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Committee on Herbal Medicinal Products (HMPC)

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

Draft – Revision 1

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)		Rosmarini folium and Rosmarini aetheroleum
Herbal preparation(s)		*Rosmarinus officinalis* L., folium a) Comminuted herbal substance b) Liquid extract (DER 1:1), extraction solvent ethanol 45% V/V *Rosmarinus officinalis* L., aetheroleum Essential oil
Pharmaceutical form(s)		*Rosmarinus officinalis* L., folium Comminuted herbal substance as herbal tea for oral use and as a bath additive. Herbal preparations in solid or liquid dosage forms for oral use. *Rosmarinus officinalis* L., aetheroleum Herbal preparations in semi-solid dosage forms for cutaneous use.
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Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monographs on *Rosmarinus officinalis* L., aetheroleum and *Rosmarinus officinalis* L., folium. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monographs. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monographs.

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1. Introduction

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

- Herbal substance(s)

According to the European Pharmacopoeia (Ph. Eur. 01/2013:1560), Rosemary leaf is defined as whole, dried leaf of *Rosmarinus officinalis* L. It contents a minimum of 12 ml/kg of essential oil and a minimum of 3% of total hydroxycinnamic derivatives, expressed as rosmarinic acid ($C_{18}H_{16}O_8$; M_r 360.3). c (Ph. Eur. 01/2008:1846) is defined as the essential oil obtained by steam distillation from the flowering aerial parts of *Rosmarinus officinalis* L.; it is clear, mobile, colourless or pale-yellow liquid with a characteristic odour.

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.

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 herbal documents 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 Ericacea and not related to rosemary (Chandler, 1985).

In the last years, a revision of *Salvia* genus based in phylogenetic, taxonomic, morphological and practical factors offered the transfer of *Rosmarinus officinalis* L. to *Salvia rosmarinus* (L.) Schleid. (Drew et al., 2017). Thus, *Salvia rosmarinus* (L.) Schleid. is included as a synonym of *Rosmarinus officinalis* L.

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). The 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 clear, mobile, colourless or pale-yellow liquid with characteristic odour and a complex chemical composition. For rosemary oil, Spanish type, the percentages are within the following ranges: α-pinene: 18-26%; camphene: 8.0-12.0%; β-pinene: 2.0-6.0%; β-myrcene: 1.5-5.0%; limonene: 2.5-5.0%; cineole: 16.0-25.0%; p-cymene: 1.0-2.2%; camphor: 13.0-21.0%; bornyl acetate: 0.5-2.5%; α-terpineol: 1.0-3.5%; borneol: 2.0-4.5%; verbenone: 0.7-2.5%. For rosemary oil, Moroccan and Tunisian type, the percentages are within the following ranges: α-pinene: 9.0-14.0%; camphene: 2.5-6.0%; β-pinene: 4.0-9.0%; β-myrcene: 1.0-2.0%; limonene: 1.5-4.0%; cineole: 38.0-

55.0%; p-cymene: 0.8-2.5%; camphor: 5.0-15.0%; bornyl acetate: 0.1-1.5%; α-terpineol: 1.0-2.6%; borneol: 1.5-5.0%; verbenone: maximum 0.4% (Ph. Eur. 01/2008:1846).

Methanolic extracts from the leaves of *Rosmarinus officinalis* L. harvested from different locations of Turkey at four different times of the year were analysed by HPLC. The amounts of carnosol, carnosic acid and rosmarinic acid, 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).

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* L. (del Baño et al., 2006).

- Herbal preparation(s)

Rosemary leaf is commonly used in the form of comminuted herbal substance, liquid extracts and expressed juice. The essential oil is also commonly used.

- 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.

Not applicable.

1.2. Search and assessment methodology

This Assessment Report resulted from a revision of that previously issued (EMA/HMPC/13631/2009) considering the decision of HMPC (July 2021) on the need of revision following relevant updates from data published in the literature between 2010 and 2021.

Search web engines used: Google, Google Scholar

Scientific databases: Web of Science; PubMed; Science Direct; Clinical Key; Cochrane Database of Systematic Reviews

Medical or Toxicological databases: Toxline

Search terms: "*Rosmarinus officinalis*" or "Rosemary" (2010-2020).

Pharmacovigilance resources: Data from EU and non-EU regulatory authorities, European database for suspected adverse drug reaction reports.

Data from EU and non-EU regulatory authorities: Assessment report on *Rosmarinus officinalis* L., aetheroleum and *Rosmarinus officinalis* L., folium - EMA/HMPC/13631/2009)

No data were provided by the interested parties.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Table 1 abridges information provided by the national competent authorities (NCAs) on medicinal products on the market in the European Union (EU)/European Economic Area (EEA) Member States containing *Rosmarinus officinalis* L. (rosemary leaf or rosemary oil) or its preparations as single active substance.

