANIMALS

Very often thymus vulgaris is used in commercial products for animals against diseases of the upper respiratory tract.

4.7 ADME / PHARMACOKINETICS

Data about pharmacokinetics of essential oils are scarcely available, although some information are reported for the phenolic terpenes, thymol and carvacrol. In an early publication Schröder and Vollmer (1932) described thymol and carvacrol to redistribute rapidly to the blood and kidneys following oral administration. The metabolism of carvacrol and thymol has been studied in rats. It was found that the urinary excretion of metabolites was rapid and only very small amounts were excreted after 24h. Although large qhantities of carvacrol and, especially, thymol were excreted unchanged (or as their glucuronide and sulphate conjugates), extensive oxidation of the methyl and isopropyl groups occured. This resulted in the formation of derivatives of benzyl alcohol and 2-phenylpropanol and their corresponding carboxylic acids. In contrast, ring hydroxylation of the two phenols was a minor reaction (Austgulen et al., 1987).
Takada et al. (1979) investigated the metabolism of thymol in rabbits and humans. Thymol glucuronide featering an intact aglycone was isolated from the urine of thymol-medicated rabbits and identified as an acetyl derivate of methyl glucuronate. The hydroxylated product of thymol, thymohydroquinone, was detected in small amounts in the urines of thymol-medicated humans. It was presumed that thymolhydroquinone is excreted as an ethereal sulfuric acid conjugate.

5. MICROBIOLOGY

Thyme in its crude herb form is carminative, antibiotic, anthelmintic, astringent, expectorant, and antitussive (Leung and Foster, 1996; Newall et al., 1996).
The Commission E reported bronchoantispasmodic, expectorant, and antibacterial activity.

5.1 ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY

In vitro studies have shown that both thyme essential oil and thymol have antifungal activity against a number of fungi, including *Cryptococcus neoformans, Aspergillus, Saprolegnia* and *Zygorhynchus* species (Tantaoui-Elaraki and Errifi, 1994; Vollon and Chaumont, 1994; Perrucci et al., 1995; Paster et al., 1995). Both the essential oil and thymol posses antibacterial activity against *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli* and a number of other bacterial species (Janssen et al., 1987; Juven et al., 1994; Dorman and Deans, 2000). As an antibiotic, thymol is 25 times as effective as phenol, but less toxic (Czygan, 1989).

6. EFFICACY

In the ESCOP monographs (2003) the application of thyme and thyme preparations is only recommended for the treatment of some clearly-defined diseases as follows:
External use: bath additive as a supporting cure of acute and chronical diseases of the upper respiratory tract; in addition against pruritus of dermatosis.
Internal use: thyme preparations can be administered against symptoms of bronchitis and whooping cough, and catarrhs of the upper respiratory tract.

7. TOXICOLOGY

Toxic effects of the vegetable parts of thyme, *T. vulgaris*, have not been published, but it is important to mention that a certain level of toxicity can be found for the essential oil of thyme (LD50=4,7g/Kg after oral administration in rats). This toxicity has been attributed by some authors to thymol and carvacrol (Dilaser, 1979), their acute oral LD50 in rats being 0,88-1,8g/Kg and 0,1-0,18g/Kg, respectively. Furthermore, these phenols cause skin irritations and especially irritations of the mucosa, which precludes patients with gastroduodenal ulcers from the use of the essential oil. Thus undiluted thyme oil was found to be severely irritating to both mouse and rabbit skin, however it produced no irritation on human subjects when tested at the concentration of 12 % (Tisserand and Balacs, 1995). Hypersensitivity reactions have also been reported for thyme herb after contact with the skin, inhalation or ingestion (Tisserand and Balacs, 1995; Benito *et al*., 1996; Lemiere *et al*., 1996); therefore it is strongly recommended to perform a tolerance test prior to attempting its use. Various essential oils from *Thymus* contain considerable quantities of camphor and other terpenic ketones, which are known to produce convulsions and neurotoxic crises (Dupeyron *et al.,* 1976; Steinmetz *et al*., 1980). Limonene, which is a common component of thyme oil, is capable of diminishing the incidence of tumors in experimental animals treated with tumor-inducing agents (Tisserand and Balacs, 1995). This carcinogenic effect as well as other renal damages observed in male rats are species and gender specific: consequently they are not predictable for human toxic effects (Meek et al., 2003). Therefore it is important to use fresh, non-oxidized essential oil of thyme in phytotherapy and aromatherapy.
Due to the toxic effects, the use of the essential oil during pregnancy and lactation is contraindicated (Peris *et al*., 1995).
Thymus vulgaris leaves fed to rats at 2% or 10% of standard diet for 6 weeks did not induce signs of toxicity (Haroun et al., 2002).

7.1 SAFETY

7.1.1 PREGNANCY AND LACTATION

The safety of Herba Thymi preparations during pregnancy and lactation has not been established. As a precautionary measure, the drug should not be used during pregnancy or lactation except on medical advice.

7.1.2 ADVERSE REACTIONS

Contact dermatitis has been reported. Patients sensitive to birch pollen or celery may have a cross-sensitivity to thyme.

7.1.3 INTERNAL USE OF HERBA THYMI/ MODE OF ADMINISTRATION

Cut herb, powder, liquid extract or dry extract for infusions and other galenic preparations. Liquid and solid medicinal forms for internal and external application. Note: Combinations with other herbs that have expectorant action could be appropriate.

Dosages
recommended daily allowance:

Cattle	25,0 - 50,0 g
Horse	6,0 - 15,0 g
Small ruminants, Pig	2,0 - 10,0 g
Dog	0,5 - 1,0 g
Cat, Fowl	0,01 - 0,3 g

(by Gachnian and Assenov, 1990]

Presently it is not allowed to use Thyme herb (*Thymi herba*) as an active agent for foodstuff delivering animals in the EU, but it is allowed to use Thyme-oil (*Thymi aetheroleum*) as an active agent for foodstuff delivering animals in the EU. [VO (EWG) Nr. 2377/90]. [Reichling et al., 2005]

Internal:
Infusion: 1–2 g of herb for 1 cup of tea, several times daily as needed.
Fluidextract 1:1 (g/ml): 1–2 ml, one to three times daily.
External:
Compress: 5% infusion for compresses.

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UNCARIA TOMENTOSA

Uncaria tomentosa

1. INTRODUCTION

The use of *Uncaria tomentosa* and *U. guianensis* by indigenous healers of tropical South America has not been traced to any particular time in history, but extends for untold past generations as part of an oral tradition of healing. In the region of the eastern incline of the Peruvian Andes to the Amazon rainforest, indigenous healers prepare a decoction of the bark of *U. tomentosa* or *U. guianensis* for internal use to treat gastric ulcers. They regard the preparations as antitumor, anti-inflammatory, antirheumatic and contraceptive. In central Peru, indigenous people primarily use the bark of *U. tomentosa* to relieve inflammations, including those associated with rheumatism, arthritis, urinary tract inflammations and gastric ulcers. Other central Peruvian indigenous uses of cat´s claw bark include the relief of side effects from antibiotics and chemotherapy, and other treatments of cancer, weakness, bone pain, and deep wounds. It is also used to cleanse the kidneys, and for recovery from childbirth. External application of the bark decoction or tea is applied to wounds using the preparation as a wash twice daily.

The Asháninka Indians of central Peru are noted to use the root bark in the treatment of cancer. The highest order of Asháninka shaman priests use the root bark of *U. tomentosa*-PC, known to them as *savéntaro* or powerful plant, to remove the main cause of disease in the Asháninka medico-religious system of healing: a disturbance between the spirit and the body, or an "anxiety". Accordingly, *U. tomentosa* -PC is used by Asháninka shamans in the treatment of psychological disorders, and is regarded as being inhabited by "good spirits". Other uses of , *U. tomentosa* by the Indians of Peru include the treatment of skin "impurities", "blood purification", "bone pains", fevers, asthma (sap with water), disease prevention, hemorrhages, menstrual

irregularity, recovery from childbirth, contraception, "loose" stomach, fevers, and for a normalizing effect on the body. The main stem (stalk) holds potable water which is obtained by cutting it open.
Traditional uses of *U. guianensis* in Peru are similar to *U. tomentosa* and include treatment of arthritis, rheumatism, gastric ulcers, gastritis, female contraception, female genitourinary tract cancers, tumors, cirrhosis, and diabetes. In Brazil, the Yanomami drink an infusion of the stem to treat diarrhea and stomachache which is also treated by wrapping the crushed stem around the waist. Indians of Columbia use the vine to treat gonorrhea and dysentery.
Compared to *U. tomentosa*, this species remains scarcely studied. The chemical composition of the plant shows similarities to *U. tomentosa,* yet, whereas *U. guianensis* is reported to contain nearly only tetracyclic oxindole alkaloids, *U. tomentosa* contains largely pentacyclic oxindole alkaloids or both forms.
Around the mid-1970s, the Austrian Klaus Keplinger began his comprehensive studies of the traditional medicine system and medicinal plants used by the Asháninka Indians of Peru. At that time *Uncaria tomentosa* (Willd.) DC was still completely unknown to Western medicine.
The convincingly positive research findings finally led to the development of patented extracts from the root of this plant, tested for the absence of TOA and with a standardised POA content, and to the regulatory approval of a medicinal drug as an adjunctive to basic therapy, and if required to pain therapy, in patients with rheumatoid arthritis (active agent: Extractum radicis Uncariae tomentosae (Willd.) DC mod. pent. titratum).

2. DESCRIPTION OF THE PLANT

Uncaria tomentosa is a massive climbing vine with curved, sharp, highly pungent, protruding spines on the braches. The main stam (stalk) can reach over 27 meters in length with a diameter of close to 15 cm for most of its length, although 20 cm and larger diameters are known.The roots are porous, bitter-tasting, and adstringent. They usually measure several meters in length and as much as 7 cm in diameter. The leaves, measuring 10 cm in length, are opposite, ovate oblong, with either to ten pairs of lateral veins; the underside of the leaves shows minute hairs, hence the term *tomentosa*. The flowers are fragrant, five-lobed, yellowish-white and tiny, occurring in globose heads at the end of the vine. *Uncaria tomentosa* is native to tropical South America and occurs in Colombia, Ecuador, Venezuela, Costa Rica, Guatemala, Panama and Peru.
The only other tropical American species, *Uncaria guianensis*(Aubl.) Gmel., also known as "uña de gato", can be readily distinguished from U. tomentosa by its alternating, recurving to spiralling spines, rather than the slightly curved, opposite spines of the former. The leaves are glabrous whereas those of *U. tomentosa* are hairy and generally less elongated. The inflorescence of *U. guianensis* is reddish-orange, spherical, three-lobed, and 2 to 4 cm in diameter, whereas those of U. tomentosa are yellowish-white, five-lobed, and 1,5 to 2,5 cm in diameter.
Two chemotypes were identified for Peruvian *U. tomentosa*: a tetracyclic oxindole alkaloid type and a pentacyclic oxindole alkaloid type. The pentacyclic chemotype (PC) contains isomeric pentacyclic oxindole alkaloids and the precursor indole alkaloids in small amounts. These differences were confirmed in analyses of progeny sprouted from the seeds of the two chemotypes.

3. INGREDIENTS / CONSTITUENTS

The ingredients of *Uncaria* are carbohydrates (Glycosides), nitrogenous compounds (alkaloids), phenolic compounds (tannins and polyphenolics) and terpenoid compounds (triterpenes and steroids).

