



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Rosmarinus officinalis* L., aetheroleum and *Rosmarinus officinalis* L., folium

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Whole or cut dried leaf of <i>Rosmarinus officinalis</i> L.
Herbal preparation(s)	Comminuted herbal substance Liquid extracts Expressed juice Essential oil
Pharmaceutical forms	Solid, semi-solid, liquid dosage forms and as bath additive.
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1. Introduction

Rosemary (*Rosmarinus officinalis* L.) belongs to the family Lamiaceae (Labiatae) and has been an important medicinal plant since earliest times. It is also a commonly used spice and flavouring agent. Its essential oil is used therapeutically, in particular in balneology.

It was recognised for its medicinal and cosmetic properties in ancient Greece and by the Romans. In the middle ages, Rosemary oil was distilled for medical purposes and the alcoholic distillate was probably the first popular perfume.

The plant is native to the Mediterranean regions but has spread to all parts of the world. The leaves are sessile, tough, linear to linear-lanceolate, 10 mm to 40 mm long and 2 mm to 4 mm wide, and have recurved edges. The upper surface is dark green and glabrous, the lower surface is greyish-green and densely tomentose with a prominent midrib (Ph Eur 2001).

There is extensive consumption of the plant. It is mentioned in the literatures that, for the period 1980-1984, 400 to 500 tons were used, with most of this in Western Europe and USA (Chandler, 1995).

The name is derived from the Latin *ros* (*roris*), meaning dew, and *marinus*, meaning the sea, being known as the 'dew of the sea'.

In the Mediterranean area, it blooms throughout the year and flowering is most abundant in spring. Rosemary is mentioned in Anglo-Saxon herbals at the 11th century and it is believed that it was grown in Britain prior to the Norman Conquest. It is widely held to be a single species with several subspecies and varieties, but there are claims for additional species. The structure of the carbon skeleton of the main constituents of the essential oils point to three biogenetic types: the eucalyptol type (Italy, Morocco and Tunisia), the camphor-borneol type (Spain) and the alpha-pinene-verbenone type (France, Corsica). Bog rosemary (*Andromeda* species) and wild or March rosemary (*Ledum palustre* L.) are members of the family Ericaceae and not related to rosemary (Chandler, 1995).

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Whole or cut dried leaf of *Rosmarinus officinalis* L. (Ph. Eur. monograph ref.:01/2008:1560)

- Herbal preparation(s)

Comminuted herbal substance

Liquid extracts

Expressed juice

Essential oil

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

N/A

1.2. Information about products on the market in the Member States

Rosmarinus Aetheroleum (Rosemary Essential oil)

Countries	Year	Preparations	Indications	Posology
Germany	1976 Trad.use	Bath additive	Traditional use to support the function of the skin	If necessary 1 x daily: 10 ml bath additive /100 ml water for 10-20 min at 34-37°C 13,5 g rosemary oil /100 ml (=104 g) bath additive Every 2-3 days: 3 ml bath additive/150 ml water for 10-30 min at 35-39°C 13 g rosemary oil/100 ml (=104 g) bath additive
	1976 WEU	Bath additive	Auxiliary treatment in conditions of exhaustion	If necessary 3-4 x / weekly 10 ml bath additive / 150 ml water for 10-20 min at 34-37 °C 48 g rosemary oil / 120 ml bath additive If necessary 2-4 x / weekly 10 ml bath additive / 100 ml water for 10-20 min at 34-37 °C 20.8 g rosemary oil / 100 ml (=104 g) bath additive If necessary 3-4 x / weekly 20 ml bath additive/100 water for 10-20 min at 34-37 °C 10 g rosemary oil /100 g bath additive Maximal 1 x daily 20 ml bath additive/150 ml water for 10-20 min at 35-38 °C 25 g rosemary oil /100 g bath additive
	1990 WEU	Bath additive	Same indications as previous	Same
		Ointment 3 cm ointment containing 6 g rosemary oil/ 100 g ointment.	For the symptomatic treatment of muscle and joint pain and in circulatory disturbance.	2-3 x daily
			Contraindications - Not to be used in bronchial asthma, whooping cough, pseudo-croup. Adverse reactions -	

Countries	Year	Preparations	Indications	Posology
			urge to cough, bronchial and laryngeal spasm	
Austria	1994 Traditional use	100 g solution contain 5 g essential oil as bath additive	Stimulation of circulation	For a full bath 30 ml

Rosmarinus folium (Rosemary leaf)

Countries	Year	Preparations	Indications	Posology
Spain	1976	Comminuted herbal substance for herbal tea	Improvement of digestion	1-2 g/250 ml, 2-3 times/day (2-4 g/day)
	1990	Powdered herbal substance (capsules)	Dispepsia, improvement of digestion	2 caps (250 mg) 3 times/day
Poland	30 years	Infusion	Dyspeptic complaints Improvement of hepatic and biliary function and in dyspeptic complaints	2 g, 1-2 times/day
		Decoction (External use)	Adjuvant therapy in rheumatic conditions and peripheral circulatory disorders. Adjuvant therapy in rheumatic conditions, myalgia and peripheral circulatory disorders	1 liter of decoction (1:20) added to bath water (twice weekly)
Germany	1976 Oral use	Extract (1:17.5-18.9), extraction solvent: liqueur wine	Traditional use to support the cardiac and circulatory function	2-3 x daily 20 ml; 100 g liquid contain 94.816 g extract; 700 ml = 721 g liquid 2-3 x daily 10 ml
		Extract (1:12.5-13.5), extraction solvent: liqueur wine	Same	1-2 x daily 20 ml
		Expressed juice (1:1.8-2.2) - Rosm herba recens	Same	2-3 x/daily, 5 ml containing 100% expressed juice

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Belgium	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	only in combinations
Bulgaria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	only in combinations
Cyprus	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Czech Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Denmark	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Estonia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Finland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
France	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Germany	<input checked="" type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Greece	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Hungary	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	only in combinations
Iceland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Ireland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Italy	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Latvia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Liechtenstein	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Lithuania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Luxemburg	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Malta	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
The Netherlands	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Norway	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Poland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Portugal	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Romania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Slovak Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Slovenia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
Spain	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Sweden	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No RP
United Kingdom	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only in combinations

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

Other: If known, it should be specified or otherwise add 'Not Known'

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

RP: registered products

1.3. Search and assessment methodology

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Rosemary was used in traditional Greek and European medicine as a tonic, stimulant, and carminative for dyspepsia, headache, and nervous tension. The ancient Greeks used rosemary to strengthen the memory.

In different regions of the world, the use varies.

In traditional Chinese medicine, rosemary was used for headaches.

In the Indian Materia Medica (Nadkarni, 1999), rosemary oil it is described to have a carminative and stimulant action.

Rosemary was used topically to treat cancer in ancient Greece and South America (Hartwell, 1982). The Eclectic physicians used the oil of rosemary in 2 to 10 drop doses for colic, nervous disorders, and painful or delayed menses (Felter and Lloyd, 1983). Women have used rosemary for minor menstrual complaints.

Rosemary is used as an abortive agent in Brazilian folk medicine. It is traditionally referred to as an emmenagogue and is generally avoided during pregnancy. It is claimed to stimulate bile. Rosemary is said to prevent baldness when used as a hair tonic.

The following uses are reported in the literature: as an antiseptic, diuretic, antidepressant and antispasmodic, as well as for cold, influenza, rheumatic pain. The oil is reported to have antimicrobial properties and to have a relaxing effect on tracheal smooth muscles. (Erenmemisoglu, 1997; Chandler, 1995).

In folk medicine, rosemary is put on dressings for healing wounds and for eczema. It is also used as an insecticide, as a preservative and antioxidant for meals and fats (Wichtl, 2004).

Regulatory status

Rosemary oil

Rosemary oil was notified for Generally Recognized as Safe (GRAS) status by the Fragrance and Essence Manufacturers Association of the USA (FEMA) in 1965 and has been listed by the U.S. Food and Drug Administration (FDA) for food use (GRAS). In 1970 the Council of Europe included rosemary oil in the list of substances, spices and seasonings deemed admissible for use, with a possible limitation of the active principles in the final product (EFSA, 2008 citing Opdyke, 1974).

2.2. Information on traditional/current indications and specified substances/preparations

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

Bibliographic sources:

A) Pharmacopée Française - Préparation Officinale, 1980 (Romarin)

Justified by the use:

Oral use:

1. Spasmolytic
 - Infusion or decoction – 5 to 10 g/l; Infusion for 15 m. Decoction for 30 min 200 to 400 ml/day
 - Liquid extract – 3 to 5 g/day
 - Essential oil – 3 to 4 drops, 3-4 times/day

Local application

2. Antiseptic and wound healing
 - Alcoholic solution 2 % V/V essential oil

Justified by the pharmacological properties:

3. Spasmolytic, cholagogic, cholaretic
 - Extracts, tinctures, essential oil: several pharmaceutical specialties

Contra-indications: Pregnancy, prostatic affections and dermatosis

B) British Herbal Pharmacopoeia - 1983

Action

1. Carminative, spasmolytic, Thymoletic, Sedative, Diuretic, Antimicrobial
2. Topically: rubefacient, mild analgesic, parasiticide
3. Specific indications: Depressive states with general debility and indications of cardio-vascular weakness
4. Combinations used – May be used with Avena, Cola and Verbena in depression; with Salvia, Gelsemium and Valerian in migraine
5. Preparations and dosage: dried leaves and twigs
6. Dose: 2-4 g or by infusion. Liquid extract 1:1 in 45 % alcohol. Dose 2-4 ml

C) ESCOP – 1997

Indications

- Oral use

Improvement of hepatic and biliary function and in dyspeptic complaints.

- External use

Adjuvant therapy in rheumatic conditions and in peripheral circulatory disorders.

Promotion of wound healing and as a mild antiseptic.