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Strength** (where relevant) **Posology** **Duration of use**	**Regulatory Status**
Rosemary essential oil			
Rosemary oil	Traditional use to support the function of the skin	As bath additive: • 1.35 g/100 l water for 10-20 min at 34-37ºC, if necessary 1 time/daily; • 0.39 g/150 l water for 10-30 min at 35-39ºC, every 2-3 days.	1976, Germany, TU
Rosemary oil	Auxiliary treatment in conditions of exhaustion	If necessary 3-4 times/weekly: • 4 g rosemary oil / 150 l water for 10-20 min at 34-37ºC; if necessary 3-4 times/weekly; • 2.08 g rosemary oil/100 l water for 10-20 min at 34-37ºC; if necessary 3-4 times/weekly; • 2 g rosemary oil/100 l water for 10-20 min at 34-37ºC; if necessary 3-4 times/weekly; • 5 g rosemary oil/150 l water for 10-20 min at 35-38ºC; max. 1 time/daily.	1976, Germany, WEU 1990, Germany, WEU
Rosemary oil	For the symptomatic treatment of muscle and joint pain and in circulatory disturbance.	Ointment: 6 g rosemary oil/100 g ointment; approximately 3 cm of ointment 2-3 time/daily.	1976, Germany, WEU
Rosemary oil	Stimulation of circulation	100 g solution contain 5 g essential oil as bath additive; for a full bath 30 ml.	1994, Austria, TU
Rosemary oil	Inflammation of the skin; small superficial wounds; strain trauma	Suspension (0.1%) for cutaneous use: 100 g contain 0.1g rosemary oil;	2010, Austria, TU

		Adults and adolescents >12 years; Diluted or undiluted as poultice; Duration: 7 days.	
Rosemary oil	Minor muscular and articular pain and minor peripheral circulatory disorders	Cream with 10% rosemary oil: 3-6 cm of cream 2-3 times/daily; Adults; Duration: 4 weeks.	2016, AT, CZ, ES, HR, HU, PL, SE, SI, SK, UK, TU
Rosemary leaf			
Comminuted herbal substance for herbal tea	Improvement of digestion	1-2 g/250 ml, 2-3 times/daily (2-4 g/day)	1976, Spain, TU
Powdered herbal substance (capsules)	Dyspepsia, improvement of digestion	2 caps (250 mg) 3 times/daily	1990, Spain, TU
Infusion	Dyspeptic complaints; Improvement of hepatic and biliary function and in dyspeptic complaints	2 g, 1-2 times/daily	1990, Poland, TU
Decoction (External use)	Adjuvant therapy in rheumatic conditions and peripheral circulatory disorders. Adjuvant therapy in rheumatic conditions, myalgia and peripheral circulatory disorders	1 l of decoction (1:20) added to bath water (twice weekly)	1990, Poland, TU
fluid extract (1:17.5-18.9), extraction solvent: liqueur wine	Traditional use to support the cardiac and circulatory function.	2-3 times/daily 20 ml; 100 g liquid contain 94.816 g extract; 700 ml = 721 g liquid 2-3 times/daily 10 ml	1976, Germany, TU
fluid extract (1:12.5-13.5), extraction solvent: liqueur wine		1-2 time/daily 20 ml	
Expressed juice (1:1.8-2.2) - Rosemary herba recens		2-3 time/daily, 5 ml containing 100% expressed juice	

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable.

Information on other products marketed in the EU/EEA (where relevant)

Not applicable.

2.1.2. Information on products on the market outside the EU/EEA

Not applicable.

2.2. Information on documented medicinal use and historical data from literature

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 (Morton, 1977).

It was recognised for its medicinal and cosmetic properties in ancient Greece and by the Romans, where it was used as a tonic, stimulant, and carminative for dyspepsia, headache, and nervous tension, and also to strengthen the memory. In the middle ages, Rosemary oil was distilled for medical purposes and the alcoholic distillate was probably the first popular perfume (Puerto, 2005).

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).

In different regions of the world, the use varies. In the Indian Materia Medica (Nadkarni's, 1999), rosemary oil it is described to have a carminative and stimulant action.

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 and an abortive agent in Brazilian folk medicine. It is traditionally referred to as an emmenagogue and is generally avoided during pregnancy (Lemonica et al., 1996). It is claimed to stimulate bile. Rosemary is said to prevent baldness when used as a hair tonic (de Oliveira et al., 2019).

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 et al., 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, 1994).

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 United States 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 2008a, EFSA 2008b, citing Opdyke, 1974).

A summary of historical data on the use of *Rosmarinus officinalis* L. (rosemary leaf or rosemary oil) is included in Table 2.