Carbohydrates:

Glycosides

Major active constituents of *U. tomentosa* are various quinovic acid glycosides, which have been identified by Cerri et al. (1998) and Aquino at al. (1989, 1991, 1997) in the barks of U. tomentosa and U. guianensis by Yepes et al. (1991). Due to manufacturing procedures (e.g., aqueous-acid), quinovic acid derivates are not extracted without undergoing chemical modification (Keplinger, 1999).

Nitrogenous Compounds:

Alkaloides

The main active constituents of the root and stalk bark are thought to be oxindole alkaloids (Wagner, Kreutzkamp et al., 1985; Reinhard, 1999; Wurm et al., 1998). The major pentacyclic oxindole alkaloids (POAs) identified in the root and stalk bark of Peruvian *Uncaria tomentosa* are pteropodine (uncarine C), isopteropodine (uncarine E), speciophylline (uncarine D), uncarine F (methyl-2-oxo-formosanan-16-carboxylic acid-methyl ester), mitraphylline, and isomitraphylline. The tetracyclic oxindole alkaloids (TOAs) rynchophylline and isorynchophylline also occur in the root. The TOAs occur in small amounts in *U. tomentosa*-PC and in relatively large amounts in *U. tomentosa*-TC. The main alkaloids in the stalk of *U. tomentosa*-PC are POAs (0,5%) (Keplinger et al., 1996). The POA content of the roots (U. tomentosa-PC) ranges from 0,5-3% (Laus and Keplinger, 1997). The leaves, stalk and root barks of *U. guianensis* contain predominantly tetracyclic oxindole alkaloids (TOAs), the major one being rynchophylline. Most cat´s claw products contain varying mixtures of TOAs and POAs (Keplinger et al., 1999), and possibly mixtures of two species of *Uncaria* indigenous to Peru: *U. tomentosa* and *U. guianensis*

Phenolic Compounds:

Tannins

The stalk bark of *U. tomentosa* contains a high amount (20%) of tannins (De Ugaz, 1995).

Polyphenolics

The root bark of U. tomentosa contains proanthocyanidins 1b, 2b, 3b, 4b and 1a and (-)-epicatechin (Montenegro de Matta et al., 1976; Wirth and Wagner, 1997).

Terpenoid Compounds:

Triterpenes

The root bark of U. tomentosa contains triterpenes (Aquino et al., 1990, 1991, 1997), including ursolic acid, oleanolic acid (Aquino et al., 1991), and hydroxylated ursenes (Aquino et al., 1991). Four hydroxyursolic acid-type triterpenes were identified in the stem bark (Kitajima, Hashimoto, Yokoya, Takayama, and Aimi, 2000).

Steroids

Stigmasterol and campesterol were identified in the bark of U. tomentosa and β-sitosterol was found to comprise 60% of the total sterolic fraction (Senatore et al., 1989).

Other Constituents:

The inner bark of the stalk contains β-sitosteryl glucoside and 7-deoxyl-organic acid, the C-8-(S)-isomer of 7-deoxyloganic acid, a newly reported natural productstructurally similar to deoxyloganic acid isomers found in Nepeta cataria (Muhammad et al., 2001).

3.1. CHEMICAL ANALYSIS

Two chemotypes were identified for Peruvian *U. tomentosa*: a tetracyclic oxindole alkaloid type and a pentacyclic oxindole alkaloid type. Researchers have noted that the pentacyclic oxindole alkaloid type is named *savéntaro* (powerful plant) by shamans of the Asháninka tribe of Peru. The pentocyclic chemotype (PC) contains isomeric pentacyclic oxindole alkaloids and their

precursor indole alkaloids in small amounts. These differences were confirmed in analyses of progeny sprouted from the seeds of the two chemotypes.

3.2. STANDARDISATION

No offici(n)al standardisation known

4. PHARMACOLOGY

In vitro tests:

Cytoprotective effects:

The decoction of *Uncaria guianensis* stalk bark was shown to limit gastric and intestinal epithelial cells death in response to oxidant stress, an effect that could have significant therapeutic potential against gastritis and enteritis, such as those induced by *Helicobacter pylori* or by non steroidal anti-inflammatory drugs intake (Miller et al., 2001). The cytoprotective effect could depend from the inhibition of transcription factors associated with apoptosis and inflammation, such as NF-kappaB, which is inhibited by *Uncaria* extracts (Sandoval-Chacón et al., 1998; Miller et al., 1999 and 2001).

Endocrine and Hormonal Functions:

An aqueous extract of dried *U. tomentosa* bark from Peru was tested for the specific interaction with the estrogen receptor of ductal carcinoma cells, in comparison with tamoxifen. The extract significantly and concentration-dependently inhibited [^{3}H] estradiol binding to the cell receptor of the estrogen-dependent tumor cytosol after 1 h incubation. The results of these tests suggest that constituents of the cat´s claw bark extract can interact with "distinct binding sites" on the estrogen receptor which undergoes "different conformational modifications" that may be related to the biological response (Salazar and Jayme, 1998).

Rodriguez and colleagues found out, that cat´s claw has the potential to selectively alter ovarian hormone production and therefore should be used with care.

Clinical studies:

Rheumatoid arthritis:

A randomized double blind clinical trial of a dry extract from the pentacyclic alkaloid-chemotype of *Uncaria tomentosa* was investigated for the treatment of rheumatoid arthritis. Forty patients with active rheumatoid arthritis undergoing sulfasalazine or hydroxychloroquine treatment were included in a randomized 52-week 2-phase-study to evaluate safety and clinical efficacy of *U. tomentosa* extract (60 mg/day, orally). During the first phase (24 weeks, double blind, placebo controlled), patients were treated with the extract or placebo. In the second phase (28 weeks) all patients received the plant extract. 24 weeks of treatment with the extract resulted in a reduction of the number of painful joints compared to placebo. Patients receiving the extract only during the second phase demonstrated a reduction in the number of painful and swollen joints and the articular index for the assessment of joint tenderness (Ritchie Index) compared to the values after 24 weeks of placebo. Only minor side effects were observed (Mur et al., 2002).

A study on the efficacy of a dried aqueous extract of *U. guianensis* bark in the treatment of osteoarthritis of the knee was carried out on forty-five patients: 30 patients were treated with the extract (100 mg/day, orally) and 15 with placebo, for four weeks. The results showed that *U. guianensis* extract reduced pain associated with activity, with benefits occurring within the first week of therapy, even though knee pain at rest or at night, and knee circumference were not significantly reduced during the trial (Piscoya et al., 2001).

Cancer:

Chemotherapy adjunct treatments. An open-label trial of a proprietary extract of cat´s claw root bark (Immodal Pharmaka, GmbH,Austria, Krallendorn a.k.a. Savéntaro) (Uncaria tomentosa-PC, 20-60 mg orally daily) as an adjuvant with conventional treatments was reported over a period of nine years in 53 patients with tumors. All patients were reported to have less side effects

from radiation and chemotherapy and increased mobility and vitality. Those with early-stage tumors remained in remission ten years later, while those with advanced tumors showed, in some cases, arrested cancer growth for several months. Preliminary results indicated promise for the adjuvant in acute myeloid leukemia, brain stem glioma, adenocarcinoma of the colon, testicular teratoma, cervical carcinoma stage IVA, relapsing melanoma, and medulloblastoma (Immodal Pharmaka, 1996).
A 12-month open-label trial therapy of the same extract at 60 mg/day as an adjuvant with conventional cancer treatments in 78 cases of brain tumors was conducted by the University of Innsbruck, Austria. The best results appeared in patients who remained in remission after treatment for some types of brain tumors (e.g. ependymoblastoma WHO II and II, astrocytoma WHO II) more than others (e.g., glioblastoma multiforme WHO IV, malignant meningioma) (Immodal Pharmaka, 1996).

Immune Disorders:
In Europe, physicians using cat´s claw in the form of 4:1 standardized, hydrochloric acid extract of the root bark (Immodal Pharmaka, GmbH, Austria, Krallendorn a.k.a Savéntaro) (Uncaria tomentosa-PC) have reported benefits in their patients with viral infections of HIV (Keplinger, 1992, 1993), herpes simplex, and h. zoster (Immodal Pharmaka, 1996).
13 HIV patients, who refused all other treatments, took voluntarily the same standardized powder extract (total POA content, 12 mg/g) for five months (20 mg/day orally). The extract appeared to decrease the levels of neutrophils in people with high levels of these cells (>9000 cells/µL), while in people with low levels of these cells (<4000 cells/µL) neutrophil levels rose to normal. There were no significant changes in T4/T8 cells ratios. However, absolute and relative lymphocyte counts showed a significant increase. (Keplinger et al., 1999).

Immune Modulation:
Lamm et al. (2001) conducted a study on the potential of a stalk bark extract of *U. tomentosa* (C-Med-100) to enhance the persistence of antibody responses to a 23 valent pneumococcal vaccine (Pneumovax). Male volunteers (n=23) ages 40-60 who had not received previous pneumococcal vaccines and had no chronic disease or were taking nutritional supplements or medications were randomly assigned to two groups: one group received the cat´s claw extract for 60 days at the oral dosage of 350 mg, twice daily and the other group did not receive the extract. After two months the cat´s claw treated group showed a significant enhancement in the ratio of lymphocytes to neutrophils, but no changes in other blood cells. No significant difference in antibody titers between the two groups were observed. Nevertheless, at five months, the cat´s claw-treated group showed no drop in antibody titer responses to pneumococcal vaccination whereas the other group had significantly decreased pneumococcal antibody titers. No reports of side effects attributable to the cat´s claw extract were reported.

5. MICROBIOLOGY

Anti-inflammatory activity:
In vivo studies showed that *U. tomentosa* possesses anti-inflammatory properties. Aguilar et al. (2002) assessed the anti-inflammatory activity of *U. tomentosa* bark, comparing the effect of a hydroalcoholic extract to that of an aqueous extract after daily oral administration to mice, for 8 days. The anti-inflammatory activity of the hydroalcoholic extract, evaluated as inhibition of the carrageenan paw oedema, was higher than that of the aqueous extract. The extracts inhibited also the transcription factor NF-kappaB, the hydroalcoholic one being the most active.
Aqueous extracts of *U. tomentosa* and *U. guianensis* bark, were shown to possess antioxidant activity and to inhibit TNF-α production in RAW 264.7 cells. Despite its higher oxindole and pentacyclic alkaloids content, *U. tomentosa* was significantly less active than *U. guianensis*, suggesting that the effect is independent from the alkaloid content. Moreover, administration of each extract to rats for 3 days in drinking water (5 mg/ml), reduced the gastric lesions induced by indomethacin, and prevented TNF-α mRNA expression and apoptosis (Sandoval et al., 2002).

Among *U. tomentosa* constituents, a quinovic acid glycoside was shown to possess anti-inflammatory properties (Aquino et al., 1991).

Antiviral activity:
Six quinovic acid glycosides from *U. tomentosa* were shown to inhibit vesicular stomatitis virus infections in CER cells, whereas a quinovic acid glycoside [(28)-β-D-glucopyranosyl ester] was reported to exhibit activity against rhinovirus type 1B infection in HeLa cells (Aquino et al., 1989). The procyanidins cinchonain Ia and cinchonain Ib, and (-)-epicatechin, isolated from the bark of *U. tomentosa*, are considered responsible for both antiinflammatory and antiviral activity (Aquino et al., 1989). These studies support the suggestion that both forms of bioactivity in *U. tomentosa* (anti-inflammatory and antiviral) are due to a combination of active compounds.