Preparations and posology

- Oral use

Infusion: 2-4 g of rosemary daily

Fluid extracts (1:1, 45 % ethanol v/v): 1.5-3 ml daily

Tincture (1:5, 70 % ethanol): 3-8.5 ml daily

- External use

Ethanolic extract (1:20)

Essential oil (2 % V/V) in ethanol, as an antiseptic

1 litre of decoction (1:20) added to bath water (twice weekly)

D) Blumenthal (The Complete German Commission E Monographs, 1998)

Indications

- Oral use – Dyspeptic complaints
- External use - Supportive treatment for rheumatic diseases; Circulatory problems
- Dosage

Internally – Daily dose

4-6 g drug, 10-20 drops essential oil; equivalent preparations

Ed. Note: The essential oil dosage appears excessive and possibly unsafe. A more reasonable dosage for internal use would be 2 drops (1 ml).

Externally

50 g to a full bath; 6-10 % essential oil in semi-solid and liquid preparations; equivalent preparations

Assessor's comments (oral use):

Comparing both proposals for the posology, the editor's note seems more reasonable:

Daily dosage: 4 – 6 g herb containing 1.2 % (V/m) essential oil; Corresponding: 48 – 72 µl essential oil/day (equal to 0.048 – 0.072 ml essential oil).

In the European Pharmacopoeia, the density of rosemary essential oil is reported as 0.895 to 0.920 (M/V) -> approximately 0.90.

Considering that comparable essential oils weigh 19 mg per drop, the following calculation should be correct:

Minimum: $48 \mu\text{L} \times 0.9 \text{ (dens.)} = 43.2 \text{ mg} \rightarrow 43.2 \text{ mg}/19 \text{ mg} = 2.27 \text{ drops}$, rounded: 2 drops

Maximum: $72 \mu\text{L} \times 0.9 \text{ (dens.)} = 64.8 \text{ mg} \rightarrow 64.8 \text{ mg}/19 \text{ mg} = 3.41 \text{ drops}$, rounded: 3 drops

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

(e.g. primary pharmacodynamics, secondary pharmacodynamics, safety pharmacology, pharmacodynamic interactions)

Composition

Rosemary leaves contain 1,2-cineole, α -pinene, apigenin, betulin, betulinic acid, caffeic acid, camphor, carnosic acid, carnosol, carnosol isomer, methyl carnosate, cirsimaritin, diosmin, hesperidin, limonene, luteolin 3'-O-beta-D-glucuronide, luteolin 3'-O-(3"-O-acetyl)-beta-D-glucuronide, oleanolic acid, rosmadial, rosmanol, rosmarinic acid, scutellarein, thymol, ursolic acid (Senorans et al., 2000; Okamura et al., 1994).

A diterpene, rosmariquinone, has been isolated from a methanolic extract of *Rosmarinus officinalis* L. (Houlihan et al., 1985).

The leaves contain 0.5 to 2.5 % of a volatile oil, consisting of 0.8-6 % esters and 8-20 % free alcohols (Chandler, 1995).

The essential oil is a colourless or pale yellow liquid with a camphoraceous taste and contains monoterpenes, phenols, sesquiterpenes, monoterpenoid ethers, monoterpenoid ketones, monoterpenoid alcohols, and monoterpenoid esters, camphor, eucalyptol, α -pinene, borneol (Fahim et al., 1999; Steinmetz et al., 1987).

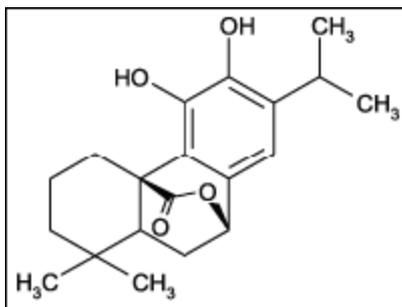
It contains 1,8-cineole (20–50 %), α -pinene (15-25 %), camphor (10-25 %), bornyl acetate (1-5 %), borneol (1-6 %), camphene (5-10 %) and α -terpineol (12-24 %), limonene, β -pinene, β -caryophyllene and myrcene (ESCAP, 1997).

The 40-day-old *in vitro* proliferating shoots of *Rosmarinus officinalis* L. var. *genuine* forma *erectus* produced an appreciable quantity of essential oil, i.e., 1.8 % fresh weight, which was similar in its constituents to that obtained from 1-year-old plants, whether naturally grown or *in vitro*-raised potted plants. The quantity of the various constituents identified was marginally less in the former case than the latter two kinds, with the exception of bornyl acetate and 1,8 cineole, where the concentration was higher (Jain et al., 1991).

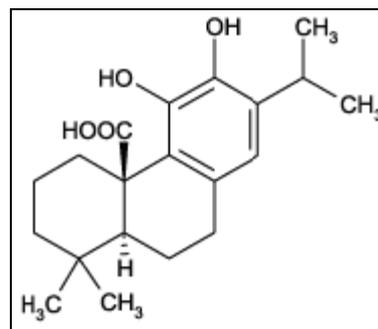
During an investigation period of 17 months, the shoot culture of rosemary accumulated varying amounts of carnosic acid and carnosol, which were also present in callus culture but about 20- to 80-fold lower than in the shoot culture. In suspension culture, only carnosic acid and no carnosol could be detected. The level of carnosic acid in suspension culture was threefold less than detected for the callus culture on average. The amount of rosmarinic acid produced in shoot culture and callus culture were comparable, whereas in suspension culture higher concentrations of rosmarinic acid could be measured than in shoot and callus culture. Thus, the content of carnosic acid, carnosol, and rosmarinic acid in the extracts depended on the differentiation grade of the cell culture type (Kuhlmann et al., 2006).

Methanolic extracts from the leaves of *Rosmarinus officinalis* harvested from different locations of Turkey at four different times of the year were analyzed by HPLC, and their radical scavenging capacities and antioxidant activities were studied by various assays. The amounts of carnosol, carnosic acid and rosmarinic acid, active constituents of rosemary, varied in different geographical regions of

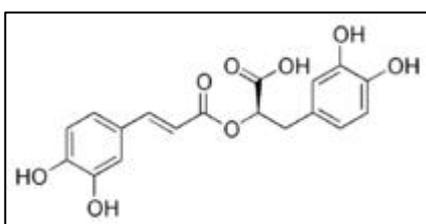
growth, and also showed a seasonal variation. The levels of the constituents were higher in the warm months of June 2004 and September 2004 (Yesil-Celiktas et al., 2007).



Carnosol



Carnosic acid



Rosmarinic acid

The principal antioxidative components of the extracts are the phenolic diterpenes carnosol (molecular formula $C_{20}H_{28}O_4$) and carnosic acid (molecular formula $C_{20}H_{28}O_4$).

The amount and nature of the oil vary with the subspecies, part of the plant used, the geographic source and the method of preparation. Phenolic diterpenes, flavones and rosmarinic acid distribution may also vary during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis* (del Baño et al., 2006).

1. *In vitro* studies

- Spasmolytic activity

A study was performed to test the antispasmodic activity of 2.5 and 10.0 ml/l of alcoholic extracts of some medicinal plants, including Rosemary, prepared from 1 part of the plant and 3.3 parts of ethanol (31% w/w). The guinea pig ileum was employed and acetylcholine and histamine were used as spasmogens. In histamine-induced contractions all plants, except Melissa exhibited a significant increase of the DE_{50} and decreased the maximal possible contractility induced by histamine (Forster and Niklas, 1980).

Three essential oils (*Mentha piperita* L., *Salvia officinalis* L., *Rosmarinus officinalis* L.) were investigated for their spasmolytic action on the longitudinal musculature of guinea-pig ileum. The concentration of the components of the oils influences their action. The three essential oils show a spasmolytic action. Pinene always induces spasms and the other components give rise to the double spasmogen-spasmolytic effect. The stimulating action of pinene, which is present at a higher content in rosemary (21.4 %), can be observed (Taddei et al., 1988).

In another study, these three plant emulsions were tested in doses between 0.1 and 1 mg/kg i.v., in male guinea pig, using the experimental method of Boissier and Chivot's. Oddi's sphincter, contracted by morphine hydrochloride (1 mg/kg i.v.) prolapses following injection of the three plants. The time to

return to normal of Oddi's sphincter is accelerated in relation to the dose of the various essences (Giachetti et al., 1988).

The spasmolytic activity (against BaCl₂ and acetylcholine) of the major components of the essential oils of several aromatic plants was studied. Camphor revealed no agonistic activity against either of the two spasmogens studied (Cabo et al., 1986).

- Antioxidant activity

A mixture of α -tocopherol and rosemary extract, as additives each at 0.035 % (total 0.07 %) expressed very strong antioxidant activity in sardine oil stored at 30°C, 50°C and dried sardine meat at 5°C (Wada and Fang, 1994).

Four diterpenoids (carnosic acid, rosmanol, carnosol and epirosmanol) isolated from the leaves of *Rosmarinus officinalis* by bioassay-directed fractionation, inhibited superoxide anion production in the xanthine/xanthine oxidase system, showing to be protective against oxidative stresses (Haraguchi et al., 1995).

Inhibition of the growth of 6 strains of food associated bacteria and yeasts by carnosol and ursolic acid, was achieved at concentrations of 150 $\mu\text{g ml}^{-1}$ for carnosol to the greatest extent. Butylated Hydroxyanisole (BHA) proved a superior inhibitor to ursolic acid which itself was more effective than Butylated Hydroxytoluen (BHT) (Collins and Charles, 1987).

A study on the variability of rosemary and sage and their volatile oils on the British market has been performed. The antioxidative properties of the various samples were determined and found to be variable based on the geographical location and type of processing (Svoboda, 1992).