Table 2: Overview of historical data

Herbal preparation	**Documented use / Traditional use**	**Pharmaceutical form** **Strength** (where relevant) **Posology** **Duration of use**	**Reference**
Rosemary essential oil			
Essential oil	Cutaneous use Antiseptic and wound healing	Alcoholic solution 2% V/V	Ph. Fr., 1980

Essential oil	Cutaneous use Adjuvant therapy in rheumatic conditions and in peripheral circulatory disorders. Promotion of wound healing and as a mild antiseptic	Essential oil (2% V/V) in ethanol, as an antiseptic	ESCOP, 1997 citing: Stahl-Biskup, 1994; Bisset, 1994; Paris, 1971; Hänsel, 1991; Velasco Negueruela et al, 1992; del Rio Hijas, 1992; Rulffs, 1984
Essential oil	Dyspeptic complaints	10-20 drops essential oil	Blumenthal et al., 1998
	External use Supportive treatment for rheumatic diseases; Circulatory problems	6-10% essential oil in semi-solid and liquid preparations	
Rosemary leaf			
Herbal substance for Infusion or decoction	Spasmolytic	5 to 10 g/l; Infusion for 15 m. Decoction for 30 min 200 to 400 ml/day	Ph. Fr., 1980
Liquid extract		3 to 5 g/day	
Dried leaves and twigs	Flatulent dyspepsia associated with pathogenic tension. Headaches, migranous or hypertensive. Topically: mylagia, sciatica, intercostal myalgia	2-4 g or by infusion, 3 times/daily 2-4 ml of liquid extract 1:1 in 45% alcohol, 3 times/daily	British Herbal Pharmacopoeia, 1983
Dried flowering tops	Improvement of hepatic and biliary function and in dyspeptic complaints.	Infusion: 2-4 g/daily Fluid extracts (1:1, 45% ethanol v/v): 1.5-3 ml/daily Tincture (1:5, 70% ethanol): 3-8.5 ml/daily	ESCOP, 1997, citing: citing: Stahl-Biskup, 1994; Bisset, 1994; Paris, 1971; Hänsel, 1991; Weiβ, 1991
	Cutaneous use Adjuvant therapy in rheumatic conditions and in peripheral circulatory disorders. Promotion of wound healing and as a mild antiseptic.	Ethanolic extract (1:20) 1 l of decoction (1:20) added to bath water (twice weekly)	
Herbal substance	Dyspeptic complaints	4-6 g drug	Blumenthal et al., 1998
	Cutaneous use Supportive treatment for rheumatic diseases; Circulatory problems	50 g to a full bath	

Assessor's comments during the 5 years revision

While the Posology for the essential oil is 10-20 drops in the Commission E Monograph (1998), the Ph. Fr. posology was only 3-4 drops. Nevertheless, this posology has been deleted from the current use. Also the reference from the British Herbal Pharmacopoeia refers to the oral use of Rosemary herbal substance and not the pure essential oil.

In summary, as there is no real bibliographic reference confirming the posology for the oral use and there are no marketed products containing rosemary essential oil.

2.3. Overall conclusions on medicinal use

A summary of evidence on period of medicinal use of *Rosmarinus officinalis* L. (rosemary leaf or rosemary oil) is included in Table 3.

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Essential oil			
Rosemary oil	For the symptomatic treatment of muscle and joint pain and in circulatory disturbance.	Cutaneous use Ointment 6 g rosemary oil/100 g ointment; approximately 3 cm of ointment 2-3 times/daily	1976, DE
Rosemary oil	Minor muscular and articular pain and minor peripheral circulatory disorders	Cutaneous use 10% rosemary oil 3-6 cm of cream 2-3 times/daily Adults Duration: 4 weeks	2016, AT, CZ, ES, HR, HU, PL, SE, SI, SK, UK
Rosemary leaf			
Comminuted herbal substance	Improvement of digestion	Oral use 1-2 g/250 ml, 2-3 times/daily (2-6 g/day) as infusion	1976, ES
Comminuted herbal substance	Dyspeptic complaints Improvement of hepatic and biliary function and in dyspeptic complaints	Oral use 2 g, 1-2 times/daily as infusion	1990, PL
Comminuted herbal substance	Adjuvant therapy in rheumatic conditions and peripheral circulatory disorders. Adjuvant therapy in rheumatic conditions, myalgia and peripheral circulatory disorders.	As bath additive 1 l of decoction (1:20) added to bath water (2 times/weekly)	1990, PL

Comminuted herbal substance	Spasmolytic	Oral use 5 to 10 g/l; Infusion for 15 min. Decoction for 30 min 200 to 400 ml/daily	1980, Ph. Fr.
Dried leaves	Flatulent dyspepsia associated with pathogenic tension. Headaches, migranous or hypertensive.	Oral use 2-4 g or by infusion, 3 times/daily 2-4 ml of liquid extract 1:1 in 45% alcohol, 3 times/daily	British Herbal Pharmacopoeia, 1983

Historical data and documented period of use in the EU support the evidences of traditional use of both Rosemary essential oil and leaf for:

- Oral administration for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract: 1-2 g of comminuted herbal substance as a tea preparation in 250 ml boiling water, 2-3 times/daily; 2-4 ml liquid extract (DER 1:1), extraction solvent ethanol 45% V/V.
- Cutaneous use and use as a bath additive for the relief of minor muscular and articular pain and in minor peripheral circulatory disorders: cutaneous use of preparations containing 6-10% essential oil in semi-solid dosage forms, 2-3 times/daily; *50 g either as decoction (in 1 L of boiling water) or direct in the bath. Usage should be 2-3 times/weekly, if necessary also daily*.