Antibacterial properties:
Although the *Uncaria* genus is not used extensively to treat infections in traditional medicine, some reports evaluating antibacterial activity have appeared as early as 1965. An extract of *U. glabrata* was observed to inhibit the growth of Streptococcus aureus and Escherichia coli as evaluated by the disk diffusion method (Arret et al., 1971).
As part of an antimutagenicity study of *U. tomentosa*, toxicity against *Salmonella typhimurium* TA 98 and TA 100 strains was evaluated. Results indicated toxicity only at the highest concentrations (1000 µg/plate) (Wurm et al., 1998). Toxicity of *U. tomentosa* towards *Photobacterium phosphoreum* was evaluated using a Microtox assay which showed no toxicity of aqueous extracts at concentrations up to 100 µg/ml (Sandoval et al., 2002).
Kloucek et al. (2005) showed an antibacterial activity for an ethanol extract of *U. tomentosa* bark against the Gram + *Bacillus cereus*, *B. subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *S. epidermidis*, as well as against the Gram – *Escherichia coli*.

6. EFFICACY

The principal action of the extract from the pentacyclic chemotype of Uncaria tomentosa (Willd.) DC is on the immune system.
Pentacyclic oxindole alkaloids *of U. tomentosa* stimulate the phagocytotic activity of the macrophages(Wagner et al., 1985).. and RES cells. Under the influence of pentacyclic oxindole alkaloids, human endothelial cells secrete a protein that regulates the proliferation of lymphocytes: while the proliferation rate of resting or weakly activated lymphocytes is significantly increased, this protein inhibits the clonal expansion of lymphoblasts and lymphoid cells lines (Keplinger et al., 1999). The pentacyclic oxindole alkaloids increase or reduce the reactivity of the immune system, depending on the initial status. These chemical constituents can thus stimulate a weak immune system and suppress an over-reactive immune system (www.uncaria.at).
This immunological action of preparations of *Uncaria tomentosa* (Willd.) DC is corroborated by its use in folk medicine as well as by recent scientific studies. The efficacy of *Uncaria tomentosa* (Willd.) DC in the treatment of rheumatoid arthritis and osteoarthritis has been clinically proven (Piscoya et al., 2001; Mur et al., 2002). This immunological action of preparations of Uncaria tomentosa (Willd.) DC is corroborated by its use in folk medicine as well as by recent scientific studies. The efficacy of Uncaria tomentosa (Willd.) DC in the treatment of rheumatoid arthritis has been clinically proven.

Efficacy recommended in Phytokodex: antiphlogistic, antiviral, immun-stimulant, immun-modulating, enhancement of the number of leucocytes and enhancement of phagocytosis (Kubelka,W.,Länger,R,2001).

No scientific studies of the efficacy of cat´s claw as birth control or for menstrual difficulties exist, though hormonal effects in animals indicate potential efficacy.

7. TOXICOLOGY

In vitro Toxicity: An aqueous extract of *U. tomentosa* bark, evaluated for cytotoxicity against Chinese hamster ovary cells and bacterial cells (*Photobacterium phosphoreum*) at concentrations ranging from 10 to 100 mg/ml, did not induce toxic effects detectable at neutral red assay, total protein content, tetrazolium assay and Microtox test (Santa Maria et al., 1997).
U. tomentosa root bark extracts and fractions did not show mutagenicity in different strains of *Salmonella typhimurium* with and without metabolic activation (Rizzi et al., 1993).
The acute median lethal dose (LD_{50}) in mice of the freeze-dried aqueous extract of the root (*U. tomentosa*-PC) administered as 40% suspension in tragacanth gruel (0,5 %) by oral route was shown to be ~~is~~ greater than 16 g/kg (Reinhard, 1999; Keplinger et al., 1999).
After single oral administration to rats, a hydroalcoholic extract of *U. tomentosa*, containing 4 % alkaloids, showed LD_{50} and maximum tolerated dose levels greater than 8 g/kg. The same extract, daily administered to rats at oral doses of 10-80 mg/kg for 8 weeks, or 160 mg/kg for 4 weeks, did not induce acute or chronic toxicity signs observable symptomatically. In addition, no body weight, food consumption, organ weight and kidney, liver, spleen, and heart pathological changes were found to be associated with the extract treatment (Sheng et al., 2000).
Further studies revealed that oral administration of an aqueous extract of *U. tomentosa* in mice at the dose of 16 mg/kg was lethal for two of ten animals and that the LD_{50} of the extract was higher than 16 mg/kg. Moreover, an aqueous extract administered to rats for 28 days (1000 mg/kg/day), did not provoke mortality of animals, but it caused a slight increase in percentage of lymphocytes, a reduction in percentage of neutrophil granulocytes, and an increase of the relative weight of the kidney (Keplinger et al., 1999).
A commercial cat´s claw extract obtained in the United States (water/ethanol extract containing 4 % alkaloids) showed a maximum tolerated dose level of 2,5-5 g/kg, with diarrhea and sedation as toxic signs (Sheng et al., 2000).

7.1. SAFETY

Recommended dosage for humans
recommended daily allowence: 60 mg extract (standardised on pentacyclic Oxindole-alcaloids, free from tetracyclic Oxindole-alcaloids.

Contraindications:
In Europe, use of root (*U. tomentosa*-PC) and preparations thereof is not advised for infants under three years of age because of a lack of clinical data.Other contraindicated patients are those waiting to undergo bone marrow or any other organ transplants (Immodal Pharmaka, 1995).

Drug interactions:
In Europe, patients are advised to avoid the root (*U. tomentosa*-PC) or extract preparations thereof if receiving or about to receive treatment with any of the following medical interventions: any treatment using immunosuppressant agents: hyperimmunoglobulin therapy (intravenously: i.v.); thymic extracts (i.v.); hormone treatments with animal peptide or protein hormones, such as insulin derived from pigs or cattle: cryoprecipitates or fresh plasma (in hemophiliacs) although standardized factor presents no problem; and passive vaccines made from animal sera which present a risk of serum reaction, although active vaccinations present no risk except for the possibility of a more severe postvaccinational reaction which may be attended with fever (Immodal Pharmaka: Kemper, 1995).
Keplinger et al. (1999) analyzed approximately fifty products of *U. tomentosa* from United States, Central America and Peru and found varying mixtures of pentacyclic and tetracyclic (up to 80 % of total) alkaloids. Two chemotypes of *Uncaria tomentosa* with different alkaloids

patterns occur in nature: one contain pentacyclic oxindoles and the other contains tetracyclic oxindoles. This difference should be considered when the plant has to be used for medicinal purposes as tetracyclic oxindole alkaloids act on the central nervous system, whereas pentacyclikic oxindole alkaloids affect the cellular immune system. Moreover, tetracyclic alkaloids exert antagonistic effects on the action of pentacyclic alkaloids (Reinhard, 1999). The pentacyclic oxindole alkaloids of *U. tomentosa* were shown to stimulate human endothelial cells to release a factor which increases the production of B and T lymphocytes and that the secretion of this factor was antagonized by tetracyclic oxindole alkaloids (Wurm et al., 1998; Keplinger et al., 1999). For these reasons, mixtures of the two types of drugs are unsuitable for medicinal uses (Reinhard, 1999).

Pregnancy and Lactation:
Use of cat´s claw root (*U.tomentosa*-PC) and extract preparations is contraindicated for pregnant or lactating women and in children less than three years old (Kemper, 1999). However, the considerations for pregnancy and lactating differ.
A number of studies have demonstrated hormonal effects of *U. tomentosa* extract that may interfere with fertility as well as caus miscarriage during pregnancy. Mice administered with an aqueous extract of cat´s claw root (*U. tomentosa*) in their drinking water (6,25mg/kg and 25 mg/kg) showed no signs of toxicity. However, when caged with male mice, the female mice produced no offspring. Cat´s claw stalk bark (*U. tomentosa*) has shown in in-vitro anti-estrogenic activity. Amounts equivalent to dietary doses caused a dose-dependent (100 pg/mL to 10 µg/mL) and statistically significant inhibition of progesterone production in vitro. In sexually mature female rats, cat´s claw bark (2mg/day or 20 mg/kg orally: p.o) reduced serum progesterone (P4) and estradiol (E2) levels by 68% and 71%, respectively. Low progesterone states are known to reduce fertility, trigger miscarriage, and contribute to menstrual difficulties.
No studies of cat´s claw in lactating animals exist. No cases of adverse reactions of infants exposed to cat´s claw are known.

The root bark decoction may, in some patients, result in temporary constipation during the first course of ingestion (Reinhard, 1999).

In patients infected with HIV and in cancer patients, however, rarely, an increase in uric acid may result from an increased lysis of tumor and virally-infected cells. In patients with tumors taking the same extract, a resultant lytic fever may persist for as long as two weeks. In patients with autoimmune diseases or tumors, intermittent constipation and/or diarrhea may occur as a result of additive lactose, both of which are readily treatable with available agents.

Interaction with chemotherapies:
Patients receiving large doses of chemotherapy and taking a standardized liquid extract of the root (*U.tomentosa*-PC, p.o.) have sometimes shown a dramatic fall in erythrocytes, which may be the result of increased elimination of damaged cells. The effect can be avoided by taking the extract up to two days prior to and every two to six days following chemotherapy (Immodal Pharmaka, 1995).

KBB

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URTICA SP

Herba/folium Urticae

1. INTRODUCTION

Nettles have been used for more than 2000 years in various ways including fiber production, the use as a nutrient and animal fodder, as a dye for fabrics and as a vegetarian rennet for cheese making. For medical purposes, in Europe mainly *U. dioica* L. and *U. urens* L. are used.
The main medicinal uses of nettles historically were internally as a tonic and highly nutrient food and for the treatment of anemia, rheumatism, and arthritis, eczema, asthma, urinary gravel, stomach complaints, skin infections and as an anti-haemorrhagic. Externally they have traditionally been used as a hair tonic/shampoo, as a styptic for nose bleeds and haemorrhoids and for stinging treatment (urtication) in arthritis and rheumatism (Randall, 2003a). Some of these traditional indications could be proven by pharmacological testings and clinical studies, but in many cases the compounds responsible for the effects attributed to nettle remain unknown.
The plant parts used for medical purposes nowadays are the leaves, the herbs, the roots and the fruits (Frank et al. 1998).
Nettle leaf and herb are used internally and externally for the adjuvant therapy of rheumatic diseases, as a mild diuretic in disorders of the urinary tract and for the prevention and therapy of renal gravel (Wichtl, 2002, Randall, 2003b). They are used as fresh plant, tea preparation, pressed juice, tincture, fluid and dried extract (Blaschek *et al*, 1998; Wichtl, 2002). There is also some evidence that nettle fruit extracts are effective in the adjuvant therapy of rheumatic diseases. Nettle roots are used as tea or extracts for the treatment of benign prostatic hypertrophy (Randall, 2003b). The present monograph will focus on nettle herbs and leaves.

2. DESCRIPTION OF THE PLANT

The genus *Urtica* L. belongs to the family Urticaceae, under the division Spermatophyta, subdivision of Angiospermae, class Dicotyledonae, group Apetalae, order Urticales. The members of the genus are annual or perennial herbs, in some cases shrubs or small trees, furnished with stinging hairs. The genus comprises about 100 species, distributed in temperate and tropical regions throughout the world. Some *Urtica* species are grown for food and nutrition purposes (Kalvali, 2003).