The concentration of phenolic diterpenes in commercially available extracts of *Rosmarinus officinalis* determined by HPLC with electrochemical detection ranged from 2.8 to 22.5 %. Antioxidant activity of extracts under simultaneous storage and thermal stress depended directly on the concentration of phenolic diterpenes. Differences in rates of degradation of individual phenolic diterpenes at different temperatures were obtained (Schwarz et al., 1992).

In a sardine oil model system, a mixture of α -tocopherol and rosemary extract showed a significantly stronger antioxidant effect, as it prolonged the induction period for 10 and 16 days longer than α -tocopherol alone and rosemary extract alone, respectively. Treatment of samples with this mixture also led to a lower rate of decomposition of highly unsaturated fatty acids, myoglobin and haemoglobin, and triglyceride compared to samples treated with tocopherol or rosemary extract alone (Fang and Wada, 1993).

To test *in vitro* conditions the action of *R. officinalis* L. infusion and ethyl acetate extract against hydroxyl radicals, which may be implicated directly or indirectly in cell damage, two different methods were performed and the results expressed as IC₅₀. Both the infusion and ethyl acetate extract have a lower activity as a scavenger of OH⁺. The antioxidant activity of the infusion is probably related to the content in flavonoids, as a major group of the polyphenols. In the ethyl acetate extract, this activity is probably related to the enrichment in phenolic acids, namely rosmarinic acid, as major group of polyphenols (Guerreiro and Cunha, 1994).

Tateo et al. (1988) made the comparison of the antioxidant power between two dry rosemary extracts obtained by a simplified extraction process, a commercial rosemary extract and BHA, and an evaluation of the mutagenic effect of four different dry rosemary extracts. Four conclusions were reached by the authors: the antioxidant activity of the extracts was comparable; the extraction treatment by supercritical CO₂, which is as efficient for deodorizing as the traditional method of steam flow distillation, gives an antioxidant product with an activity comparable to the product deoleated by

steam flow distillation; the antioxidant activity of rosemary extracts in general is less evident in regards to soy oil, even at considerably higher concentrations than those active in solid fat; the antimutagenic activity is higher for the rosemary extract obtained by hydroalcoholic extraction (ethyl alcohol 50 % v/v).

Various experimental models were used for the characterisation of the antioxidant activity of four commonly consumed herbs belonging to the Lamiaceae family, i.e. oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.), including iron reduction capacity, 2,2-diphenyl-1-picrylhydrazyl DPPH_•, ABTS_{•+} and _•OH radical-scavenging activities and the capacity of the extracts to inhibit copper-induced oxidation of human low-density lipoproteins (LDL) *ex vivo*. The extracts showed varying degrees of reductive and radical scavenging capacity, and were capable of a marked prolongation of the lag-time in the LDL oxidation assay. The hierarchy of the observed antioxidant activity of the extracts was dependent on the type of assay used. The observed antioxidant characteristics were not fully related to the total phenolic contents of the extracts in any of the assays, but were presumably strongly dependent on rosmarinic acid, the major phenolic component present in this type of Lamiaceae extract (Dorman et al., 2003).

The DPPH radical scavenging method, Folin–Ciocalteu method and HPLC chromatography were used to study the distribution and levels of antioxidants (AOXs). A good correlation between the AOX activities and total phenol content in the extracts was found. All rosemary extracts showed a high radical scavenging activity (Moreno et al., 2006).

On other study, Kuhlmann et al. (2006) were able to demonstrate that the DPPH radical-scavenging activity of the extracts of rosemary depended on the amount of all three phytochemicals, carnosic acid, carnosol, and rosmarinic acid, in particular the last one. The anti-inflammatory character of the extracts was mainly based on their carnosic acid content.

R. officinalis L. essential oil showed greater activity than three of its main components (1,8-cineole, α -pinene, β -pinene) by means of DPPH assay and β -carotene bleaching test. The antioxidant activities of all the tested samples were mostly related to their concentrations (Wang et al., 2008).

The results of a study performed with methanolic extracts from the leaves of *Rosmarinus officinalis* harvested from different locations of Turkey at four different times of the year, were analyzed by HPLC, and their radical scavenging capacities and antioxidant activities were studied by various assays. The results indicated that the plants harvested in September possess higher levels of active constituents and had superior antioxidant capacities compared to those collected at other times (Yesil-Celiktas et al., 2007).

- Relaxation activity

The effects of the volatile oil of *R. officinalis* on the tracheal smooth muscle of rabbit and guinea pig were tested *in vitro* using tracheal strips. The contractions of rabbit tracheal smooth muscles induced by acetylcholine were inhibited as well the contractions of guinea pig tracheal muscle, induced by histamine stimulation. The oil also inhibited contractions of both tracheal muscles induced by high potassium solution, which was dose dependent and reversible. It inhibited the contractions of both tracheal muscles induced by acetylcholine and histamine in Ca²⁺ free solution. This result suggests possible calcium antagonistic properties of rosemary oil (Aqel, 1991).

- Chemopreventive effect

Rosemary extract, carnosol, carnosic acid

Some of the molecular mechanisms involved in the chemopreventive action were investigated using *in vitro* human liver and bronchial cell models. Rosemary extract and some of its active components,

carosol and carosic acid, are potent inhibitors of DNA adduct formation induced by benzo(a)pyrene or aflatoxin B₁. According to the authors, two mechanisms among others are involved in the anticarcinogenic action: the inhibition of the metabolic activation of the procarcinogens catalysed by the phase I cytochrome P450 enzymes and the induction of the detoxification pathway catalysed by the phase II enzymes such as glutathione S-transferase (Offord et al., 1997).

- Cardiovascular activity

Rosemary oil

A study has been performed to investigate if rosemary and its constituents affect the contractility of isolated guinea pig atria and if there are quantitative differences when compared with the guinea pig ileum. The rosemary oil used consisted of 40.9 % 1,8-cineole, 5.2 % bornyl acetate, 13.9 % α -pinene and 7.1 % β -pinene. The method consisted of a modification of the one of Magnus. In guinea pig ileum, half-maximal inhibition of acetylcholine-induced contractions was achieved by 465 nl/ml 1,8-cineole (2.5×10^{-3} M), 112 nl/ml bornyl acetate (5.7×10^{-4} M). Half maximal inhibition of contractility of the non-stimulated atria was observed at 250 nl/ml rosemary oil, 100 nl/ml 1,8-cineole (6×10^{-4} M), 400 nl/ml bornyl acetate (2×10^{-3} M). α -pinene and β -pinene increased contractility of the isolated guinea pig ileum. The contractility of the heart was not influenced up to 300 nl/ml, for both substances. The authors concluded that rosemary oil, 1,8-cineole and bornyl acetate depress contractility of the cardiac muscle and inhibit acetylcholine-induced contractions of guinea pig ileum (Hof and Ammon, 1989).

In another study, the effect of the oil was investigated on the vascular smooth muscle of rabbit, using isolated aortic segments (rings). The oil inhibited the contractions induced by norepinephrine stimulation in Ca²⁺ containing and free solution and high K⁺ solutions. The effects were shown to be dose-dependent and reversible. It suggests that the oil has a direct vascular smooth muscle relaxant effect (Aqel, 1992).

Aqueous extract

The potential effects of an aqueous extract of the leaves of *R. officinalis* on certain cardiovascular parameters on isolated rabbit heart, such as left ventricular pressure, coronary flow and heart rate, were investigated. In conclusion, some of the active constituents were shown to be associated with coronary vasodilatation and positive inotropic effects (Khatib et al., 1998).

- Immunological effects

Rosmarinic acid

The effects of caffeetannins and related compounds isolated from medicinal plants were investigated on the arachidonate metabolism in human peripheral polymorphonuclear leukocytes (PMN-L). Rosmarinic acid strongly inhibited the formation of 5-hydroxy-6, 8, 11, 14 - eicosatetraenoic acid (5-HETE) and leukotrienes B₄ (LTB₄) (5-lipoxygenase products) at concentrations of 10^{-5} - 10^{-3} M. The formation of LTB₄, induced by calcium ionophore A 23 187 in human PMN-L was inhibited by 3.5-, 4.5-, and 3.4-di-*O*-caffeoylquinic acid, caffeoylmalic acid, caffeoyltartric, rosmarinic acid and caffeic acid (Kimura and Okuda, 1987).

The phenolic compound, rosmarinic acid, obtains one of phenolic rings from phenylalanine via caffeic acid and the other from tyrosine via dihydroxyphenyl-lactid acid. It is well absorbed from the gastrointestinal tract and from the skin. It increases the production of prostaglandin E₂ and reduces the production of LTB₄ in human PMN-L and inhibits the complement system (Al-Sereiti et al., 1999).

Rosmarinic acid and fragments of human gamma globulins, an inhibitor of complement activation, were tested on endotoxin-induced hemodynamic and haematological changes in the rabbit. Their

effects were compared with complement depletion by cobra venom factor (CVF) pre-treatment. Rosmarinic acid (20 mg/kg) inhibited the activation of complement after endotoxin injection by about 70 %. The complement-dependent features of endotoxin, i.e. the stimulation of prostacyclin and thromboxane biosynthesis, both hypotensive phases and the primary thrombocytopenia, were largely reduced after treatment with rosmarinic acid. The complement-independent effects of endotoxin (leucopenia, formation of lung oedema) were hardly influenced by rosmarinic acid (Bult et al., 1985).