The uses as "auxiliary treatment in conditions of exhaustion", "to support the cardiac and circulatory function" and "for depressive states with general debility" and indications of "cardiovascular weakness" cannot be accepted for traditional use as they are not suitable for use without the need for medical diagnosis, prescription and supervision. The topical use of the liquid extract cannot be accepted for traditional use as no data regarding the preparation and dosage for this indication are found. The cutaneous use of rosemary oil as antiseptic and wound healing based in the French Pharmacopoeia is not considered as this reference has been deleted and not in use anymore.

The use "to support the function of the skin" cannot be considered as a therapeutic indication.

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

- **Spasmolytic/relaxant activity**

Ex vivo studies

Rosemary leaf extracts

A study was performed to test the antispasmodic activity of 2.5 and 10.0 ml/l of alcoholic extracts (not further specified) 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).

Essential oil

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%), could 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).

Isolated compounds

The spasmolytic activity (against $BaCl_2$ 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 spasmogen agents studied (Cabo et al., 1986).

Aqueous extract

The potential effects of an aqueous extract of the leaves of *Rosmarinus 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).

Essential oil

The effects of the volatile oil of *Rosmarinus 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 Ca^{2+} free solution. This result suggests possible calcium antagonistic properties of rosemary oil (Aqel, 1991).

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 x 10-3 M), 112 nl/ml bornyl acetate (5.7 x 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 x 10-4 M), 400 nl/ml bornyl acetate (2 x 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 Ca^{2+} 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).

3.1.2. Secondary pharmacodynamics

***In vitro* studies**

As a general activity, the **antioxidant** potential of rosemary extract, essential oil and isolated compounds has been assessed.

- **Antioxidant effect**

In a number of studies antioxidant activity of rosemary leaf extracts and rosemary essential oil with different conditions and test models could be shown (Tateo et al., 1988; Schwarz et al., 1992; Svoboda, 1992; Fang and Wada, 1993; Wada and Fang, 1994; Guerreiro and Cunha, 1994; Dorman et al., 2003; Moreno et al., 2006; Kuhlmann et al., 2006; Yesil-Celiktas et al., 2007; Wang et al., 2008; Abuashwashi et al., 2017). This activity was attributed to several different components such as carnosic acid, carnosol, rosmanol, carnosol and epirosmanol and rosmarinic acid (Haraguchi et al., 1995; Kuhlmann et al., 2006).

- **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 macrorrhisum* and *Urtica dioica* grown in Bulgaria. It was concentration and time of incubation dependent.

- **Chemopreventive effect**

Some of the molecular mechanisms involved in the chemo-preventive action were investigated using *in vitro* human liver and bronchial cell models. Rosemary extract and some of its active components, carnosol and carnosic acid, are potent inhibitors of DNA adduct formation induced by benzo(α)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).

- **Immunological effects**

Rosmarinic acid

In several test systems 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 (Kimura and Okuda, 1987; Al-Sereiti et al., 1999), increased the production of prostaglandin E_2 and inhibited the complement system (Al-Sereiti et al., 1999; Bult et al., 1985).

- **Renal activity**

An *in vitro* study suggested that rosmarinic acid may prevent mesangial cell proliferation. Murine mesangial cells were isolated from mice glomeruli and incubated. Quiescent cells were stimulated for 24 hours with platelet-derived growth factor (PDGF) or tumour necrosis factor-alpha (TNFα) 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 reduced the basal deoxyribonucleic acid (DNA) synthesis and inhibited PDGF- and TNFα-induced DNA synthesis. 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 (Makino et al., 2000).

- **Anti-viral effect**

Some rosemary extracts as well as fractions and the isolated compounds (carnosic acid and carnosol) showed inhibitory effects in virus replication with and without significant signs of cytotoxicity (Romero et al., 1989; Paris et al., 1993).

- **Antimicrobial, fungicide and insecticidal action**

Rosemary extracts

Methanol and aqueous extracts, as well as carnosic acid, carnosol, ursolic acid and rosmarinic acid, respectively were found to show antimicrobial activity against Gram positive bacteria , Gram negative bacteria and yeast. 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 (Collins and Charles, 1987; Moreno et al., 2006).

Essential oil

Essential oils from *Rosmarinus officinalis* L*. were evaluated on its* antimicrobial and fungicide activities by several authors. It was seen active against a broad spectrum of microorganisms and also insecticidal activity at different concentrations (Benjilali et al., 1986; Héthelyi et al., 1989; Konstantopoulou et al., 1992; Panizzi et al., 1993; Boatto et al., 1994). Larrondo and Calvo (1991) could not detect activity against *Candida albicans*.

In vivo studies

- **Antimutagenic and hepatoprotective effect**

Rosemary extracts

Administration of rosemary ethanolic extract (0.15 g/100 g body weight) (no further information) to rats for 3 weeks produced a hepatoprotective effect, using carbon tetrachloride and cyclophosphamide as mutagenic and hepatotoxic compounds. 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).

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 homogenised. 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 peroxidability 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).