2.1 SYSTEMATICS

U. dioica is a perennious plant reaching a height of 30-150 cm; the stems are tetragonal and bear stinging hairs; the leaves are greygreen, opposite, ovate-oblong, tapering, with serrate leaf edges, cordiform at the leaf base and on both sides covered with stinging hairs. The plant is usually dioecious. In contrast, *U. urens* is annual, monoecious and smaller than *U. dioica* (10-60 cm), also the leaves are smaller, more roundish and incised more deeply than in U. *dioica*. (Frank *et al.,* 1998; Schomakers *et al.*, 1995). *U. dioica* is indigenous to Africa and western Asia, but is nowadays found in all temperate regions of the world. Due to the difficulty in botanical differentiation between *U. dioica* and *U. urens* in the wild, they are often harvested together. Altough both species have a similar distribution, *U. urens* has become less widely distributed due to the reduction of its habitat (WHO, 2002).

2.2 PLANT PARTS USED

Nettle leaves consist of the whole or cut dried leaves of *U. dioica*, *U. urens* or a mixture of the 2 species. The material complies with the monograph of the European Pharmacopoea (EAB, 2005).

3. CHEMICAL CONSTITUENTS

The chemical constituents of the upper plant parts of *U. dioica* and *U. urens* are rather similar with exception of the caffeic acid derivatives*: U. dioica* contains up to 1.6% caffeoylmalic acid, which is absent in *U. urens*; besides, up to 0.5% chlorogenic acid and small amounts of several other organic acids are present in both species (WHO, 2002; Frank *et al*., 1998). The EAB specifies at minimum 0.3% for the sum of caffeoylmalic acid and chlorogenic acid, expressed as chlorogenic acid (dried drug) (EAB, 2005).

Apart from that, in both species flavonoids, coumarins (mainly scopoletin) and steroids (β-sitosterol and β-sitosterolglucoside) can be found (Frank *et al*., 1998). A TLC method with scopoletin and chlorogenic acid as reference compounds is used for examination of identity according to EAB. The quantification of chlorogenic and caffeoylmalic acid can be performed by HPLC (EAB, 2005).

The aerial parts are rich in inorganic minerals (about 18% expressed as ash) including potassium (1.8-2%) and silica (0.9-1.8%). (ESCOP, 2003). The plant accumulates nitrate dependent on the nitrogen content of the soil. Marketed drugs contain on the average 1.5% nitrate, but high variation is possible. The leaves are rich in vitamins, especially vitamin C and α-tocopherol.

As 100 g of fresh herb contain 3.82 g protein, the yield in protein per hectare is higher than in most of the traditional fodder plants. Its biological quality is similar to that of yeast or oilseed protein (Frank *et al.,* 1998).

The stinging hairs contain acetylcholine, histamine, serotonine, choline and small amounts of leukotrienes (ESCOP, 2003). These compounds are responsible for the typical skin reactions.

4. PHARMACOLOGY

4.1 IN VITRO AND ANIMAL TESTING

4.1.1 ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

A *U. dioica* leaf hydroethanolic extract and its main phenolic constituent, caffeoylmalic acid, were tested for inhibitory potential on the biosynthesis of arachidonic acid metabolites in RBL-1 cells. Both showed inhibitory activity against 5-lipoxygenase dependent synthesis of LTB_4 and COX-derived prostaglandin synthesis. The same extract was shown to reduce the LPS stimulated release of two proinflammatory cytokines in human whole blood. An aqueous nettle leaf extract significantly modulated the cytokine production of human Th1 and Th2 T helper

cells which indicates that the extract may inhibit the inflammatory cascade in autoimmune diseases such as rheumatoid arthritis (ESCOP, 2003).
Inflammatory joint diseases are characterized by enhanced extracellular matrix degradation, which is predominatly mediated by cytokine stimulated upregulation of matrix metalloproteinase (MMP) expression. Besides TNF-α, IL-1β produced by articuar chondrocytes and synovial macrophages is the most important cytokine stimulating MMP expression. A 95% isopropanolic extract from nettle leaf and 13-hydroxyoctadecatrienoic acid which is present in the extract significantly suppressed IL-1β induced expression of MMP proteins on cultured human chondrocytes at a concentration of 10 µg/ml (Schulze-Tanzil *et al.,* 2002).
The analgesic activity of aqueous and ethanolic nettle extracts has been investigated *in vivo* in rats and mice with inconsistent results. Local application of a nettle herb aqueous extract to the rat tail led to a local anaesthetic effect in the tail flick test comparable to that of lidocaine (ESCOP, 2003).

4.1.2 DIURETIC AND HYPOTENSIVE EFFECTS

Continuous intravenous perfusion of isotonic solutions of an aqueous nettle herb extract led to a significant diuretic and natriuretic effect which was in the higher dose comparable to that of furosemide. This effect was accompanied by a dose dependent hypotensive effect which was reversible within about 1 hour at the lower dose but persistent after the higher dose of nettle extract indicating a possible toxic effect at this dose level.
Other authors did not find diuretic effects of nettle herb extracts in rats. Due to these contradictory results, the efficacy of nettle herbs as a diuretic is not yet fully documented (ESCOP, 2003, Wichtl, 2002).

4.1.3 INFLUENCE ON THE LEVEL OF BLOOD SUGAR

An 80% ethanolic extract and an aqueous decoction of nettle herb produced hyperglycaemic effects in an oral glucose tolerance test in mice (Neef *et al.,* 1995). Roman *et al*. (1992), who tested the antidiabetic effect of an *U. dioica* decoction in rabbits, also found a slight increase in glycemia. In contrary to that, Bnouham *et al*. (2003) found a strong blood glucose lowering effect in rats for an aqueous nettle herb extract which was administered 30 minutes prior to glucose loading. According to the authors this effect may be caused in part by the reduction of intestinal glucose absorption.

4.2 VETERINARY STUDIES

The number of studies concerning the effect of nettle herbs and leaves on productive livestock is very small and mainly concerning herbal mixtures that contain Urtica among other plants.
Grela *et al.* (2003) investigated the influence of dietary supplementation with two different herbal mixtures, both containing *U. dioica* leaves, on the general performance and blood parameters of piglets. After 40 days, they compared the results to a control group receiving standard feed and a positive control receiving 20 mg avilamycin/kg diet. The group fed the mixture containing U. *dioica* leaves, *Plantago lanceolata* herbs, lyophilized *Allium sativum* bulbs, *Pimpinella anisum* seeds and *Echinacea purpurea* fruits had the best daily gains, feed utilization and blood indices; the production gains were even better than with the antibiotic additive.
Urbańczyk *et al*. (2002) studied the efficacy of a different multi-component herb mixture as an antibiotic substitute in pig feeding: A mixture consisting of *Melissa officinalis*, *Mentha piperita*, *U. dioica, Thymus vulgaris*, *Agropyron repens*, *Allium sativum*, *Capsicum annuum*, *Origanum majorana*, *Coriandrum sativum*, *Taraxacum vulgare* and *Silybum marianum* was compared to a control group fed standard feed and a positive control receiving 20 mg/kg flavomycin. Also in this study pigs fed the herb mixture obtained the best average body weight gains. There were no statistical differences between the experimental groups in meat quality (colour, acidity, water holding capacity).
Due to the complexity of the mixtures used in these studies it is impossible to estimate the contribution of *U. dioica* to the observed effects.
Dügenci and coauthors (2003) studied the immunostimulant effect of a nettle leave hot water extract in fish (rainbow trout, *Onchorhynchus mykiss*). General growth performance and non

specific defense mechanisms (respiratory burst activities of blood leukocytes inside and outside the cells production, phagocytosis of blood leukocytes, total plasma protein level) were determined at the end of a feeding period of three weeks. The nettle extract only showed moderate activity.
Pohorecka (2004) performed a study on the effect of standardized plant herb extracts on the general condition of the honeybee (*Apis mellifera* L.) and found that the administration of a syrup preparation containing 5% of a standardized nettle extract significantly alleviated the impact of adverse caging conditions. The worker bees of the nettle group showed better condition than on the sampling day and than bees fed syrup containing other herbal supplements or syrup alone.

4.3 HUMAN STUDIES

4.3.1 ADJUVANT TREATMENT OF ARTHRITIS, ARTHROSES AND/OR RHEUMATIC CONDITIONS

Five open, multicentric, post marketing surveillance studies have been performed on patients with arthritic or rheumatic complaints using a preparation containing a dry hydroethanolic extract of nettle leaf (6.4-8:1) at a daily dosage of 2x670 mg. In each study a proportion of the patients also continued other therapies such as non steroidal anti-inflammatory drugs, while others only received the nettle extract. Assessments were carried out through patient questionnaires and consultations with physicians. Overall, 80-95% of the patients rated the efficacy, and 93-95% rated the tolerability of the extract as good or very good.
In an open, randomized 14-day study, patients with acute arthritis received daily either 2x100 mg of diclofenac or 50 mg of diclofenac and 50 g of nettle leaf puree (caffeoylmalic acid content 20 mg). The elevated serum levels of C-reactive protein, which was the main criterion of the study, decreased by about 70% in both groups. Assessment of physical impairment, subjective pain and stiffness showed improvements in the range 52-77%, with no significant differences between the groups (ESCOP, 2003).
The efficacy of the external application of fresh nettle leaves was studied in a randomized, double-blind, crossover study involving 27 patients with persistent oseoarthritic pain at the base of the thumb or index finger. As a placebo *Lamium album* was used. Treatments were applied for 1 week each, interrupted by a 5-week washout period. Compared to placebo, significantly greater reductions in scores were observed with nettle leaf on a visual analogue scale for pain. No serious side effects were reported (Randall *et al*., 2000).

4.3.2 DIURETIC EFFECTS (ESCOP,2003)

In an open 2-week study, 32 patients suffering from myocardial or chronic venous insufficiency were treated daily with 3x15 ml nettle herb pressed juice. A significant increase in the daily volume of urine was observed throughout the treatment. Minor decreases in body weights and systolic blood pressure were also observed.

4.3.3 EFFECT AGAINST ALLERGIC RHINITIS

A randomized, double blind, placebo-controlled trial assessed the effects of a freeze dried nettle herb preparation in subjects with allergic rhinitis. At the onset of symptoms, 600 mg of the preparation or placebo were ingested daily for 1 week. Nettle herb was rated higher than placebo in the global assessment, but rated less high on the basis of data from the symptom diaries (Barnes *et al*., 2002).

4.4 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

No data available

5. MICROBIOLOGY

Steenkamp *et al*. (2004) found no antibacterial activity for *U. urens* aqueous and methanolic extracts against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Gülçin *et al*. (2004) found antimicrobial activity for an *U. dioica* aqueous extract (250 µg per disc) against nine microorganisms (*Pseudomonas aeruginosa*, *Eschericia coli*, *Proteus mirabilis*, *Citrobacter koseri*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Staphylococcus epidermidis* and *Candida albicans*), but no MICs were determined.

Keleş and coauthors (2001) tested an 80% ethanolic *U. dioica* herb extract against several pathogens of veterinary importance, namely *Staphylococcus aureus*, *Streptococcus agalactiae*, *Straptococcus dysgalactiae*, *Salmonella gallinarum*, *Escherichia coli* and *Klebsiella pneumoniae*, using the agar diffusion and dilution method. They only found activity against *E. coli, S. aureus* and *S. agalactiae*, with rather high MIC´s (4 mg/ml).