- Kidney action

Rosmarinic acid

An *in vitro* study suggested that rosmarinic acid, a constituent of rosemary, may prevent mesangial cell proliferation. Glomerular mesangial cell proliferation is one of the major histological findings in various renal diseases and is mediated by various humoral factors. Murine mesangial cells were isolated from mice glomeruli and incubated. Quiescent cells were stimulated for 24 hours with 10 nanograms per millilitre platelet-derived growth factor (PDGF) or 100 units per millilitre of tumour necrosis factor- α (TNF α) together with one of several different concentrations of rosmarinic acid. After stimulation, a pulse of [³H] thymidine was added to the culture. Cell viability was assessed by measuring the extent of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction by the cells and by the amount of lactate dehydrogenase (LDH) released by the cells. Rosmarinic acid significantly reduced the basal deoxyribonucleic acid (DNA) synthesis ($p < 0.001$). Rosmarinic acid significantly and dose-dependently inhibited PDGF- and TNF α -induced DNA synthesis ($p < 0.01$ to 0.05). Rosmarinic acid at 1.5 $\mu\text{g/ml}$ inhibited 50 % of the PDGF-induced proliferation, and at 3.8 μg inhibited 50 % of the TNF α -induced proliferation. A time course study showed that rosmarinic acid was effective when added up to 8 hours after the growth stimulus and suggested that rosmarinic acid suppressed the entry of mesangial cells into the S phase. The authors note that the concentrations of rosmarinic acid used in this *in vitro* study can be achieved by moderate ingestion of plants in the Lamiaceae family and may be a promising way to protect against the chronic aggravation of renal diseases (Makino et al., 2000).

- Anti-viral effect

Carnosic acid, carnosol, rosmanol

Two compounds, carnosic acid and carnosol isolated from rosemary and rosmanol, as well semi synthetic derivatives (7-O-methylrosmanol, 7-O-ethylrosmanol and 11, 12-O, O-dimethylcarnosol) were tested in order to find HIV protease inhibitors. The carnosic acid showed the strongest inhibitory effect ($\text{IC}_{90} = 0.08 \mu\text{g/ml}$). The cytotoxic TC_{90} on H9 lymphocytes was 0.36 $\mu\text{g/ml}$ for the same compound, very close to the effective antiviral dose (Paris et al., 1993).

The activity of some rosemary extracts and four fractions derived from rosemary against HVS₂ replication by plaque reduction assay, showed 50 % of inhibition of virus plaque formation for the extract. The results indicate that one fraction has antiviral activity without significant signs of cytotoxicity in tissue cultures (Romero et al., 1989).

- Antimicrobial, fungicide and insecticidal action

Essential oil

Essential oils from *Satureja montana* L., *Rosmarinus officinalis* L., *Thymus vulgaris* L. and *Calamintha nepeta* were chemically analysed and their antimicrobial and fungicide activities evaluated on the basis of their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). *Rosmarinus* was the least effective, but all showed a wide spectrum of action (Panizzi et al., 1993).

A modified method of Beylier-Maurel was applied to study the antimicrobial properties of essential oils from thyme, rosemary, eucalyptus and mugwort. Thyme was the most effective, followed by mugwort, rosemary and eucalyptus oil (Benjilali et al., 1986).

Rosmarinus officinalis oil showed an appreciable activity in inhibiting bacterial growth in a study using also *Inula helenium* oil, which possesses a higher activity compared to *Rosmarinus*, against Gram-positive *Staphylococcus aureus* and *Streptococcus pyogenes* (Boatto et al., 1994).

The chemical composition of 10 essential oils (including rosemary oil) and their antimicrobial activity have been investigated. The antimicrobial activity was evaluated by agar diffusion and atmospheric methods with respect to Gram+ and Gram- bacteria, hypomycetes and *Saccharomyces* (a total of 40 microorganisms). It was found that the oils were Gram+ selective, as the oil of *Artemisia dranunculus*, type 19 of *Tanacetum vulgare*, *T. patula* and one of *Rosmarinus officinalis* oils resulted in 100 % inhibition in the multiplication of strains (Héthelyi et al., 1989).

Essential oils from eleven aromatic plants belonging to the Lamiaceae family were examined on three different development stages of *Drosophila auraria*. All showed insecticidal effects, either by preventing egg hatching, or by causing the death of larvae and adult flies. Malformation and/or prohibition of puparium formation were also observed (Konstantopoulou et al., 1992).

The effect of essential oils from three common herbs of the family Lamiaceae, *Lavandula officinalis*, *Melissa officinalis* and *Rosmarinus officinalis*, on the morphology of *Candida albicans* was examined by scanning electron microscopy. The results showed significant inhibitory effects on *Candida albicans* by *Melissa officinalis* (100 %) but *Rosmarinus officinalis* oil did not inhibit *C. albicans* (Larrondo and Calvo, 1991).

Methanol extract

A methanol extract containing 30 % of carnosic acid, 16 % of carnosol and 5 % of rosmarinic acid was found to be the most effective antimicrobial against Gram positive bacteria (MIC between 2 and 15 mg/ml), Gram negative bacteria (MIC between 2 and 60 mg/ml) and yeast (MIC of 4 mg/ml). By contrast, a water extract containing only 15 % of rosmarinic acid showed a narrow activity. MIC value of the methanol and water extracts is in a good correlation with the values obtained with pure carnosic acid and rosmarinic acid, respectively (Moreno et al., 2006).

- Antimutagenic and hepatoprotective effect

Administration of rosemary ethanolic extract (0.15 g/100 g body weight –BW–) to rats for 3 weeks produced a hepatoprotective effect, using carbon tetrachloride and cyclophosphamide as mutagenic and hepatotoxic compounds. This effect was comparable to silymarin (reference), and there were amelioration of the serum and liver parameters, confirmed by histopathological examination of the liver tissue. Rosemary oil (1.1 mg/g BW) used as pre-treatment for 7 days, followed by i.p. injection with cyclophosphamide reduced the mitodepression in the bone marrow. According to the author, this effect is due to the high percentage of phenolic compounds with antioxidant activity (Fahim et al., 1999).

Tert-butyl hydroperoxide induces in freshly isolated rat hepatocytes malonaldehyde formation and lactic dehydrogenase and aspartate aminotransferase leakage. The demonstration of both anti-lipoperoxidant and antihepatotoxic activity of reference products such as quercetin and silymarin and plant extracts such as *Rosmarinus officinalis* and *Escholtzia californica* was possible using this model, adapted to the crude extracts. The results confirm the antihepatotoxic action of *Rosmarinus officinalis* young sprouts, *in vivo*, on carbon tetrachloride-induced toxicity in rats (Joyeux et al., 1990)

Carnosic acid and carnosol against chromosomal damage induced by γ -rays, were compared with those of L-ascorbic acid and the S-containing compound dimethyl sulfoxide (DMSO), and demonstrated to be the only compounds that showed a significant antimutagenic activity both before and after γ -irradiation treatments. These results are closely related to those reported by other authors on the antioxidant activity of the same compounds, and the degree of effectiveness depends on their structure (del Baño et al., 2006).

2. *In vivo* studies

- Choleric and diuretic action

One of the main traditional indications of rosemary is related with the hepato and kidney axes, particularly the hepatobiliary problems. A study was performed to evaluate the influence of rosemary and compare its different parts on the rat bile secretion and its hepatoprotective effect. The authors conclude that Rosemary presents choleric, diuretic and hepatoprotective activity. The new sprouts are choleric at doses of 500, 1000 and 2000 mg/kg and diuretic at 50 mg/kg. The solvent hydro-alcoholic is needed for the diuretic effect. The others parts of the plant stimulates the biliary flux. The entire plant at 1500 mg/kg didn't show hepatic protection or diuretic effect (Fleurentin et al., 1986).

After the administration of the essential oil (donated by the company of Drey's Italia of Calderara di Reno-Bologna), the increase of the secreted bile and of the cholates present in it did not produce in rats the expected dose-effect linearity (Taddei and Giachetti, 1993).

Two medicinal plants used in Morocco, *Rosmarinus officinalis* L. and *Centaureum erythraea* L., reported for the treatment of urinary ailments, were tested for their diuretic effect. Aqueous extracts of both plants were administered orally to Wistar rats for 1 week. The urinary volume, the excretion of sodium, potassium and chloride were determined, as well the concentration of electrolytes and urea in plasma and creatinine clearance. The dose of 10 mg/kg of 8 or 16 % extract in distilled water enhanced diuresis in rats compared with the control group from the day five. *R. officinalis* at the dose of 8 % reached at the day six the peak of urinary excretion of sodium, potassium and chloride ($p < 0.01$). At 16 %, it induced slight increases of sodium and chloride excretion on day seven and potassium on day six ($p < 0.05$) (Haloui. et al., 2000).

For the evaluation of the choleric and protective activities in the rat, lyophilised and aqueous extracts of *Rosmarinus officinalis* young sprouts and total plant were tested. *R. officinalis* ethanol extracts prepared from young sprouts and total plant show a significant dose-related choleric activity and are more active than the total plant extract. Aqueous extract of young sprouts show a significant hepatoprotective effect on plasma GTP levels when given as pre-treatment before tetrachloride intoxication, while the whole plant extract was inactive (Hoeffler et al., 1987).

An aqueous alcoholic extract (15 %) of *R. officinalis* in blossom has been investigated by experimental biliary fistula in guinea pig. The increase of the biliary flux happens because of a rapid cholagogic activity and a slowest choleric activity. An acute toxicity in mice and rats did not reveal any signs of toxicity at the dose used (2 g/kg i.p.) (Mongold et al., 1991).

- Liver action

Rosemary extract exhibited an antioxidative effect in mice. The hexane extract of rosemary (containing about 1.5 % carnosol) was fed to a group of 18 mice. Another 18 mice (eating a normal diet) served as controls. At the end of one week, the animals were fasted overnight and heparinized blood was withdrawn from 6 mice in each group. The animal's livers were weighed and homogenized.