An extract of rosemary (no further information) 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 study was performed to evaluate the hepatoprotective effect of the aqueous extracts from the whole plant and young sprouts. The authors concluded that the extract obtained from young sprouts exerted an hepatoprotective activity in rat when administered orally at 1000, 150O and 2000 mg/Kg (Fleurentin et al., 1986).

Tert-butyl hydroperoxide induces in freshly isolated rat hepatocytes malonaldehyde formation and lactic dehydrogenase and aspartate aminotransferase leakage. The results showed the antihepatotoxic action of an extract of *Rosmarinus officinalis* young sprouts (no further information) on carbon tetrachloride-induced toxicity in rats (Joyeux et al., 1990).

Essential oil

The protective effect of the oral administration of the isolated essential oil after CCl_4-induced injury was tested in rats. The administration of 5 and 10mg/kg BW for 7 days showed a hepatoprotective effect. Interestingly, pre-treatment with the studied essential oil for 7 days significantly reversed the activities of antioxidant enzymes catalase, peroxidase, glutathione peroxidase and glutathione reductase in liver homogenates, especially in the dose of 10 mg/kg (Rašković et al., 2014).

Isolated compounds

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).

- **Choleretic and diuretic activity**

Rosemary extracts

A study was performed to evaluate the influence of rosemary and compare its different parts on the rat bile secretion. The authors conclude that Rosemary presents choleretic and diuretic activity. The new sprouts are choleretic 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 other parts of the plant stimulate the biliary flux (Fleurentin et al., 1986).

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

An aqueous alcoholic extract (15 %) of *Rosmarinus officinalis* L. 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 choleretic 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).

Essential oil

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).

- **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 sulfhydryl 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**

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

- **Hypoglycaemic effect**

Some reviews summarise the positive effect in lipidic and glycaemic profiles of essential oil, leaf extracts and leaf (Hasani et al., 2016; Andrade et al., 2018; de Oliveira et al, 2019; Bao et al., 2020).

A leaf extract was obtained from the fresh leaves (5 g) in 50 ml of boiled water with 1 h of stirring at room temperature. Then, the supernatant was decanted, and the residue was macerated for two more days with distilled water. The pooled supernatants were combined and filtered. Diabetic rats received 200 mg of the rosemary extract/kg BW for 21 days. An hypoglycaemic effect, together with restoration of elevated liver enzymes function close to normal levels was observed (Ramadan et al., 2013).

In a study on normo- and hyperglycaemic mice, the effect of a hot infusion of *Rosmarinus officinalis* L. 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 given orally 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, *Rosmarinus officinalis* L. 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 *Rosmarinus officinalis* L. has hyperglycaemic and insulin inhibitory effects in rabbits (Al-Hader et al., 1994).

- **Immunological effect**

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 butylated hydroxytoluene (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 immunopotentiation 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**

A study was performed to evaluate the activity of rosemary extract (no further information), carnosol and ursolic acid in inhibiting the *in vivo* formation of mammary 7, 12-dimethylbenz[α]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).

A methanol extract of the leaves of *Rosmarinus officinalis* L. (no further information) was evaluated for its effects on promotion and initiation of mouse skin tumour. (Ho et al., 1994). According to the authors studies of the effects of 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. 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).

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).

- **Anticonvulsant activity**

Effects of the aqueous extract of leaves and stems of *Rosmarinus officinalis* L. on the Picrotoxon-induced seizures in mice was performed. The mortality rate, onset of convulsion and gamma aminobutyric acid (GABA) content were monitored. The extract was found to delay the onset of picrotoxin-induced seizures and to decrease the mortality rate (Abdul-Ghani et al., 1987).

- **Antinociceptive activity**

The effect of the aqueous and ethanol extracts of *Rosmarinus officinalis* L. 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 pre-treatment. Phytochemical study indicated that only the aqueous extract of *Rosmarinus officinalis* L. has an alkaloid component. The authors concluded that the aqueous and ethanol extracts of *Rosmarinus officinalis* L. aerial parts could diminish morphine withdrawal syndrome (Hosseinzadeh and Nourbakhsh, 2003).

Other studies

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 O_2 consumption and the loss of electrolytic gradient of Na^+ and K^+ (Steinmetz et al., 1987).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

None reported.

3.1.5. Conclusions

The data obtained with some herbal preparations and pure secondary metabolites show spasmolytic effects *ex-vivo* and *in-vitro*, respectively. The data available allow only very limited conclusions on the plausibility of the therapeutic effects of the traditional use preparations of the monograph.

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

There are no data available on the pharmacokinetics of Rosemary preparations. Just some aspects of the pharmacokinetics of rosmarinic acid, rosemary oil and camphor are known, depending on the preparation used.

Isolated compounds

- Rosmarinic acid

In *ex vivo* experiments, permeation of rosmarinic acid across excised rat skin was about 8 times higher from alcoholic solution than from water. After topical application, rosmarinic acid 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.) is rapidly eliminated from the circulation (i.v. $t_{1/2}$=9 min) and has a low toxicity (LD_{50} 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).

There are no data on the transfer into human milk.

- **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) by different Rosemary extracts (Barceloux, 2008; Debersac et al., 2001).