6. EFFICACY

The leaves of *Urtica* species are excellent sources of some important minerals and vitamins. Moreover they have a higher level and a better quality of protein compared with many other green leafy vegetables and fodder plants. Because of their high nutritional value they are used in husbandry as a fodder for pigs and poultry (Frank *et al.* 1998; Wetherilt, 2003). About the efficacy of nettle as sensory and flavouring agent for animal feed no data are available. Concerning the use in humans, nettle leaves and herbs is listed by the Council of Europe as a natural source of food flavouring in category 1 (no restrictions for use proposed; not considered as a health risk in the quantities used). Nettle is used as a food in soups or herbal teas. In the USA, the plant is listed by the FDA as Herb of Undefined Safety (Barnes *et al.*, 2002).

Concerning the medical efficacy in humans, *U. dioica* and *U. urens* herb and leaf preparations are considered to be effective in the internal and external adjuvant therapy of rheumatic diseases and as diuretics in the therapy of inflammatory diseases of the urinary tract and for the prevention and therapy of renal gravel (Wichtl, 2002).

7. TOXICOLOGY

7.1 SAFETY

7.1.1 PRECLINICAL SAFETY DATA

The intraperitoneal LD_{50} of an aqueous *U. dioica* herb extract has been determined in mice as 3.625 g/kg b.w (ESCOP, 2003) and 3,5 g/kg b.w. respectively (Bnouham *et al*., 2003), suggesting a low toxicity of the water extract.
An ethanolic *U. dioica* herb extract showed low toxicity in rats and mice after oral and intraperitoneal administration at the equivalent of up to 2 g of dried drug per kg body weight (ESCOP, 2003).

7.1.2 CLINICAL SAFETY DATA

No serious adverse effects were reported from 5 clinical studies in which a total of 10,368 patients took 2x 670 mg of a dry hydroethanolic nettle leaf extract (6.4-8:1) daily for periods of 3 weeks up to 12 months; the incidence of minor adverse effects (mainly gastrointestinal upsets or allergic reactions) was 1.2-2.7%. In a study where 19 patients received 50 g of a stewed nettle leaf puree daily for 14 days, 3 patients reported meteorism (ESCOP, 2003)
.

For productive livestock, no safety data are available.

7.2 DOSAGE

7.2.1 INTERNAL USE

Adults: hydroalcoholic extracts corresponding to 8-12 g of nettle leaf daily, divided into 2-3 doses; 3-5 g drug as an infusion up to 3 times daily; tincture 1:5 (25% ethanol) 2-6 ml 3 times daily; 15 ml of fresh juice up to 3 times daily (Frank *et al*., 1998; ESCOP, 2003).

External use:

Adults: fresh nettle leaf applied to the skin in the area of pain for 30 seconds once daily (ESCOP, 2003).

EMW

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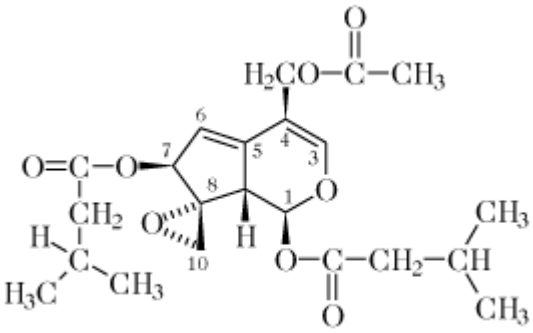
VALERIANA OFFICINALIS



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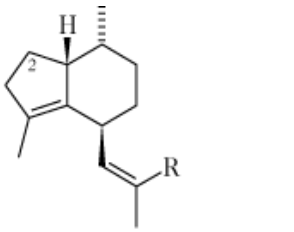

Valeriana officinalis

1. INTRODUCTION

The use of valerian species has a very long tradition in many parts of the world. The original use as a perfume or as a source of food was totally different to its modern application as a sedative and calming agent. The most important commercial *Valeriana* species are *V. officinalis*, *V. wallichii*, *V. fauriei* and *V. edulis* (Dweck, 1997). The present monograph will focus on *Valeriana officinalis* L., the species commonly used in northern European medicine.

Valerian is stated to possess sedative, mild anodyne, hypnotic, antispasmodic, carminative and hypotensive properties. Traditionally, it has been used for hysterical states, excitability, insomnia, hypochondriasis, migraine, cramp, intestinal colic, rheumatic pains, dysmenorrhoea and specifically for conditions presenting nervous excitability. Modern interest in valerian is focused on its use as a sedative and tranquillizing agent. In a draft Community Herbal Monograph on valerian root, the European Medicines Evaluation Agency Commitee on Herbal Medicinal Products (EMEA HMPC) designates the use of aqueous or aqueous/ethanolic valerian root preparations for the relief of mild nervous tension and difficulty in falling asleep to be well-established (EMEA, 2005). The plant is also used as a sedative for animals in herbal veterinary medicine (Bentz *et al.*, 1989). However, for some animals such as cats or rats, it is an attractant and has an excitatory effect (Dweck, 1997)

Despite intensive efforts it is not possible so far to attribute the quite well documented sedative activity of the drug to a single compound or a class of compounds; current thinking is that the activity of valerian is based on a synergistic effect of the active classes of compounds and not caused by a single substance (Wichtl, *2002*).

2. DESCRIPTION OF THE PLANT

Valeriana officinalis is a tall perennial herb whose underground portion consists of a vertical rhizome bearing numerous rootlets and one or more stolons. The aerial portion consists of a cylindrical hollow, channeled stem attaining 2 m in height, branched in the terminal region, bearing opposite exstipulate, pinnatisect, cauline leaves with clasping petioles. The inflorescence consists of racemes of cymes whose flowers are small, white or pink. The fruits are oblong-ovate, 4-ridged, single-seeded achenes (WHO, 1999). The morphology can vary in detail depending on the subtype *(*Reichling *et al*., 1994).

2.1 SYSTEMATICS

Valeriana is the major genus of the Valerianaceae, a family represented in all the temperate and subtropical areas in the world (Dweck, 1997). The genus Valeriana is extremely rich in species, it comprises more than 250 species.
V. officinalis L. s.l. is a very diverse composite species occurring all over the temperate regions of Eurasia, which is split up in several ecotypes and geographic races. Additionally, differences exist concerning the number of chromosomes, ranging from diploid to octaploid races. They are partly restricted to certain regions, partly they are spread over wide areas. Nowadays, in middle and southern Europe they are grouped in 4 base types depending on morphology: *exaltata*, *collina*, *procurrens* and *sambucifolia* (Reichling *et al*., 1994).

Other *Valeriana* species used: *Valeriana celtica*, celtic valerian, Speick (for fragrance);
V. wallichii, Indian or Himalayan valerian; *V. edulis ssp. procera,* Mexican valerian

2.2 PLANT PARTS USED

For medical purposes, the underground parts of the plant are used. Valerian root (Valerianae radix) consists of the gently dried (not > 40 °C), whole or cut underground parts of *V.officinalis* L. *s.l.*, including the rhizome surrounded by the roots and stolons. The material complies with the monograph of the European Pharmacopoea (EAB, 2005, WHO, 1999). The drug has a characteristic smell like isovaleric acid and camphor. The taste is sweetish at the beginning, later aromatic and weekly bitter (Reichling *et al*., 1994).
The drug is prepared as a tea or processed to tinctures, fluid and dry extracts or pressed juices and other galenic preparations for internal use. The extracts and/or the essential oil of valerian root can also be used externally as bath essence (Reichling *et al.,* 1994; Wichtl, 2002). According to the EMEA HMPC draft monograph (EMEA, 2005), the use of aqueous and hydroethanolic extracts and tinctures is well-established, whereas the use of dried valerian root, fresh plant juice and valerian root oil is based on tradition.
As the drug nowadays is mostly derived from cultures, adulterations and confusions are rare (Reichling *et al*., 1994).

3. CHEMICAL CONSTITUENTS

Due to the many subtypes and varieties of *V. officinalis* L. *s.l.,* the spectrum and amount of constituents can vary considerably depending on geographic origin and subtype (Reichling *et al.*, 1994). The classes of compounds the sedative activity of valerian root is nowadays mainly attributed to are the volatile oil and the valepotriates and their degradation products, but latterly also a contribution of lignans and flavonoids is discussed.

Current thinking is that these classes of compounds contribute to the overall activity of the drug by different mechanisms of action. Therefore, the quality of valerian preparations will depend on the presence and concentrations of several classes of compounds (Barnes *et al.*2002).
The volatile oil consists of monoterpenes mainly present as esters, sesquiterpens and the sesquiterpene acids acetoxyvalerenic acid, hydroxyvalerenic acid and valerenic acid. Acetoxyvalerenic acid and valerenic acid are characteristic compounds for *V. officinalis* and do not occur in non European Valeriana species, so they are used for the identification of the drug (Barnes *et al.*, 2002, Reichling *et al*., 1994). The European Pharmacopoea specifies a minimum volatile oil content of 5 ml/kg for the whole drug and 3 ml/kg for the cut drug. The content of sesquiterpene acids has to be at least 0.17%, calculated as valerenic acid. The volatile oil content is determined by steam distillation, and the sesquiterpene acids are quantified by HPLC (Ph. Eur. 5, 2005).
The dried crude drug contains about 0.5 to 2% valepotriates (iridoids) with valtrate as the main compound. Those compounds are unstable and decompose by high drying temperature, long storage and processing. Valepotriates are not present in significant amounts in aqueous or dilute alcoholic extracts after a few days. They are also metabolized in the gastrointestinal tract. The main degradation products are baldrinal and homobaldrinal. There is evidence that these compounds are partly responsible for the sedative activity of the drug (Reichling *et al*., 1994, Houghton, 1997).
Additionally the drug contains organic acids such as chlorogenic acid, caffeic acid and 1-2% isoferulic acid, which serves as a marker compound for aqueous extracts, because it is only present in *V. officinalis* root. Recently several lignans have been isolated, and some of them have also shown effects in radioligand binding assays on several CNS-receptors (Schumacher *et al.*, 2002; Bodesheim and Hölzl, 1997). Small amounts of alkaloids are present, and the concentration of amino acids is quite high in the drug. Especially γ-aminobutyric acid (GABA) is present in quite high quantities and could therefore also contribute to the sedative effect of the drug (Reichling *et al.*1994, Houghton, 1997).

4. PHARMACOLOGY

4.1 IN VITRO TESTING

Sedative effects

The sedative activity of *V. officinalis* has been demonstrated both *in vitro* and *in vivo* and has been attributed to the volatile oil and the valepotriate fractions (Barnes *et al.*, 2002). Recent studies also revealed effects of other compounds in several *in vitro* and animal models.
Many potential mechanisms for the sedative activity of valerian have been proposed, including agonistic activities on the GABA, adenosine, benzodiazepine and barbiturate receptors (Barnes *et al.*, 2002). However, in some older reports the concentrations used in these investigations were very high, and more recent reports put some of these data into question (Dietz *et al.*, 2005). So, the discussion about the mechanism of sedative action is still going on.
Aqueous and hydroalcoholic extracts were found to show affinity to the $GABA_A$ receptors; also an inhibition of the uptake and a stimulation of the release of radiolabelled GABA in synaptosomes isolated from rat brain cortex was shown. This effect was attributed to GABA itself, which was present in the extract in high concentrations. Additionally, valerenic acid was shown to inhibit the enzyme system responsible for the central catabolism of GABA (Barnes *et al.*, 2002).
Wasowski *et al.*(2002) found that 6-methylapigenin, a flavone present in valerian, is a ligand for the benzodiazepine binding site of the $GABA_A$ receptor. Recently, Granger *et al.* (2005) found, that the essential oil compounds (+)-and (-)-borneol are positive modulators of GABA action at human recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors, enhancing the action of low GABA concentrations by more than 1000%. The enhancing effect was independent to flumenazil, indicating that the compounds do not act at classical benzodiazepine sites.
Schumacher *et al.* (2002) performed radioligand binding assays at various receptors of the central nervous system (CNS) and found that an olivil derivative acts as a partial agonist at adenosine A_1 receptors exhibiting A_1 affinity and activity in low micromolar concentrations.