Phosphatidylcholine hydroperoxide and phosphatidylethanolamine hydroperoxide in the plasma, red blood cells (RBC), and livers were determined by chemiluminescence's-high performance liquid chromatography. Phospholipid hydroperoxides (PLOOH) were assessed to reflect products of oxidative

injury in membranous phospholipids layer in the plasma, RBC, and liver of mice. An aliquot of the liver homogenate was incubated with ferrous sulphate and ascorbic acid and PLOOH and thiobarbituric acid reactive substances (TBARS) measured. The addition of rosemary did not affect food intake or liver weight and did not change the *in vitro* liver lipid peroxidizability compared to controls. However, the α -tocopherol concentrations in plasma, RBC, and liver were significantly lower in mice fed rosemary. Rosemary resulted in a lower level of PLOOH in RBC but did not affect PLOOH levels in plasma or the liver compared to controls. The authors stated that the decrease in α -tocopherol concentrations was unexpected and is unexplained; however, the PLOOH levels remained similar to that of controls, suggesting that some component in rosemary had an antioxidant effect in the liver and partially made up for the loss of α -tocopherol (Asai et al., 1999).

- Antiulcerogenic effect

The crude hydroalcoholic (70 %) extract (CHE) of *Rosmarinus officinalis* L. decreased the ulcerative lesion index in different experimental models in rats, produced by some ulcerogenic products like indomethacin, ethanol and reserpine. The pharmacological mechanism seemed not related with nitric oxide, or with prostaglandins. The results of the experiments suggested that the CHE increases the mucosal nonprotein sulphhydryl group's content or, as another hypothesis, the activity of the antioxidant compounds of the CHE react with N-ethyl-maleimide (Dias et al., 2000).

- Anti-inflammatory activity

Rosmarinic acid

In preliminary studies, at short term, ebselen and rosmarinic acid were effective by reducing both gingival inflammation and plaque accumulation when topically applied in the Rhesus monkey model (Van Dyke et al., 1986).

- Hypoglycaemic effect

In a study on normo- and hyperglycaemic mice, the effect of a hot infusion of *R. officinalis* leaves (two handful of leaves in 1 l of boiling water) was investigated, as well the chronic toxicity. The mixture was cooled to the temperature room and 200 ml was drunk 30-60 min before each meal. The normo- and alloxan-induced hyperglycaemic group taking the infusion presented lower levels of glucose plasma levels than the control ($p < 0.05$, 0.01 respectively). The author mentions the hyperglycaemic and insulin release inhibitory results in alloxan-diabetic rabbits, using the volatile oil, from the study of Al-Hader et al. The interpretation of the author about this controversial effect is the small content of volatile oil on the leaves infusion and the presence of other components (Erenmemisoglu et al., 1997).

In alloxan diabetic rabbits, *R. officinalis* volatile oil increased fasting plasma glucose levels by 17 % ($p < 0.05$) above those of untreated animals 6 h after administration. The author concludes that these data suggest that the volatile oil of *R. officinalis* has hyperglycaemic and insulin inhibitory effects in rabbits (Al-Hader et al., 1994).

- Immunological effect

Rosemary may only have an immune enhancing effect *in vivo* in stressed conditions, such as protein or antioxidant deficiency. Male rats were fed an experimental diet for 8 weeks. Test diets contained either 10 % or 20 % casein with rosemary (0, 100, 200, or 400 parts per million (ppm)) or BHT, 400 ppm, as a positive control. The mitogenic reactivity of isolated splenic mononuclear cells from the test animals against concanavalin A (Con A), phytohemagglutinin (PHA), and lipopolysaccharide (LPS) were assessed as were plasma uric acid and tocopherol levels in blood and liver. Rosemary treatment had no effect on feed consumption or growth of the animals and did not affect uric acid or tocopherol levels. Rosemary only had a significant effect on mitogenic reactivity to Con A and PHA in rats fed a 10 %

casein diet with 200 ppm rosemary ($p < 0.05$ compared to controls). Rosemary had no impact on mitogenic reactivity to LPS. The authors conclude that rosemary may not have any significant immunopotential in healthy situations but that its effectiveness in a more oxidative stressed model, such as decreased dietary antioxidants and/or severe protein insufficiency, deserves further study (Babu et al., 1998).

- Antitumorigenic activity

This study was performed to evaluate the activity of rosemary extract, carnosol and ursolic acid in inhibiting the *in vivo* formation of mammary 7, 12-dimethylbenz[*a*]anthracene (DMBA)-DNA adducts and the initiation of DMBA-induced mammary tumorigenesis in female rats. A significant decrease in the *in vivo* formation of rat mammary DMBA-DNA adducts, compared to controls resulted after the supplementation of diets for two weeks with rosemary extract (0.5 % by weight), but not with carnosol (1.0 %) or ursolic acid (0.5 %). After injecting intraperitoneally for 5 days at 200 mg/kg BW, rosemary and carnosol, but not ursolic acids, significantly inhibited adduct formation by 44 % and 40 %, respectively, compared to controls. A significant decrease of 74 % and 65 % in the number of DMBA-induced mammary adenocarcinomas per rat was associated with injection of this dose of rosemary and carnosol, respectively. Ursolic acid had no effect (Singletary et al., 1986).

An extract of rosemary was given to female A/J mice for 4 weeks at concentrations of 0.3-0.6 % (by weight) prior to determination of the activities of detoxification enzymes glutathione S-transferase (GST) and NAD(P)H:quinone reductase (QR) in lung, liver and stomach. Liver activities of GST and QR, and stomach GST activity were significantly increased in animals fed diets containing rosemary extract but did not affect lung GST and QR activities (Singletary and Rokusek, 1997).

A methanol extract of the leaves of *Rosmarinus officinalis* L. was evaluated for its effects on promotion and initiation of mouse skin tumour. Rosemary extract application to the mouse skin inhibited the covalent binding of benzo (*a*) pyrene [B (*a*) P] to epidermal DNA and inhibited tumour initiation by B (*a*) P and DMBA. It also inhibited TPA-induced ornithine decarboxylase activity (Ho et al., 1994).

Topical application of carnosol or ursolic acid isolated from rosemary inhibited TPA-induced ear inflammation, ornithine decarboxylase activity and tumour promotion (Huang et al., 1994).

According to Ho et al. (Ho et al., 1994), studies of the effects of a fraction of green tea polyphenols, an extract of leaves of rosemary and the pure phytochemicals on the carcinogenic process in short-term animal studies (biochemical markers) and long term animal tumour studies, indicate that they have potent inhibitory effects on biochemical marker changes associated with tumour initiation and promotion, and anticarcinogenic activity in several animal models.

After 13 weeks, post-DMBA tumour incidence for rats fed the 1.0 % rosemary diet (33.3 %) was significantly lower than for rats fed the control diet (53.6 %). But by 20 weeks, incidence for rats fed 0, 0.5 and 1.0 % rosemary was 72.2, 69.6 and 58.3 % respectively ($p < 0.5$). Rosemary extract can inhibit DMBA-induced mammary tumorigenesis when fed prior to and after DMBA dosing (Singletary, 1992).

- Antimutagenic and hepatoprotective effect

Administration of rosemary ethanolic extract (0.15 g/100 g BW) to rats during 3 weeks produced a hepatoprotective effect, using carbon tetrachloride and cyclophosphamide as mutagenic and hepatotoxic compounds. This effect was comparable to silymarin (reference), and there were amelioration of the serum and liver parameters, confirmed by histopathological examination of the liver tissue. Rosemary oil (1.1 mg/g BW) used as pre-treatment for 7 days, followed by i.p. injection with cyclophosphamide reduced the mitodepression in the bone marrow. According to the authors, this

effect is due to the high percentage of phenolic compounds with antioxidant activity (Fahim et al., 1999).

Tert-butyl hydroperoxide induces in freshly isolated rat hepatocytes malonaldehyde formation and lacticodehydrogenase and aspartate aminotransferase leakage. The demonstration of both anti-lipoperoxidant and antihepatotoxic activity of reference products such as quercetin and sylimarin and plant extracts such as *Rosmarinus officinalis* and *Escholtzia californica* was possible using this model, adapted to the crude extracts. The results confirm the antihepatotoxic action of *Rosmarinus officinalis* young sprouts, *in vivo*, on carbon tetrachloride-induced toxicity in rats (Joyeux et al., 1990).

- Cytotoxic effect

A well expressed direct cytotoxic effect on L1210 leukaemia cells in hybrid-BDF1 mice was demonstrated by Ilarionova et al. (1992), on a study about the essential oils extracted from *Rosmarinus officinalis*, *Geranium macrorrhizum* and *Urtica dioica* grown in Bulgaria. It was concentration and time of incubation dependent.

- Anticonvulsivant activity

To study the effects of the aqueous extracts of leaves and stems of *Rosmarinus officinalis*, *Pimpinella anisum*, *Matricaria chamomilla*, *Artemisia vulgaris*, *Origanum vulgare*, *Lapinus albus* and *Olea europea*, on the Picrotoxin-induced seizures in mice, this test were performed. The mortality rate, onset of convulsion and GABA content were monitored. The extracts of these plants were found to delay the onset of picrotoxin-induced seizures and to decrease the mortality rate. Extracts of *Origanum vulgare*, *Lapinus albus* and *Olea europea* had no effect on the onset of convulsions or on the mortality rate (Abdul-Ghani et al., 1987).

- Antinociceptive activity

The effect of the aqueous and ethanol extracts of *Rosmarinus officinalis* aerial parts on morphine withdrawal syndrome was investigated in mice. The aqueous and ethanol extracts induced a significant antinociceptive activity in the writhing test. This activity was inhibited by naloxone pretreatment. Phytochemical study indicated that only the aqueous extract of *R. officinalis* has an alkaloid component. The authors concluded that the aqueous and ethanol extracts of *R. officinalis* aerial parts could diminish morphine withdrawal syndrome (Hosseinzadeh and Nourbakhsh, 2003).

- Enzymes induction

Rodent studies suggest the possibility of the induction of CYP1A, CYP2B, CYP2E1, and CYP3A along with some phase II enzymes (e.g. glutathione S-transferase, UDP-glucuronosyltransferase) (Barceloux, 2008).