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

3.3.1. Single dose toxicity

In a study to evaluate the acute toxicity in Wistar rats, two representative rosemary leaf extracts obtained by supercritical fluid extraction followed by fractionation 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).

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

Rosemary extract (no further information) showed no mortality at intragastric doses up to 1.2 g/100 g of BW in rats, classified as a very low lethality (Fahim et al., 1999).

- Essential oil

Essential oil of rosemary had a lethal dose 50 (LD_{50}) 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).

3.3.2. Repeat dose toxicity

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 no-observed-adverse-effect level (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).

3.3.3. Genotoxicity

- Rosemary extract, carnosic acid and carnosol

No guideline conform tests are available for rosemary extracts or the essential oil from rosemary.

A rosemary extract (no further information) with carnosic acid and carnosol as the two major 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; no further information) 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 dl-camphor in strains TA 97a, TA 98, TA 100 and TA 102 with and without metabolic activation (Gomes-Carneiro et al., 1998).

3.3.4. Carcinogenicity

- 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 (Stoner et al., 1973).

3.3.5. Reproductive and developmental toxicity

- Aqueous extract

An aqueous extract of *Rosmarinus officinalis* L. was given to pregnant rats during the preimplantation period (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 (pre-implantation 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 16α-hydroxylation of estradiol was inhibited by 50%. It also stimulated the liver microsomal glucoronidation of estradiol by 54-67% and estrone by 37-56%. In ovariectomized CD-1 mice, it inhibited the uterotropic action of estradiol and estrone by 30-50% compared with the group control (Zhu et al., 1998).

- Ethanolic extract

Nusier et al. (2007) performed 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.

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 aqueous extract did not interfere with normal foetal development after implantation in rats. Mated female rats were randomly assigned to groups and treated either during the pre-implantation or post-implantation 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 post-implantation loss was the same in both groups (Lemonica et al., 1996).

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

None reported.

3.3.8. Conclusions

Acute toxicity by different rosemary extracts was not observed in animal tests.

Teratogenicity data on rosemary oil is not available. 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. Nevertheless, it is use is not recommended during pregnancy.

Although some toxicological data exist for preparations from *R. officinalis*, for most extracts listed in the monograph no toxicological data are available.
Tests on genotoxicity, reproductive toxicity and carcinogenicity have not been performed for the preparations listed in the monograph.

3.4. Overall conclusions on non-clinical data

Results from *in vitro* and *in vivo* experimental studies on Rosemary leaf and isolated constituents to support the proposed indications are very limited. Nonetheless, the reported pharmacological effects, mainly as spasmolytic/ relaxant activity may be in line with the oral use of rosemary leaf preparations for the symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.

Specific data on pharmacokinetics and interactions are not available.
Non-clinical information on the safety of *Rosmarinus officinalis* L. leaf and essential oil is scarce.
There is scarce and incomplete information on reproductive and developmental toxicity, thus the use during pregnancy and lactation cannot be recommended.

Oral, cutaneous use and use as a bath additive administration of preparations from *Rosmarinus officinalis* L. folium can be regarded as safe at traditionally used doses. *Rosmarinus officinalis* L. aetheroleum is intended for cutaneous use only.

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed.

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 electroencephalogram (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

The effectiveness of a hydrophilic fraction (Rosm1) from an alcoholic extract of rosemary 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 lipoperoxidation chain reactions than did lipids from the controls. The authors suggested that the hydrophilic rosemary extract may be an important natural antioxidant for the skin (Calabrese et al., 2000).

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

None reported.

4.2. Clinical efficacy

4.2.1. Dose response studies

None reported.

4.2.2. Clinical studies (case studies and clinical trials)

Cutaneous use

The study performed by Panahi et al. (2015) revealed positive effects for the cutaneous use of the essential oil in the treatment of androgenetic alopecia (AGA).This study investigated the clinical efficacy of rosemary oil in the treatment of AGA and compared its effects with minoxidil 2%. It was a randomized study including a rosemary oil group (n=50) or minoxidil 2% group (n=50) for a period of 6 months. After a baseline visit, patients returned to the clinic for efficacy and safety evaluations every 3 months. A standardized professional microphotographic assessment of each volunteer was taken at the initial interview and after 3 and 6 months of the trial. No significant changes were obtained after 3 months, but both groups experienced a significant increase in hair count at the 6-month endpoint compared with the baseline and 3-month endpoint ($p<.05$). Scalp itching was more frequent in the minoxidil group at both assessed endpoints ($p<.05$). According to the authors, these findings could be in favour of the efficacy of rosemary oil in the treatment of AGA. Nevertheless, no clinical relevance can be derived from the study, due to several limitations such as the sample size or the lack of use of a validated score.

The review by Dhariwala and Ravikumar (2019) pointed to caffeic acid, 1, 8-cineole, and rosmarinic acid as potential therapeutic agents in rosemary oil. The observed effects on AGA may be due to the improvement shown in blood circulation and vascularity, this helping the regeneration of follicles.