Another lignan, namely 8´-hydroxypinoresinol, was shown to exhbit affinity to serotonin 5-HT_{1A} receptors (Bodesheim and Hölzl, 1997). Dietz and coauthors (2005) tested valerian root extracts of different polarities in radioligand binding studies for 8 serotonin receptor subtypes and some other CNS receptors and showed that lipophilic valerian root extracts and valerenic acid act as partial agonists of the 5-HT_{1A} receptor. This serotonin receptor is believed to be involved in circadian (sleep-wake) rhythms, anxiety and explorative behavior.

Spasmolytic activity
The spasmolytic activity of the valepotriates is principally due to valtrate or dihydrovaltrate. These agents can act on centres of the CNS and through direct relaxation of smooth muscle, apparently by modulating Ca^{2+} entry into the cells or by binding to smooth muscle (WHO, 1999; Barnes *et al.*, 2002). The antispasmodic activity of valerian oil was shown on isolated guinea pig uterine muscle, but could not be confirmed *in vivo* (Barnes *et al.*, 2002).

4.2 ANIMAL STUDIES

Also many animal studies were performed to prove the *sedative activity* of valerian root:
A screening of the volatile oil components for sedative activity concluded valerenal and valerenic acid to be the most active compounds, causing ataxia in mice at a dose of 50 mg/kg (i.p.). Valerenic acid also acts as a general CNS depressant at high doses (100 mg/kg i.p.); a dose of 400 mg/kg however resulted in muscle spasms, convulsions and death. Valerenic acid was also reported to prolong pentobarbitone induced sleep in mice, resulting in a hangover effect (Barnes *et al.*, 2002).
CNS-depressant activities in mice have also been documented for the valepotriates and for their degradation products, the activity being higher after i.p. than after p.o. administration. A specific valepotriate fraction has been documented to exhibit tranquillizing, central myorelaxant, anticonvulsant, coronary-dilating and anti-arrhythmic actions in mice, rabbits and cats (Barnes *et al.*, 2002).
The central depressant activity of a valerian root extract was assessed *in vivo* using an autoradiographic tracer method to measure glucose transformations in more than 30 areas of the rat brain after i.p. injection of test substances, as a measure of neuronal activity. A dichloromethane extract and a particular fraction from it were demonstrated to be active at 50 and 10 mg/kg respectively, but no activity was found for the essential oil, pure valerenic acid, valeranone, valtrate, didrovaltrate and homobaldrinal (ESCOP, 2003).
Recently, some flavonoids present in valerian were shown to exhibit CNS-activity in mice and rats: 2S(-) hesperidin showed sedative and sleep enhancing properties, and 6-methylapigenin was found to have anxiolytic properties and to potentiate the sleep enhancing activities of 2S(-) hesperidin (Marder *et al*., 2003). The flavone glycoside linarin also showed sedative and sleep – enhancing properties that were potentiated by simultaneous administration of valerenic acid (Fernandez *et al.*, 2004).
To summarize, several mechanisms of action possibly contributing to the clinical effect have been identified for diverse valerian root constituents (sesquiterpenoids, lignans, flavonoids) and include interactions with the GABA-system, agonism at the A1 adenosine receptor and binding at the 5-HT_{1A} receptor (EMEA, 2005).

4.3 HUMAN STUDIES

Numerous studies have explored the effects of valerian preparations on subjective and/or objective sleep parameters. Collectively, the findings of these studies are difficult to evaluate, as different studies have assessed different valerian preparations at different dosages, and some have involved healthy volunteers whereas others have involved patients with diagnosed sleep disorders. In addition, some studies have been performed in sleep laboratories and others with test persons sleeping at home. Overall, several, but not all studies have documented a sleep enhancing effect of valerian preparations with regard to subjective measures of sleep quality, and some have documented effects on objective measures of sleep structure (Barnes *et al.*, 2002).

Stevinson and Ernst (2000) included randomized, placebo controlled double blind clinical trials that had measured the effect of valerian monopreparations on sleep in human participants in a systematic review. From the evaluation of 9 studies that met the inclusion criteria, the authors conclude that the evidence of the efficacy of valerian for improving sleep is promising, but not fully conclusive. The results of some trials suggest that valerian may have both acute and cumulative effects on sleep, but not all studies have produced positive findings. These discrepancies may be a result of inconsistencies between trials in experimental design.
In a recent trial, Diaper and Hindmarch (2004) compared the acute effects of two doses of valerian (single dose 300 and 600 mg of the commercial valerian extract Sedonium™, with a washout period of 6 days after each dose) in 16 elderly patients with mild sleep disorders. Tests were performed in a sleep laboratory, and the sleep EEG was recorded form 23 to7 h each night. Psychometric tests and mood scales were performed after the test nights. No significant differences were observed between placebo and verum. The authors conclude that valerian at these doses is ineffective as an acute remedy for sleep disorders. Also Glass *et al.* (2003) found no acute sedative effect for a commercial valerian extract (400 or 800 mg), which was ingested to elderly healthy volunteers. Effects were assessed 0.5, 1, 2, 3, 4, 6 and 8 hours after medication using validated measures of subjective sedation, mood and psychomotor performance. No sedation and no impairment of psychomotor performance were observed at any time studied. A study involving healthy young volunteers provided similar results (Guiterrez *et al*., 2004).

4.4 Absorption, distribution, metabolism, excretion

There are some pharmacokinetic data available concerning valtrate and valerenic acid derivatives:
The *in vivo* distribution and metabolism of ^{14}C-valtrate after p.o. and i.v. administration was investigated in NMRI-mice. The results of p.o. administration indicate that the stomach is the most important resorption area for valepotriates. The relative specific activity in blood and different organs was rather low with the highest activity in the liver. Intact valtrate could only be found in very low concentrations, the major part of radioactivity was found in metabolites.
After i.v. application, the highest relative activity was found in the liver, followed by lung and spleen (Reichling *et al.,* 1994).
In order to study the pharmacokinetics of valerenic acid, 6 healthy volunteers received a single 600 mg oral dose of valerian (Sedonium™, Lichtwer Pharma), and blood samples were collected at particular times after administration. Valerenic acid was quantified in the serum using derivatization of valerenic acid followed by LC-MS determination. The maximum serum concentration for 5 of the 6 subjects occurred between 1 and 2 hours after administration.
Valerenic acid was detectable in serum for at least 5 hours after intake. The elimination half life for valerenic acid was 1.1±0.6 hours (Anderson *et al.*, 2005).
To investigate the occurrence and the effect of metabolic changes of valerian constituents in the liver *in vitro*, a valerian 50% methanolic extract was incubated with freshly prepared rat hepatocytes and subsequently analyzed phytochemically by HPLC and pharmacologically by testing for the binding affinity of the extract to the benzodiazepine and picrotoxin binding sites of the $GABA_A$ receptor. HPLC analysis revealed considerable metabolic changes concerning the sesquiterpenes and the valepotriates: The amount of acetoxyvalerenic acid decreased 9-fold, while the hydroxyvalerenic acid content increased 9-fold due to deacetylation. The valepotriates didrovaltrate, isovaltrate and valtate decreased 2-, 18-, and 16-fold respectively. However, the binding affinity to the $GABA_A$ receptor remained unchanged, indicating that other or multiple compounds are responsible for this action (Simmen *et al.*, 2005).

5. MICROBIOLOGY

Valerian oil is generally regarded as lacking in antimicrobial activity. However, one study exists that showed mild antimicrobial activities of certain valerian cultivars against some microorganisms such as *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*. The authors demonstrate that the antimicrobial activity of the essential oil may depend on its biological origin (cultivar) and on the developmental stage of the plant, both factors causing variations in the amount and composition of the essential oil (Letchamo *et al.*, 2004).

6. EFFICACY

Concerning its medical indications, valerian root is stated to be effective for the relief of temporary nervous tension and difficulties in falling asleep (Blumenthal, 1998, EMEA, 2005). The efficacy in these indications is quite well documented in comparison to other herbal drugs, however the active compounds could not yet be fully identified and also the exact mechanisms of action remain to be investigated.
Because of its gradual onset of efficacy, the drug is obviously not suitable for acute interventional treatment of stress symptoms or reactive sleep disturbances, so a continued use over 2 to 4 weeks is recommended to achieve an optimal effect (EMEA, 2005).
As far as the use as flavouring agent is concerned, the legal status depends on the country. It is listed by the Council of Europe as a natural source of food flavouring with some restrictions (root: category 5, products for which additional toxicological and/or chemical information is required. These could temporarly be acceptable provided that any limits set for the active principles or the other chemical components are not exceeded). In the USA, valerian is permitted for use in food (Barnes *et al.*, 2002).

6.1 VETERINARY USE

The transport of pigs can cause stress, thereby raising the issue of animal welfare. The use of pharmacological (synthetic) sedatives before transport is forbidden in the European Union to avoid risky food residues.
The effect of a herbal preparation containing *V. officinalis* L. and *Passiflora incarnata* L. (Sedafit™) on responses of pigs during a transport simulation was investigated. Sedafit supplementation (2.5 g/l drinking water for 2 days) resulted in smaller increases of the investigated heart variables (minimum heart rate, ventricular ectopic beats, ST elevation) during and after stress evocation compared with the control group, suggesting sedative and antianxiety effects (Peeters *et al.*, 2004).

The use as a sedative in herbal veterinary medicine for horses and small animals has also been described (Bentz *et al.*, 1989), but it is not yet sufficiently documented by studies.

7. TOXICOLOGY

7.1 SAFETY

Preclinical safety data:
A low order of toxicity was reported for an ethanolic valerian root extract (LD_{50} after i.p. injection in mice 3.3 g/kg b.w.). The same extract was administered daily to rats for 45 days in doses ranging from 400- 600 mg/kg b.w. i.p. daily, no significant changes in weight, blood or urine were observed in comparison with control animals. A similar result was obtained in a 30

day study in rats when growth, arterial pressure, weight of key organs, haematological and biochemical parameters were compared (daily administration of 300 or 600 mg/kg/day ethanolic valerian root extract). The LD_{50} of valerian essential oil was determined as 15 g/kg b.w. in rats. In acute oral toxicity tests the LD_{50} of valeranone was greater than 3 g/kg b.w. in rats and mice.
Valerenic acid caused reduction of spontaneous motility at a dose of 50 mg/kg b.w. i.p. in mice, at a dose of 100 mg/kg it caused ataxia, 150-200 mg caused muscle spasms and 400 mg/kg led to heavy convulsions resulting in the death of 6 out of 7 mice within 24 hours (ESCOP, 2003).
In vitro cytotoxicity (inhibition of DNA and protein synthesis and potent alkylating activity) has been reported for the valepotriates, with valtrate stated to be the most toxic compound. However, the valepotriates are known to be unstable in both acidic and alkaline media and it has been suggested that their *in vivo* toxicity is limited due to poor absorption and distribution. Due to their low stability it is also unlikely that they are present at significant concentrations in finished products (Barnes *et al.*2002, Reichling *et al.*, 1994).
Baldrinal and homobaldrinal, decomposition products of the valepotriates, have exhibited direct mutagenic activity *in vitro* in the Ames test and the SOS-Chromotest. Their *in vitro* cytotoxicity was lower than that of the valepotriates (Barnes *et al.*2002, Reichling *et al.*, 1994). Due to the poor resorption of the valepotriates and the high first pass effect and the rapid metabolism of the baldrinals, the systemic distribution of these compounds is very low. Thus, the mucosa of the gastrointestinal tract and to a lower extent the liver might be the target organs for a potential mutagenic, genotoxic and cytotoxic risk of the valepotriates and their decomposition products (Reichling *et al.*, 1994).
In vivo, the following LD_{50} values were determined for valepotriates and baldrinals in mice:
For i.p. injection 62 mg/kg for valtrate, 150 mg/kg for acevaltrate, 125 mg/kg for didrovaltrate and 460 mg/kg for baldrinal. For p.o. administration, 4600 mg/kg for valtrate, acevaltrate and didrovaltrate (Reichling *et al.*, 1994).