3.1.1. Assessor's overall conclusions on pharmacology

The essential oil content of rosemary and the pharmacological effects of the constituents support the claims for carminative and digestive ailments, such as flatulence and feelings of distension. Documented antibacterial and anti-inflammatory effects are also attributable to the essential oil.

Antihepatotoxic action and diuretic activity have been shown with young sprouts of *Rosmarinus officinalis*, *in vivo*. The diuretic action was achieved with traditional doses but the hepatoprotection needed very high doses.

The antioxidant properties of rosemary results primarily from the actions of phenolic diterpenes, such as carnosic acid, carnosol and rosmanol. The *in vitro* studies indicate that the main diterpene

antioxidant is carnosic acid. The concentration of carnosic and rosmarinic acid depends on drying techniques and distillation processes.

Rosemary leaf diterpenes have been shown to have lipid peroxidase inhibiting activity and to exert an influence on glucose levels in mice. They also appear to have an antitumour effect and may increase the accumulation of chemotherapeutic agents in drug resistant mammary tumour cells *in vivo* and *in vitro*.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

(e.g. absorption, distribution, metabolism, elimination, pharmacokinetic interactions with other medicinal products)

- Rosmarinic acid

In *ex vivo* experiments, permeation of rosmarinic acid (RA) across excised rat skin was about 8 times higher from alcoholic solution than from water. After topical application, RA concentration in muscle and bone tissue beneath the application site was comparable to those after systemic administration. Upon intravenous administration, the classical two-compartment open pharmacokinetic model is followed, given the indication for extensive peripheral tissue distribution, which becomes 7 to 13 times higher in the soft tissue than in blood concentrations (Ritschel et al., 1989).

Rosmarinic acid (i.v.) produced moderate inhibition of plaque-forming cell (B-cell) activity in mice and was mildly antihyperpetic. The compound is rapidly eliminated from the circulation (i.v. $T_{1/2}$ =9 min) and has a low toxicity (LD₅₀ in mice=561 mg/kg i.v.), transient cardiovascular actions becoming pronounced at ≥ 50 mg/kg i.v. (Parnham and Kesselring, 1985).

- Rosemary oil

In mice, inhalation of 0.5 ml of volatile oil released into the breathing air resulted in detectable levels of 1,8-cineole in the blood and was biphasic, with a short half-life of about 45 min during a second phase, indicating elimination by a two compartment model (Kovar et al., 1987).

- Camphor

Camphor's cyclic terpene structure makes it highly lipophilic, explaining both its rapid movement across mucous membranes and large volume of distribution. Once absorbed, it is rapidly oxidized to camphorol, which is then conjugated in the liver to the glucuronide form. As a result of their lipophilic nature, active metabolites are stored in fat deposits and cleared over a prolonged period of time. Most camphor is ultimately excreted in the urine (Sage leaf AR, HMPC, 2008).

Breastfeeding

Scientific evidence for the safe use of rosemary during lactation is not available. Neither the German Commission E nor the American Herbal Products Association note any contraindications to its use during lactation (Blumenthal et al., 2000; McGuffin et al., 1997).

3.2.1. Assessor's overall conclusions on pharmacokinetics

Just some aspects of the pharmacokinetics of rosmarinic acid, rosemary oil and camphor are known, depending on the preparation used.

There are no data on the transfer into human milk.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

(e.g. single/repeat dose toxicity, genotoxicity, carcinogenicity and reproductive and developmental toxicity, local tolerance, other special studies)

1. Acute and sub-chronic toxicity

- Rosemary extracts

In a study to evaluate the acute toxicity, in Wistar rats, two representative rosemary leaf extracts were used, with different concentrations of phenolic diterpenes, representing medium and high values found in commercial supercritical extracts. At a single dose of 2.0 mg/kg of BW, no deaths or negative clinical signs were observed during the 2 weeks observational period, with no significant differences in weight gain, food and water consumption, clinical chemistry parameters or histological changes (Anadón et al., 2008).

- Alcoholic extract

A 15 % alcoholic extract showed no signs of toxicity when i.p. administered to rats at doses of 2 g, 7 kg (Wichtl, 1994).

Antioxidant rosemary extracts have low acute and sub-chronic toxicity in the rat. Sub-chronic studies on five solvent extracts (rosemary extract produced from dried rosemary leaves by acetone extraction; rosemary extract prepared by extraction of dried rosemary leaves by means of supercritical carbon dioxide; rosemary extract prepared from a partially deodorized ethanolic extract of rosemary; extract prepared from a deodorized ethanolic extract of rosemary; extract which is a decolorized and deodorized rosemary extract obtained by a two-step extraction using hexane and ethanol) reveal that the only effect at high doses of these rosemary extracts is a slight increase in relative liver weight. This effect has been shown to be reversible and may be the result of Phase I and II enzyme induction. The effect was not accompanied by increases in plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP). Considering the low magnitude, reversibility and the nature of the hepatic changes, and the absence of increases in plasma ALT, AST and AP, the Panel concluded that the minor increase in the liver weight reported, accompanied by minimal centrilobular hypertrophy and microsomal enzyme induction, represent an adaptive response and are not of toxicological concern. Overall, the 90-day feeding studies in rats with the different rosemary extracts tested, reveal NOAEL values in the range of 180 to 400 mg extract/kg BW/day equivalent, depending on the carnosol and carnosic acid content of the respective extracts, to 20-60 mg/kg BW/day of carnosol plus carnosic acid (EFSA, 2008).

- Essential oil

The results from the study of the action of rosemary essential oil, eucalyptol and camphor on the cortex of mice *in vitro* showed an inhibition of O₂ consumption and the lost of electrolytic gradient of Na⁺ and K⁺ (Steinmetz et al., 1987).

With the ingestion of large amounts of rosemary oil, there is a danger of gastroenteritis and nephritis (Wichtl, 1994)

Rosemary extract showed no mortality at intragastric doses up to 1.2 g/100 g of BW in rats, classified as a very low lethality. Essential oil of rosemary had a lethal dose 50 (LD₅₀) of 5.5 g/kg BW intragastrically in rats, and a lethal effect on all animals at an intragastric dose of 0.9 g/100 g BW (Fahim et al., 1999).

2. Genotoxicity

- Rosemary extract, carnosic acid and carnosol

A rosemary extract with carnosic acid and carnosol as the two major active ingredients were shown to exhibit strong antimutagenic effects in Ames tester strain TA102. This property was attributed to their antioxidant capacity. Carnosic acid was held responsible for the antimutagenic effect (Minnunni et al., 1992).

According to EFSA report (EFSA, 2008) four of the five rosemary extracts considered (rosemary extract produced from dried rosemary leaves by acetone extraction; rosemary extract prepared by extraction of dried rosemary leaves by means of supercritical carbon dioxide; rosemary extract prepared from a partially deodorized ethanolic extract of rosemary; extract prepared from a deodorized ethanolic extract of rosemary; extract which is a decolorized and deodorized rosemary extract obtained by a two-step extraction using hexane and ethanol) were tested for genotoxicity. Several *in vitro* genotoxicity studies were performed in both prokaryotic and eukaryotic test systems and an *in vivo* mouse micronucleus test performed with the last above-mentioned rosemary extract. The Panel concluded that these do not give rise to safety concerns with respect to genotoxicity of the rosemary extracts.

- Camphor

Camphor did not show mutagenic activity in *Salmonella typhimurium* strains TA 1535, TA 1538, TA 98 and TA 100 with and without S9 activation. No mutagenic effect was found with d,l-camphor in strains TA 97a, TA 98, TA 100 and TA 102 with and without metabolic activation (Sage leaf AR, HMPC, 2008).

3. Carcinogenicity

Several studies reported that rosemary may be protective at various stages of carcinogenesis in animal models *in vivo*.

- Camphor

No oral studies on chronic toxicity or carcinogenicity from camphor are available. In a pulmonary tumour response test *d*-camphor injected intraperitoneally into strain A/He mice (groups of 15 males and females) three times a week for 8 weeks in total doses of 3.6 and 18 g/kg BW induced no increase in primary lung tumours and was not considered by the authors to be carcinogenic for lung (Sage leaf AR, HMPC, 2008).

4. Reproductive toxicity

- Aqueous extract

In Central America, a tea prepared with *R. officinalis* and "ocean Artemisia" is used to control fertility, producing temporary sterility. To assess if rosemary induces abortion or interferes with the normal development of the conception, an aqueous extract of *R. officinalis* was given to pregnant rats during the preimplantation period, as the same dose as used by women to induce abortion (doses of 26 mg of a 30% w/v aqueous extract – 13 mg solids/ml, made with leaves, flowers and stems, administered by gavage during two different periods of Wistar rats pregnancy). One group (N=12) received the extract from days 1 to 6 of pregnancy (preimplantation period) and another group (N=14) received the same extract from days 6 to 15 of pregnancy (organogenic period), against control groups (N=12) which received saline solution. The animals were sacrificed at term. The results suggest that rosemary extract may present an anti-implantation effect (the difference was not significant compared to the control), without interfering with the normal development of the concept after implantation (Lemonica et al., 1996).

- Methanolic extract

A methanolic extract (2 %) from the leaves of *Rosmarinus officinalis* L. was given to female CD-1 mice, in AIN-76A diet for 3 weeks. The liver microsomal 2-hydroxylation of estradiol and estrone were increased 140-180 %, 6-hydroxylation was increased by 30 % and 16 α -hydroxylation of estradiol was inhibited by 50 %. It also stimulated the liver microsomal glucuronidation of estradiol by 54-67 % and estrone by 37-56 %. In ovariectomized CD-1 mice, it inhibited the uterotrophic action of estradiol and estrone by 30-50 % compared with the group control (Zhu et al., 1998).