Two other clinical studies have been performed with rosemary preparations in other therapeutic areas.

The study by Nematolahi et al., (2018) was a randomized double-blind study performed in healthy students at University level (20-25 years of age, n=68) to assess the effect of rosemary powdered leaf (500mg, oral administration, 1 month) on memory performance, anxiety, depression and sleep quality. The main outcomes were a significant effect on memory performance, decreasing anxiety and depression and improving sleep quality. Although a statistical analysis based on different tests was performed, there is no clinical relevance for the results due to several limitations of the study such as the sample size, the short duration, the use of only one dose or the scales used for the depression score.

The randomized trial conducted by Moss et al. (2018) was aimed to assess the acute effect on cognition and cerebrovascular levels of one daily dose of 250 ml rosemary water versus mineral water. 80 healthy volunteers were recruited who completed a series of cognitive tasks, followed by subjective measures of alertness and fatigue. Near-infrared spectroscopy monitored levels of total, oxygenated and deoxygenated haemoglobin at baseline and throughout the cognitive testing procedure. An increase of deoxygenated haemoglobin level during test performance was found. Also, a statistically significant, small, beneficial effects of rosemary water on cognition was assessed. Nevertheless, this study was not conducted with a medicinal product; the clinical relevance of these results is unclear due to several limitations of the study, such as the sample size, the short duration or the use of only one dose of the above cited rosemary water.

A summary of clinical studies on humans, in dermatological area, with *Rosmarinus officinalis* L. (rosemary leaf or rosemary oil) is included in Table 4.

Table 4: Clinical studies on humans, in dermatological area

Type	**Study**	**Test Product(s)**	**Number of subjects**	**Type of subjects**	**Outcomes**	**Statistical analysis**	**Clinical relevance**
Dhariwala et Ravikumar, 2019	Review on androgenetic alopecia	Essential oil (cutaneous use) Leaf (oral use): 300-1400 mg/daily	-	Androgenetic alopecia	positive effects	-	
Randomized, comparative trial Panahi et al., 2015	Androgenetic alopecia: (through validated	Essential oil (cutaneous use)	N=100 (50 in each group)	Androgenetic alopecia	hair count at the 3- and 6-month endpoint frequencies of dry and greasy hair and dandruff frequency of scalp itching at the 3- and 6-month trial points Similar efficacy than minoxidil after 6-months' treatment	Percentage of satisfaction with treatment showed marginally significant difference favouring the rosemary group over the 2% topical minoxidil group. The proportion score for hair loss decrease for rosemary was significantly superior to 2% topical minoxidil group at months 3 and 6 with regard to decrease in hair loss ($p<0.05$). Hair growth was equally increased in both groups during the study according to the patients' answers ($p>0.05$)	No clinical relevance due to several study limitations

A summary of clinical studies on humans, in other therapeutic areas, with *Rosmarinus officinalis* L. (rosemary leaf or rosemary oil) is included in Table 5.

Table 5: Clinical studies on humans, in other therapeutic areas

Type	**Study**	**Test Product(s)**	**Number of subjects**	**Type of subjects**	**Outcomes**	**Statistical analysis**	**Clinical relevance**
Randomized double-blind (placebo group, starch) Nematolahi et al., 2018	Effect on memory performance, anxiety, depression and sleep quality (through validated corresponding questionnaires)	Powdered leaf: 500 mg of dried powdered aerial parts 1 month	n=68	Healthy volunteers (University students) (20-25 y')	Significant effect on enhancing memory performance, decreasing anxiety and depression and improving sleep quality (no significant changes in sleep latency and duration). Side effects: diuretic, increased libido and appetite	Normal distribution based on Kolmogorov-Smirnov test; two-tailed t-test and chi-square (or Fisher's exact test) (differences in demographic data and grades of anxiety, depression and sleep quality). Mixed model analysis of variance and paired sample t-test (changes over time).	No clinical relevance due to many limitations: small sample size, short duration, only one dose
Randomized trial Moss et al., 2018	Acute effect on cognition and cerebrovascular levels	Verum: 250 ml rosemary water* Placebo: mineral water	N= 80	Healthy volunteers	Volunteers completed a series of computerised cognitive tasks, followed by subjective measures of alertness and fatigue. Near-infrared spectroscopy monitored levels of total, oxygenated and	G*Power was used to calculate the required sample size for a global multivariate analysis of variance (MANOVA) effect with two groups and 21 outcome variables for a study with an alpha level set at 0.05, power of 0.8	No clinical relevance

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
					deoxygenated haemoglobin at baseline and throughout the cognitive testing procedure. Positive effects An increase of deoxygenated haemoglobin level during test performance Statistically significant, small, beneficial effects of rosemary water on cognition	and effect size f 2=0.3.	