Clinical safety data
Only few controlled clinical trials of valerian preparations have provided detailed information on safety. Where adverse event data were provided, randomised, placebo-controlled trials involving healthy volunteers or patients with diagnosed insomnia reported that they were mild and transient and that the types and frequency were similar to those for placebo (Barnes *et al.*, 2002, Stevinson and Ernst, 2000). Slight or no physical or cognitive impairment was reported after single or repeated administration of valerian, and serious hang over effects obviously do not occur. There have been case reports about hepatotoxic reactions in individuals taking herbal products containing valerian, and a report of cardiac complication and delirium following sudden valerian withdrawal. At high doses, valerian has been associated with cardiac function disturbance and depression of the central nervous system, making potentiation of other central nervous depressants a possibility. Addiction to valerian preparations has not been reported. For evaluation of the safety of long term use, more studies would be necessary (Stevinson and Ernst, 2000). About the safety of valerian intake during pregnancy and lactation, no data exist. For this reason, the use is not recommended (EMEA, 2005).

Interactions
Investigating valerian´s potential for herb-drug interactions, Defevbre *et al.*(2004) studied the potential of 9 commercially available valerian preparations to inhibit a major human drug metabolism enzyme and transport protein, namely cytochrome P450 3A4 and P-glycoprotein, *in vitro*. Most of the extracts tested exhibited a moderate to high inhibitory activity that was not correlated with the valerenic acid content.
A clinical study dealing with this problem focused on the activity of the two P450 isoforms CYP3A4 and CYP2D6. Together, these isoforms are involved in the metabolism of an estimated of 70% of prescription and non-prescription medications. Probe drugs dexometorphan (CYP2D6 activity) and alprazolam (CYP3A4 activity) were administered to 12 healthy volunteers at baseline and after supplementation with tablets containing 1000 mg valerian root extract

(containing 11.2 mg valerenic acid) nightly for 14 days. Dexometorphan and alprazolam metabolic rates were determined at baseline and after treatment. The results indicate that although a modest increase was observed in c_{max} of alprazolam, typical doses of valerian are unlikely to produce clinically significant effects on the disposition of medications dependent on the CYP2D6 or CYP3A4 pathways (Donovan *et al.*, 2004).
Gurley and coworkers (2005) performed a study evaluating the ability of valerian and 3 other herbal medications to modulate CYP activity in 12 health volunteers. Each supplementation period was 28 days, followed by a washout period of 30 days. The study was randomized for supplementation sequence. Concerning valerian, capsules containing 125 mg valerian root extract were administered 3 times daily. The activity of CYP phenotypes CYP1A2, CYP2D6, CYP2E1 and CYP3A4/5 was assessed before and at the end of each supplementation phase by determination of the metabolic rates for caffeine, midazolam, chlorzoxazon and debrisoquin. No significant changes in phenotypic ratios were observed during valerian supplementation. The authors conclude that valerian is not very likely to produce CYP-mediated herb-drug interactions, but as the valerenic acid content of the used valerian preparation was below the limit of detection, the authors admit that this result may not be representative of products containing greater amounts of valerenic acid.
For general conclusions, more data from larger studies would be necessary.

7.2 Dosage

Humans
Adolescents over 12 years of age, adults elderly: tinctures (1:5), aqueous or hydroethanolic extracts equivalent to 2-3 g of the drug for nervous tension up to 3 times daily; as an aid to sleep, a single dose ½ to 1 hour before bedtime, with an earlier dose during the evening if necessary.
0.3 to 1 g dried valerian root (powdered or as a herbal tea), 15 ml of fresh plant juice, 14 mg of valerian root oil up to 3 times daily (based on traditional use).
Because there is no experience available, the use of the product is not recommended in children below the age of 12 years (EMEA, 2005).

Animals
Valerian root p.o.: horse 20-50 g, cattle 30-100 g, small ruminants 5-15 g, pigs 5-10 g
Valerian tincture p.o.: pig 2-5 g, dog 0,5-3 g (Bentz et al., 1989).
Scientific studies to confirm the safety and efficacy of these doses are missing.

EMW

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CONCLUSIONS

Taking into account the large number of herbs and herbal products ("botanicals") widely available for animal nutrition through several distribution channels and marketed actually for a variety of uses, several issues have arised mainly relating to the quality assurance and safety:

1. Identification of the starting material
2. Natural variation of the plant material
    a. biodiversity and genetic variation
    b. ontogenetic variation (development stage)
    c. morphogenetic variation (plant parts)
3. Agricultural and harvesting practices
4. Contaminations
    a. agrochemicals (plant protection products)
    b. microbiological contamination
5. Identification of the "active substance(s)"
6. Product consistency and stability
7. Hazard identification and characterisation
8. Risk characterisation

AD 1. IDENTIFICATION OF THE STARTING MATERIAL

One of the problems when dealing with herbs and herbal products is the correct identification of the species and chemotype. Quite often, narrowly related species are used with different chemical outfit, as e.g. yarrow (*Achillea millefolium* aggr.) or oregano (*Origanum sp.*) consisting of a number of small and/or subspecies of different ploidy level and with distinct chemical composition. Sometimes also chemotypes occur within a species of which only one is appropriately effective. This is problematic, especially if the chemical variation results in adverse effects. For example, bitter substances vary considerably within *Labiatae* and the acceptance by animals (espec. chicken) might not be given causing minor uptake and losses.

Exotic herbs are often known under different common/vernacular names and the correct botanical source is sometimes unclear. Consequently mixtures with poisonous plants might and do occur. A specific problem in this respect is related to wild collected herbs. To avoid confusion a clear description to ensure the authenticity is therefore an indispensable prerequisite.

AD 2. NATURAL VARIATION OF THE PLANT MATERIAL

It is of common knowledge that crops vary from year to year. Prerequisite for a batch to batch conformity is therefore a uniform plant material. Special attention has to be paid in first line to the correct (chemo)variety since many plants, especially essential oil bearing species, show not only a high inter-specific phytochemical diversity but also an intra-specific chemical variation. Moreover, the respective phytochemicals are not equally distributed in the plant but can be concentrated in specific plant parts, organs and tissues. A clear description and specification of the plant part used is therefore necessary. For example, milk thistle (*Silybum marianum*) contains the effective flavonolignans in the fruit husk, thistle oil and thistle herb being totally different products.

Another typical characteristic of secondary plant products is their phase dependence. They are rarely quantitatively or qualitatively present uniformly over the life cycle of the plant but depend on the development stage, the latter being influenced by the environment, especially day length, temperature, water and nutrition. As a result, the quality of the product depends on the harvest date and the ecological influences at the production site.

AD 3. AGRICULTURAL PRACTICES AND HARVESTING

Since the above problematic of batch to bach conformity is well recognized for medicinal and aromatic plants, the European Herb Growers Association (EUROPAM/EHGA) elaborated, already in the 1990ies, 'Guidelines for Good Agricultural Practice' and 'Guidelines for Good Wild Collection Practice of Medicinal and Aromatic Plants' (GAP/GCP-Guidelines,), adopted meanwhile by EMEA and WHO The respective guidelines refer to the following items (which must not all be dealt with in detail in the present context):

- seeds and propagation material
- Cultivation, including soil and fertilisation, irrigation, crop maintenance and plant protection (products)
- Wild collection
- Harvest
- Primary processing and packaging
- Storage and distribution

and also to:

- Quality assurance
- Personnel and education
- Building and facilities
- Equipment
- Documentation

Therefore the principles stated on these guidelines should also be applied at plants/herbs used as additives in animal nutrition.

AD 4. CONTAMINATIONS

A rather crucial problem represent the different possible contaminations which might occur independent if the plants are cultivated or wild collected. They can be divided into the following main groups:

- admixtures with undesired plant material
- insects and microbiological load
- residues of plant protection products
- toxic heavy metals and radioactive isotopes

Admixtures with wrong plant material are caused on one side by lacking knowledge or performance of the plant collector, on the other side by deficiencies in crop maintenance, harvest and post-harvest processing. In field crops, weed control is done manually, mechanically or by application of herbicides causing probably respective residues. Impurities with wrong plant material will not only reduce the quality of the product, but may also result in severe problems if toxic plants/plant parts are concerned.

Pest and disease infected material is in general of lower quality but could also be hazardous if toxic substances are produced by one of the organisms. Apart from crop management special attention has to be paid to storage conditions to avoid insect and microorganism proliferation. Insect (and rodent) control is possible by gazing with CO_2 or liquid N_2 at low temperatures and /or high pressure. A certain reduction of the microbial load can be obtained through steam disinfection or irradiation with ionizing rays, fumigants as e.g. ethylenoxide or methylbromide are prohibited in Europe but respective residues might be found in imported material from outside the EU. Although indicating the tidiness of the production process, not the total number

of germs is decisive but the absence of pathogens and a low load with aflatoxine/ochratoxine producers.

In systematic plant production and in storehouses plant protection products are used to avoid phytosanitary problems. There are no specifically developed products for herbs on the market, but herbicides, insecticides and fungicides commonly used in agriculture can be applied in specific cases according to European and national legislations. Residues of plant protection products have not to exceed the respective maximum residue levels: for herbs in general according the food legislation, for medicinal plants according to the European Pharmacopoea (Ph Eur 5). In organic production, special attention has to be paid to the EU Directive 2092/91. Critical points for the users are herbs and products from outside Europe contaminated with HCH or DDT as well as herbal mixtures, extracts and essential oils with uncontrolled residues.

Contaminations with heavy metals originate from the soil, from overloaded fertilizers or from immissions. There are limits for heavy metal contaminations in food as well as pharmaceutical legislation, but some of the species as e.g. linseed (*Linum usitatissimum*) or St. Johns wort (*Hypericum perforatum*) exceed quite often the general limits. Contaminations with radionuclides are rare and problematic only at herbs of certain regional origin.

In general, residues of (agro)chemicals and contaminations with abiotic and biotic toxicants have to be controlled also at herbs and herbal products not to exceed the limits to avoid a carry over into the food chain.