- Ethanolic extract

The EFSA panel (EFSA, 2008) reports the recently Nusier *et al.* (2007) published results from a study on the effects of a 70 % ethanol: 30 % water extract of rosemary on reproductive function in adult male Sprague Dawley rats, ingesting rosemary extracts dissolved in water at levels of 250 and 500 mg/kg BW/day for 63 days. Body weight and absolute and relative testes weights were not affected, but in the highest dose group the average weight of the epididymides, ventral prostates, seminal vesicles, and preputial glands significantly decreased. A significant decline in spermatogenesis in testes due to a decrease in the number of primary and secondary spermatocytes and spermatids in the high dose group was observed and attributed to a significant decrease in testosterone. In rats of the highest dose group, sperm motility and density were also significantly decreased in the caudal epididymis and in the testes. For the high dose group the treatment also markedly increased the number of foetal resorptions in female rats impregnated by the high dose males, thereby reducing their fertility. For the 250 mg/kg BW dose groups no statistically significant decreases in these parameters were observed and it can therefore be concluded that

250 mg extract/kg BW/day is the NOAEL in this study. Analytical details on the extract used in the study were not provided.

5. Teratogenicity

- D-Camphor

D-Camphor showed no evidence of teratogenicity after oral administration during the foetal period of organogenesis to pregnant rats at doses up to 1000 mg/kg BW/day, and to pregnant rabbits at doses up to 681 mg/kg BW/day. The NOEL for the foetal organism of the rat was above 1000 mg/kg BW, and for the rabbit above 681 mg/kg BW. No increased incidence in variations, retardations or malformations was observed at any of the treated dose levels. The daily maximum therapeutic camphor dose in humans is 1.43 mg/kg BW. The author concluded that the present test conditions the therapeutic ratio is above 450 for the endpoint embryo toxicity reflecting a wide margin of safety (Leuschner, 1997).

- Aqueous extracts

Rosemary did not interfere with normal foetal development after implantation in rats. Mated female rats were randomly assigned to groups, and treated either during the preimplantation or postimplantation period. Either, 26 mg daily of a 30 % (w/v) boiled aqueous extract of rosemary (stems, leaves, and flowers) or an equal amount of saline solution was administered either from the 1st to 6th day (preimplantation) or the 6th to 15th day (organogenic period). On day 21, the rats were sacrificed and the foetuses were examined for external malformations. No differences were noted in the term foetuses and the rate of postimplantation loss was the same in both groups (Lemonica et al., 1996).

3.3.1. Assessor's overall conclusions on toxicology

Serious poisoning by rosemary or its oil is not reported. The potential problems of gastroenteritis and nephritis, after the ingestion of large amounts of the oil are mentioned in the literature, but do not relate to actual cases. Acute toxicity by rosemary extract was not observed in animal tests.

Teratogenicity data on rosemary oil is not available. One study of rats showed no foetotoxic effects from the administration of a rosemary aqueous extract at various stages of pregnancy.

Rosemary may have an anti-implantation effect in rats but it does not interfere with normal foetal development after implantation. Rosemary, which is known to have a relaxing effect on smooth muscles, may have interfered with the movements of the oviducts and ovum transport or may have interfered with the uterine conditions related to ovum implantation. These findings may explain the use of rosemary extract as an abortive in Brazilian folk medicine but the results of the studies were not conclusive. Nevertheless, it seems prudent to avoid consumption during pregnancy.

The existing data on rosemary extracts are insufficient to establish a numerical ADI. The absence of effects in the 90-day studies on reproductive organs and lack of genotoxicity do not give reason for concern.

However, as the minimum required data on mutagenicity (Ames' test) are not available for herbal preparations of rosemary leaf and rosemary oil, inclusion in the Community list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products is not recommended.

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

- Essential oil

A study was performed in 40 adults to assess the EEG activity, the alertness and the mood after 3 min of aromatherapy, with lavender and rosemary. The lavender group showed increased beta power, less depressed mood and felt more relaxed performing the math computations faster and accurately. The rosemary group showed decreased frontal alpha and beta power, suggesting increased alertness, lower state anxiety scores and was faster but not accurate on the math computations (Diego et al., 1998).

- Rosemary extract

A study was performed to determine the effect of phenolic-rich extracts from green tea or rosemary on nonheme-iron absorption. The rosemary extract was commercially available (Herbor®; FIS SA, Chatel -St-Denis, Switzerland). Each extract was diluted (10 % w/v) using an ethanol / water solution (2:1 v/v). Women aged 19-39 years consumed identical test meals on 4 separate occasions, except for the absence or presence of a phenolic-rich extract from green tea (study 1; $n=10$) or rosemary (study 2; $n=14$). The meals were extrinsically labelled with either ^{55}Fe or ^{59}Fe . The presence of phenolic-rich extracts resulted in decreased nonheme-iron absorption. Absorption decreased from $12.1\pm 4.5\%$ to $8.9\pm 5.2\%$ ($p<0.01$) in the presence of the green tea extract and from $7.5\pm 4\%$ to $6.4\pm 4.4\%$ ($p<0.05$) in the presence of the rosemary extract. The authors concluded that the phenolic-rich extracts used as antioxidants in foods reduce the utilization of dietary iron (Samman et al., 2001).

A hydrophilic fraction (Rosm1) from an alcoholic extract of rosemary had strong antioxidant activity and inhibited oxidative alterations to skin surface lipids. The effectiveness of Rosm1 was tested in humans to assess its ability to prevent lipid peroxidation of skin surface lipids with vitamin E used as a control. Thirty adult males were randomly divided into 5 groups, and a sample of skin surface lipids was obtained from the forehead of each volunteer as an internal control. Group 1 applied the vehicle for one week; groups 2 through 5 applied a 3 ml 5 % ethanol solution containing 50, 100 or 500 µg/ml of Rosm1 fraction. Samples of skin lipids were taken the morning after the last day of treatment and resistance to oxidative stress was assessed by chemiluminescence. The rosemary extract dose-dependently protected the skin lipids from oxidative stress *in vitro* in a test of the skin samples from volunteer foreheads. Lipids extracted after topical treatment with the rosemary extract showed a significantly higher resistance towards lipoperoxidative chain reactions than did lipids from the controls. The authors suggested that the hydrophilic rosemary extract may be an important natural antioxidant that may prove beneficial as an anti-aging treatment of the skin (Calabrese et al., 2000).

4.1.1.1. Assessor's overall conclusions on pharmacodynamics

Some authors conclude that rosemary extract may be an important natural antioxidant that may prove beneficial as an anti-aging treatment of the skin.

Some studies conclude that the phenolic-rich extracts used as antioxidants in foods reduce the utilization of dietary iron.

The lack of human data limits conclusions regarding the clinical relevance of the potential interactions.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

There are no clinical pharmacokinetic data.

4.2. Clinical Efficacy

4.2.1. Dose response studies

None.

4.2.2. Clinical studies (case studies and clinical trials)

Topical application of a combination of essential oils, including rosemary oil, significantly improved the symptoms of alopecia areata in a randomized, double-blind, controlled trial of 84 patients. The active test group received a combination of essential oils from *Thymus vulgaris* (88 mg), *Lavandula angustifolia* (108 mg), rosemary (*Rosmarinus officinalis* 114 mg), and *Cedrus atlantica* (94 mg) mixed in a carrier oil of 3 ml jojoba oil and 20 ml grape seed oil. The control group used the carrier oils. The oils were massaged into the scalp for 2 min each night and a warm towel was then wrapped around the head. Assessments were made initially, at 3 months, and at 7 months. Independent scoring by two dermatologists who were unaware of the treatments and who evaluated sequential photographs of the volunteers assessed primary outcome. A significant improvement was noted in the treatment group ($p < 0.05$). The responses were variable but showed a clear and statistical advantage to treatment. A secondary outcome measure, tracing hair growth where alopecia occurred in patches, and a computerized image analyzer, was used to calculate changes in the areas of alopecia. This secondary measure could only be performed in 32 patients but showed a mean reduction of approximately $104 \pm 140 \text{ cm}^2$ in the test group versus $-1.8 \pm 155 \text{ cm}^2$ in the control group. The authors noted that the

essential oil treatment caused no adverse events and showed a better therapeutic ratio compared to other available treatments for alopecia (Hay et al., 1998).

4.2.3. Clinical studies in special populations (e.g. elderly and children)

None.

Paediatric use requires a careful assessment as often medical advice and supervision should be sought, for safety reasons.

4.3. Overall conclusions on clinical pharmacology and efficacy

A single clinical study suggested that rosemary applied topically may protect skin cells from oxidative stress and another single clinical study showed that rosemary essential oil combined with other essential oils might be a moderately effective treatment for alopecia areata.

These studies are not sufficient to support therapeutic indications on the basis of WEU. The therapeutic indications can therefore only be based on traditional use.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

Commission E: Leaf permitted for oral use. No contraindications (CI), adverse events (AE), interactions (I) [BA nz nr. 223 30.11.85].

Standardzulassungen: Leaf permitted as herbal tea. CI: pregnancy. No AE, I.

French Guideline: Leaf and flowering top permitted for oral use (toxicological category 1 for powder, herbal tea, aqueous extract, low strength aqueous-alcoholic extracts, high strength aqueous-alcoholic extracts, tinctures (De Smet, 1993).

Germany (essential oil) – External use - urges to cough, bronchial and laryngeal spasm, local hypersensitive reactions.

Regarding the adverse reactions reported by Germany, these can be considered as a form of hypersensitivity to the medicinal product.

5.2. Patient exposure

Rosemary has a long history of consumption in the human diet. Rosemary extract is a component of some perfumes, disinfectants and insecticides. Rosemary leaves are widely used as a seasoning for meat dishes, sauces and salami.

- Carnosol and carnosic acid

The main potential sources of exposure to rosemary extracts used as antioxidants were reported as 'fine bakery wares', 'dehydrated soups and broths' and 'seasonings and condiments' in UK adults and 'fine bakery wares' and 'meat, poultry and fish/seafood products (non-processed)' in pre-school children (EFSA, 2008).