- `Shot strength rosemary water was supplied in 250 mL bottles. The water contains a hydrolat containing volatile compounds produced by steam distillation, and an extract containing botanical soluble compounds infused into the solvent (water and ethanol) of fresh rosemary sourced from Campania, Italy. No other ingredients are added to the product. Production and analysis of the batch hydrolat and extract used in the production of the treatments employed here was undertaken by. The two elements have different constituent profiles with the extract containing number of terpenes predominantly 1,8-cineole (0.025 mg/mL), and also rosmarinic acid (0.13 mg/mL). The hydrolat contains substantially lower levels of terpenes including 1,8-cineole (0.012 mg/mL), no rosmarinic acid, but quinic acid and glucosamine-like compounds were detectable.

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

None reported.

4.4. Overall conclusions on clinical pharmacology and efficacy

No clinical studies for the indications of the monographs could be found.

One clinical study showed that rosemary essential oil might provide evidence with respect to the efficacy of rosemary oil for the treatment of alopecia. Nevertheless, this study is not well designed and cannot be considered sufficient to support therapeutic indications, i.e. due to the small sample size. Furthermore, there are no products in the market with that indication.

5. Clinical Safety/Pharmacovigilance

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

There are no clinical safety data available from clinical trials.

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 (Wichtl, 1994).

5.2. Patient exposure

Aside from market presence and data from studies, there are no concrete data concerning patient exposure.

5.3. Adverse events, serious adverse events and deaths

- Rosemary extracts

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).

- Carnosol

A case of contact dermatitis to carnosol, , 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. The 226 controls were negative (Hjorther 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 et al., 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).

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 Pediatrics 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 (American Academy of Pediatrics, 1978).

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

No data available.

5.5.1. Use in children and adolescents

The use in children under 12 years of age is not recommended (see section 'Special warnings and precautions for use').

5.5.2. Contraindications

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

Essential oil
Cutaneous use:

Do not apply to broken or irritated skin.

Rosemary leaf

Use as bath additive:

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

5.5.3. Special warnings and precautions for use

Essential oil

Cutaneous use:

The use in children and adolescents under 18 years of age is not recommended due to lack of adequate data.

Articular pain accompanied by swelling of joint, redness or fever should be examined by a doctor.

If there is inflammation of the skin or subcutaneous induration, ulcers, sudden swelling of one or both legs particularly associated with redness and heat, cardiac or renal insufficiency, or a sudden sharp pain in the leg when at rest, a doctor should be consulted.

Contact with eyes should be avoided. Semi solid form should not be applied near mucous membranes.

Rosemary leaf

The use in children under 12 years of age is not recommended due to lack of adequate data.

Oral use:

Obstruction of bile duct, cholangitis, liver disease, gallstones and any other biliary disorders that require medical supervision and advice.

Use as bath additive:

Articular pain accompanied by swelling of joint, redness or fever should be examined by a doctor.

If there is inflammation of the skin or subcutaneous induration, ulcers, sudden swelling of one or both legs particularly associated with redness and heat, cardiac or renal insufficiency, or a sudden sharp pain in the leg when at rest, a doctor should be consulted.

In cases of hypertension, a full hot bath should be used with caution.

5.5.4. Drug interactions and other forms of interaction

There are no data available.

5.5.5. Fertility, pregnancy and lactation

The data available are not sufficient to conclude the safety or the danger of rosemary preparations used during pregnancy and lactation. Nevertheless, therapeutic doses are not recommended for use during pregnancy due to the lack of data for some components (McGuffin et al., 1997; Wichtl, 1994).

Regarding 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).

The safety during pregnancy and lactation has not been established and thus rosemary preparations are not recommended.

5.5.6. Overdose

None reported.

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

None reported.

5.5.8. Safety in other special situations

Not applicable.

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.

Due to the lack of data, use is not recommended 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, a special warning is included for rosemary leaf preparations in cases of obstruction of the bile duct, cholangitis, liver disease, gallstones and any other biliary disorders that require medical supervision.

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 (benefit-risk assessment)

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

The pharmacological studies reported in the literature, together with the long use of different preparations in the EU, give plausibility to the traditional indications set out in the monographs:

Rosmarinus officinalis L., folium

- Oral use
  Traditional herbal medicinal product for the symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.
- Use as bath additive
  Traditional herbal medicinal product for the relief of minor muscular and articular pain and in minor peripheral circulatory disorders.

Rosmarinus officinalis L., aetheroleum

- Cutaneous use
  Traditional herbal medicinal product for the relief of minor muscular and articular pain and in minor peripheral circulatory disorders.

The oral use of rosemary oil does not fulfil the Specific provisions applicable to traditional herbal medicinal products as laid down in article 16a 1(e) the data on the traditional use of the medicinal product are sufficient; in particular the product proves not to be harmful in the specified conditions of use and the pharmacological effects or efficacy of the medicinal product are plausible on the basis of long-standing use and experience. In particular, the originally included reference to the oral use of the essential oil was misunderstood and there is no real bibliographic reference to support its oral use; moreover, there is no proof of the existence of marketed products containing this essential oil for oral use in the EU.

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.

A European Union list entry is not supported due to lack of adequate data on genotoxicity.

As the minimum required data on mutagenicity (Ames' test) are not available for herbal preparations of rosemary leaf and rosemary oil, a European Union list entry cannot be supported due to lack of adequate data.

Annex

List of references