AD 5. IDENTIFICATION OF THE 'ACTIVE' SUBSTANCES

Botanicals used for health purposes or as sensory food ingredients are in general well identified and characterised with regard to their major compounds. Sometimes, however, the active principle is not definitely identified yet, as in the case e.g. of valerian, or some of the compounds acting as pro-drug (for example salicin from willow bark being metabolised first to active salicylic acid derivatives). In addition, in several cases, marker substances, without direct relation to the activity but characteristic for the species, are used for product identification. In plant preparations, quite often, not only a single compound is active, but the interaction of a number of substances is evident, and additive or adverse effects may occur, complicating the situation even more. According to the European Pharmacopoeia standardized extracts based on a level of several active constituents are therefore regarded as the 'active principle', and especially constituents with known adverse effects should be included as standardisation criteria. In cases where neither appropriate marker compounds nor active constituents are known, a chemical fingerprint will be required with limits on its variability.

As methods for quality assessment, chromatographic techniques are normally used to adequately characterise the material. In a first approach TLC (thin layer chromatography) will be the appropriate analytical method for confirming authenticity and to detect adulterations. But for more detailed information, including determination of residues, a battery of analytical micro- (and nano-) techniques may be useful (IR, UV, GLC, HPLC and coupled with MS.).
For identification of herbal compounds in feed mixtures, new approaches are available based on molecular biological methods as e.g. PCR.

AD 6. PRODUCT CONSISTENCY AND STABILITY

Consistent quality for products of herbal origin can only be assured if the starting materials are adequately defined (as already mentioned under 1. – 4.). In particular, the specific botanical and phytochemical identification of the plant material used should be defined. Critical elements are the reproducibility of the batches, when originating from different sources (environments, varieties, etc.), and the inconsistency, if different plant parts are present in the product (herb consisting of more or less leaves/stems, flowers with/without vegetation).

Product stability depends on the degree of comminution (cutting, crushing and milling), the storage conditions and the type of active substances. No herbal product should be stored above room temperature and the humidity of the product has not to exceed 14% moisture to avoid microbial growth. Essential oils, as volatile compounds, will be reduced during the storage this being more relevant for herbs and flowers than fruits/seeds and roots. The more the product is processed/comminuted and the smaller the particle size is, the higher are the losses of volatiles. Used as additives in industrially processed animal feed, essential oils and extracts should be micro-encapsulated, otherwise a short term use (consumption) is recommended. Non-volatile compounds (flavonoids, glycosides, alkaloids) show in general a better stability, but oxidation processes might also alter the quality.

AD 7. HAZARD IDENTIFICATION AND CHARACTERIZATION

Identification of the inherent biological activity of the material and assessment of relevance to animals and humans are necessary before the exposure depending risk can be characterised. Of most of the herbs in question, human safety data are available and cited in the respective chapters. But much less information is known on the activity and metabolism in target animals and especially on the carry over of single compounds in the food chain. ADME (absorption, distribution, metabolism and excretion) of herbal products in food producing animals is scarce and will be of significance only if possibly hazardous substances are contained in the starting material. Especially, matrix effects will play an important role in the safety assessment of herbs and herbal preparations used in animal nutrition. The herbs themselves consist of a complex mixture of components of macro- and micro-molecules of nutritive value, flavour compounds and other secondary metabolites being responsible for beneficial and/or adverse effects. Interactions due to the complex matrix will result in only partly comparable data to results from ADME studies performed on individual active substances. Therefore, the extent of the necessary experimental investigations depends on the adequacy of the use history and on the product comparability to traditional food/feed ingredients.

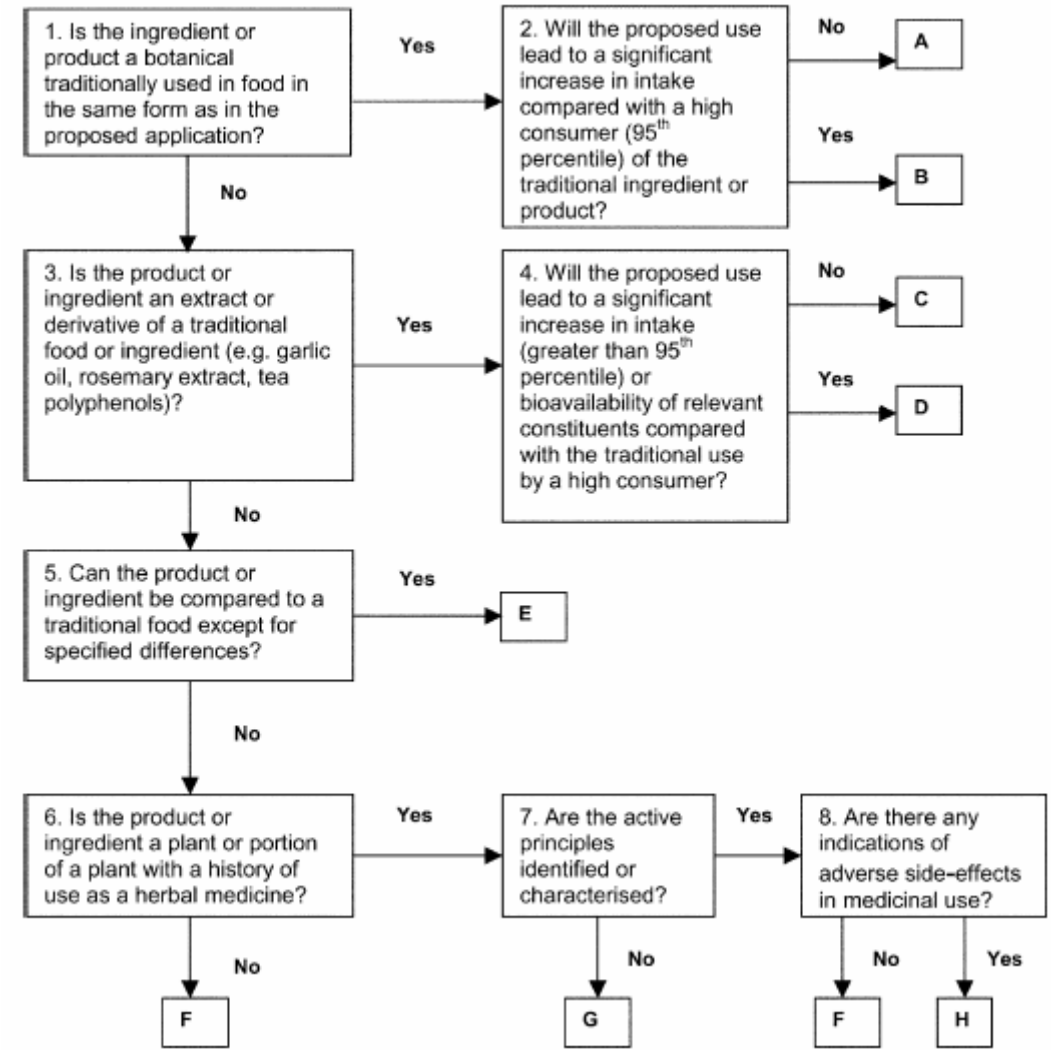
A special problem might be the safety in handling herbs and herbal products due to their allergenic potential. Since this is well documented for herbs of human use, the respective precautions should be taken also with herbal feed additives.

AD 8. RISK CHARACTERISATION

Any risk characterisation has to be considered predominantly from the poin of view of safety for the target animal and the consumer of food of animal origin. Although a couple of data may exist on pharmacological effects in laboratory animals, toxicology on target animals is seldom to be found. There is obviously a wide range of knowledge on the activity and safety of herbs and herbal products to the target animals from 'traditional use' (from field experience to experimental data up to well documented scientific results). But most of published data sets are presumably reduced to zootechical parameters (i.e. weight gain and feed conversion) not to risk further use or future authorisation by demonstrating metabolic, microbial or even pharmacological effects.

Regarding the consumer, safety data of herbs and herbal preparations used in food or food supplements may be used. A decision tree designed as guidance tool for the safety evaluation of functional plant products (Schilter *et al*., 2003) might also be useful in the present context (Scheme 1). Depending on the history of use, different levels of information could be requested to decide on the risk : benefit of a herbal product.

In conclusion, results and information on herbs and herbal products for human use – either as food, food supplement or herbal medicinal product – can also be applied for decisions and regulations of herbs in animal nutrition. In addition to the existing guidelines on feed additives, a specific guidance for the application/assessment of herbs and herbal products used as additives in animal nutrition is needed.



Scheme 1. Decision tree to assist in determining the information that needs to be considered for the safety evaluation of a botanical ingredient or product. (Schilter *et al.* 2003)

(for explanation see next page)

Information required on a herbal product as indicated by exit point of the decision tree:

A – I, II, III, IV;
B – I, II, III, IV, VI, VII, VIII, (IX);
C – I, II, III, IV, V;
D – I, II, III, IV, V, VI, VII, VIII (IX);
E – I, II, III, IV, V, VI, VII, VIII, IX
F – I, II, III, IV, V, VI, VII, VIII, IX;
G – The product needs to be characterised before a specification can be produced and a safety assessment made for its use
H – Do not add to food/feed or use in supplements at intakes comparable to medicinal use without a comprehensive risk: benefit analysis.

Summary of information required to demonstrate safety of botanical food supplements:

I – Specification of the ingredient or product;
II – Details of the source organism (Genus, species, portion of the plant consumed);
III – Evidence from previous human exposure through food and/or other sources;
IV – Extent of use and estimated intake;
V – Technical details of any processing and stability in formulation;
VI – Nutritional assessment (including assessment of any effects of the active principles on the bioavailability of other dietary components);
VII – Toxicological assessment (including assessment of any effects of other dietary components on the bioavailability of the active components
VIII – Human clinical data including variability of response, adverse effect reports and contraindications;
IX – Ancillary data (mode of action of beneficial effect to allow consideration of special studies)

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LIST OF ACRONYMS

ACSO	alkenyl-L-cysteine sulphoxides
ADME	Adsorption Distribution Metabolism Excretion
AE	anethole epoxide
All	allyl-
AUC	area under curve
BCO	blackcurrant seed oil
CADs	caffeic acid derivatives
CHE	chelerythrine
CNS	Central Nervous System
COX	cyclooxygenase
CRP	C-reactive protein
DAB	German Pharmacopoeia
DHSA	dihydroxystearic acid
DMBA	dimethylbenzanthraeene
EAB	European Pharmacopoeia
EMEA	European Medicines Agency
ESCOP	European Scientific Cooperative on Phytotherapy
EUROPAM/EHGA	European Herb Growers Association
FA	fagaronine
FEMA	Flavour and Extract Manufacturer Association
$FeSO_4$	ferrous sulphate
GABA	y-aminobutyric acid
GRAS	Generally Recognized As Safe
GSH	intracellular glutathione
HMPC	Committee on Herbal Medicinal Products
IgG, IgA and CRP	Immunglobuline G, Immunglobuline A, and C-reaktives Protein
LC/NMR and LC/APCI-MS (/MS)	Liquid chromatography/nuclear magnetic resonance spectrometer and Liquid chromatography/atmospheric pressure chemical ionization
LD_{50}	lethal dose (50%)
LDH	lactate dehydrogenase
MAO	mitochondrial monoamine oxidase
MBC values	minimum bactericidal concentration

Me	methyl-
MIC values	minimum inhibitory concentration
MMP	Matrix metalloproteinase
MRT	Magnetic Resonance Tomography
NF-kappaB	nuclear factor-kappa B
Ph Eur 5	European Pharmacopoeia, 5th Edition
PKC	protein kinase C
Pr	propyl-
RBL-1	retinoblastoma- like 1
SA	sanguinarine
SCOP	structural classifications of proteins
SERM	selective estrogen receptor modulator
sIgA	secretory immunoglobuline A
TNF	tumour necrosis factor
WHHL	Watanabe heritable hyperlipidemic
WHO	World Health Organization

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