Dietary exposure to carnosol plus carnosic acid has been estimated for adults and pre-school children (aged 1.5 to 4.5 years) and amounts to mean values of respectively 0.04 and 0.11 mg carnosol plus carnosic acid/kg bw/day, 0.10 and 0.20 mg carnosol plus carnosic acid/kg bw/day at the 95th

percentiles and 97.5th percentile values of 0.12 and 0.23 mg carnosol plus carnosic acid/kg bw/day (EFSA, 2008).

On the EFSA panel report, it is noted that the margin between the NOAEL range in the 90-day rat studies with all five extracts of 180 to 400 mg extract/kg BW/day equivalent to 20-60 mg/kg BW/day of carnosol plus carnosic acid, and the dietary exposure estimates for adults would amount between 500-1500 for the mean intake values, between 200-600 for the 95th percentile values and between 167-500 for the 97.5th percentile values. For pre-school children these margins would amount to respectively at least 182-546, 100-300 and 87-261. The Panel noted that these margins of safety are worst case estimates since the NOAELs from the different studies were generally the highest dose levels tested, and that the estimates of dietary exposure were conservative.

The EFSA Panel (2008) was of the opinion that the margin of safety is high enough to conclude that dietary exposure resulting from the proposed uses and use levels are not of safety concern. To achieve these levels of dietary exposure, high level consumers would need to select a diet that was entirely composed of foods containing rosemary extracts for those food categories in which it was permitted. In reality not all processed foods will contain added antioxidants and it seems unlikely that these extracts would be used at the maximum usage level in all the proposed food in each category or that some consumers would systematically always choose all foods containing rosemary extract.

- Camphor

Dietary exposure to camphor arises from the consumption of foods flavoured by using herbs, their essential oils or the chemically defined flavouring substance *d*-camphor. The dietary exposure to camphor was estimated to be 1.5 mg/person/day (Council of Europe, 2001). Assuming an average body weight of 60 kg, this corresponds to an exposure of 25 µg/kg BW/day. Limits for *d*-camphor, suggested by the Council of Europe were 10 mg/kg in beverages (including alcoholic drinks), 25 mg/kg in food in general, 100 mg/kg in candies, 140 mg/kg in fresh cheese, 150 mg/kg in sauces and condiments (Sage leaf AR, HMPC, 2008).

According to the EFSA report, exposure to camphor should not exceed 2 mg/kg BW on a single day in any age group.

5.3. Adverse events and serious adverse events and deaths

5.3.1. Adverse events

- Carnosol

A case of contact dermatitis to carnosol, the main constituent of Rosmanox®, made from the leaves of rosemary, developed in a 56-year old man on his hands, forearms and face, after it was introduced in a food processing factory where he was working. 226 controls were negative (Hjorth et al., 1997).

A rosemary leaf plaster caused contact dermatitis in a 56-year-old man. A man applied a rosemary leaf plaster to treat a pain in his knee. Three days later, he developed an itchy, vesicular exudative dermatitis that improved within 10 days of withdrawing the plasters. Patch tests were only positive for rosemary. The authors note that this is the first report of a rosemary-induced case of contact dermatitis (Fernandez et al., 1997).

Further case reports related to allergic contact dermatitis show, from patch testing, that carnosol (CAS RN: 5957-80-2) is a major allergen in rosemary. The chronic use of rosemary as a culinary spice in food was associated with the development of chronic contact cheilitis. Exposure to rosemary extracts also has been associated with occupational asthma (Barceloux, 2008).

- Camphor

Rosemary oil contains 20-50 % camphor; orally, camphor readily causes epileptiform convulsions if taken in sufficient quantity (Barnes, 2002).

According to EFSA (EFSA, 2008), in humans, the intoxication of camphor includes central nervous stimulation, oral and gastric irritation, nausea and vomiting, excitement, hallucinations, delirium, muscular excitability, tremors, convulsions and urinary retention. Locally, it can produce irritation of the skin, eyes and mucous membranes of the respiratory tract.

In the same report, it is mentioned that the intoxications present in the literature, in general, involve accidental intake of camphorated oil (20 % camphor in cottonseed oil).

No acute toxicity was reported after doses lower than 2 mg/kg BW. Clinically insignificant signs of toxicity may be seen in sensitive individuals at doses of 5 mg/kg BW and higher. Clinical manifest signs in these individuals require doses higher than 30 mg/kg BW (EFSA, 2008).

5.3.2. Serious adverse events and deaths

The report of a hepatic abscess secondary to ingestion of a rosemary twig was considered to be serious (Karamarkovic et al., 2007). However, this case is considered to be not relevant for the safety assessment of authorised medicinal products containing rosemary.

- Camphor

20 children aged 1 to 4 years became ill with seizures, after ingestion of 1 to 1.5 tablespoons of camphorated oil equivalent to about 3 to 4.5 g of camphor (EFSA, 2008).

In a literature review of 64 cases, 6 reports of death were found. In a 19-month old child, the ingestion of 1 g of camphor in camphorated oil was fatal (EFSA, 2008).

In a recent published case report, a 10-year old boy presented at the emergency room with symptoms of lethargy, nausea, vomiting and rigors. 24 h previously, he had chewed three over-the-counter cold remedy transdermal patches containing 4.7 % (95.4 mg/patch) camphor and 2.6 % menthol as active ingredients (EFSA, 2008). Assuming a body weight of 30 kg, this would correspond to 10 mg/kg BW of camphor.

The American Academy of Paediatrics concluded that although adults recovered from ingestion of up to 43 g of camphor, the ingestion of 2 g generally produces dangerous effects. In children, ingestion of 0.7 to 1.0 g of camphor has proved fatal.

On the basis of the data reviewed, a probable lethal dose was estimated to be in the range of 50 to 500 mg/kg BW, with a large variation on the sensitivity of humans to the acute toxicity of camphor.

5.4. Laboratory findings

Not available.

5.5. Safety in special populations and situations

Not available.

5.5.1. Intrinsic (including elderly and children)/extrinsic factors

Not to be used in cases of hypersensitivity to the active substance.

5.5.2. Drug interactions

- Essential oil

Limited data available. Cineol induction of CYP450 enzymes is possible.

The lack of human data limits conclusions regarding the clinical relevance of these potential interactions (Barceloux, 2008).

5.5.3. Use in pregnancy and lactation

Therapeutic doses are not recommended for use during pregnancy (McGuffin et al., 1997).

Preparations of rosemary should not be used during pregnancy due to the toxic effects of some components (Wichtl, 1994).

The data available are not sufficient to conclude the safety or the danger of rosemary preparations used during pregnancy and lactation. The only conclusion possible is that the safety during pregnancy and lactation has not been established and thus rosemary preparations should be avoided.

5.5.4. Overdose

Not reported.

5.5.5. Drug abuse

None.

5.5.6. Withdrawal and rebound

None.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

Not available.

5.6. Overall conclusions on clinical safety

In general, it can be concluded that rosemary preparations are safe and devoid of toxic effects if taken in recommended doses.

Use must be avoided during pregnancy and lactation, as the safety has not been established. Use is contraindicated in hypersensitive patients.

Although rosemary preparations contain variable quantities of camphor, there are no human data to support the development of seizures as a complication of the ingestion of rosemary extracts.

Hot and full baths are contraindicated in cases of open wounds, large skin injuries, acute skin diseases, high fever, severe infections, severe circulatory disturbances and cardiac failure.

With regard to oral use, rosemary preparations are contraindicated in cases of obstruction of the bile duct, cholangitis, liver disease, gallstones and any other biliary disorders that require medical supervision and advice.

With regard to cutaneous use, the warning to avoid contact with the eyes and mucous membranes is included in the monograph of *Rosmarinus aetheroleum*, due to potential irritation of the essential oil.

Where symptoms such as articular pain accompanied by swelling of the joint, redness or fever occur medical advice should be sought.

6. Overall conclusions

Rosemary (*Rosmarinus officinalis* L.) belongs to the family Lamiaceae and has been an important medicinal plant since earliest times. It is also a commonly used spice and flavouring agent for foods and its essential oil is used therapeutically, in particular in balneology.

It was mentioned for its medicinal and cosmetic properties in ancient Greece and by the Romans. In the middle ages, rosemary oil was distilled for medical purposes and also used as a perfume.

The pharmacological studies reported in the literature give plausibility to the traditional indications set out in the monographs:

- Oral use

Traditional herbal medicinal product for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.

- Cutaneous use and use as a bath additive

Traditional herbal medicinal product as an adjuvant in the relief of minor muscular and articular pain and in minor peripheral circulatory disorders.

A single clinical study suggested that rosemary applied cutaneously may protect skin cells from oxidative stress and another clinical study showed that rosemary essential oil combined with other essential oils might be a moderately effective treatment for alopecia areata.

However, these studies are not enough to support the indication on the basis of WEU. The therapeutic indications are based solely on the traditional uses and are suitable for use without the need for medical diagnosis, prescription and supervision.

Some proposals for therapeutic indications from the interested parties (see the "overview of comments received during the public consultation") were not accepted for safety reasons.

Due to the lack of sufficient data to assure the safety, the use in children (*Rosmarini folium* and preparations thereof including *Rosmarini aetheroleum*), in adolescents (*Rosmarini aetheroleum*) and during pregnancy and lactation are not recommended.

The preparations proposed in the monograph are based on those which are known to be on the market for 30 years. Some others are stated in the literature, but either information on the period of use or the posology for these preparations are missing or they do not comply with the requirement for 30 years of traditional use evidence.

As the minimum required data on mutagenicity (Ames' test) are not available for herbal preparations of rosemary leaf and rosemary oil, inclusion in the Community list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products is not recommended.

Annex

List of references