Safety Assessment of *Rosmarinus Officinalis* (Rosemary)-Derived Ingredients as Used in Cosmetics

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All interested persons are provided 60 days from the above release date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. Lillian J. Gill.

The 2013 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A.
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TABLE OF CONTENTS

ABSTRACT

The Expert Panel assessed the safety of 10 Rosmarinus officinalis (rosemary)-derived ingredients and concluded Rosmarinus Officinalis (Rosemary) Leaf Extract is safe at ≤0.2% in leave-on products and safe as used in rinse-off products and that the data are insufficient to support the safety of Rosmarinus Officinalis (Rosemary) Flower Extract as used in cosmetics. The other eight ingredients are safe as used in cosmetics. These ingredients are most frequently reported to function in cosmetics as skin conditioning agents or as fragrance ingredients. The Panel reviewed the available animal and clinical data to determine the safety of these ingredients. Because formulations may contain more than one botanical ingredient, caution was urged to avoid reaching levels of toxicity for constituents. Industry should use good manufacturing practices to limit impurities.

INTRODUCTION

This report reviews the use and safety data of the following 10 *Rosmarinus officinalis* (rosemary)-derived ingredients as used in cosmetics:

Rosmarinus Officinalis (Rosemary) Extract
Rosmarinus Officinalis (Rosemary) Flower Extract
Rosmarinus Officinalis (Rosemary) Flower/Leaf Stem Extract
Rosmarinus Officinalis (Rosemary) Flower/Leaf/Stem Water
Rosmarinus Officinalis (Rosemary) Leaf
Rosmarinus Officinalis (Rosemary) Leaf Extract
Rosmarinus Officinalis (Rosemary) Leaf Oil
Rosmarinus Officinalis (Rosemary) Leaf Powder
Rosmarinus Officinalis (Rosemary) Leaf Water
Rosmarinus Officinalis (Rosemary) Water

Most of the ingredients included in this review are extracts, oils, powders, or solutions derived from a defined part of the *Rosmarinus officinalis* (rosemary) plant.

While *Rosmarinus officinalis* (rosemary)-derived ingredients are reported to have a number of functions, the most common functions in cosmetics are as a skin conditioning agent or use as a fragrance ingredient.[1] Two of the ingredients, i.e., rosmarinus officinalis (rosemary) flower extract and rosmarinus officinalis (rosemary) leaf extract, are reported to function as antioxidants. Rosmarinus officinalis (rosemary) leaf powder is reported to function only as a flavoring agent.

Normally, the CIR does not review ingredients that only function as fragrance ingredients because, as fragrances, the safety of these ingredients is evaluated by the Research Institute for Fragrance Materials (RIFM). Three of the *Rosmarinus officinallis* (rosemary)-derived ingredients, namely, rosmarinus officinalis (rosemary) flower/leaf/stem water, rosmarinus officinalis (rosemary) leaf water, and rosmarinus officinalis (rosemary) water, function only as fragrance ingredients, according to the *International Cosmetic Ingredient Dictionary and Handbook*. The CIR is in the process of confirming with the RIFM that these ingredients are fragrance ingredients; if confirmed, these ingredients will be deleted from this safety assessment.

CHEMISTRY

Definition

The definition and chemical class of each *Rosmarinus officinalis* (rosemary)-derived ingredient included in this report are provided in Table 1. The definition indicates what part(s) of the plant from which the ingredient is obtained. In some cases, the definition also gives insight as to the method of manufacture.

General Characterization

The *Rosmarinus officinalis* L. plant, from the botanical family Lamiaceae, is a scented, evergreen shrub with a very pungent odor that is native to the Mediterranean region and Portugal; the odor is sometimes defined as camphor-like.[2,3] It has a spicy, harsh, bitter, aromatic taste. Bluish labiate flowers grow on the upper green part of the branches. The oil is produced mostly in Spain, France, and Tunisia.[4]

Rosmarinus officinalis L. is generally recognized as safe (GRAS) as a spice and other natural seasoning and flavoring. (21CFR182.10) Rosemary has traditional or folk medicine uses, some with reported side effects.[2,5,6] The flowering dried twig tips, the dried leaves, the fresh leaves, the fresh aerial parts, and the flowering branches are considered to be the medicinal parts.[5]

Chemical and Physical Properties

Rosmarinus officinalis (rosemary)-derived ingredients are strongly aromatic. Chemical and physical property data are provided in Table 2.

Preparation/Extraction

Food-grade rosmarinus officinalis (rosemary) extract is prepared by extraction from the leaves of *Rosmarinus officinalis*. Food-grade acetone, ethanol, hexane, or a combination of hexane and ethanol (in a two-step process) are used as extraction solvents; the extract can also be prepared from a deodorized or partially deodorized ethanol extract of rosemary.[7,8] Food-

grade rosmarinus officinalis (rosemary) extract may also be extracted using supercritical carbon dioxide (CO_2). Subsequent production steps include filtration, purification, solvent evaporation, drying, and sieving. The extract may be deodorized, decolorized, and standardized using diluents and carriers that are permitted in foods.

Supplier-provided data sheets report production of rosmarinus officinalis (rosemary) leaf extracts by supercritical fluid extraction with natural CO_2 and a small amount of ethanol as a solvent.[9-11] One supplier reported that the essential oil is removed by multistep separation.[11]

An additional method includes extraction with absolute ethanol (resulting in what has been called "an absolute") or a collection of the insoluble waxes (resulting in what has been called "a concrete").[12]

Food-grade rosmarinus officinalis (rosemary) leaf oil is the volatile oil obtained by steam distillation from the fresh flowering tops or dried crushed aerial parts of *Rosmarinus officinalis* L.[13] The oil from *Rosmarinus officinalis* is also obtained by hydrodistillation of dried crushed aerial parts.[14] Essential oils prepared by a steam distillation process yields two distinct fractions, a water-insoluble fraction and a water-soluble fraction.[1] The water-insoluble fraction contains the term oil in the name and the water-soluble fraction contains water in the name. So, rosmarinus officinalis (rosemary) leaf water is the water-soluble fraction of the steam distillation of *Rosmarinus officinalis* (rosemary) leaves.

One supplier reported their rosmarinus officinalis (rosemary) leaf oil is produced by supercritical fluid extraction with natural CO_2 and a small amount of ethanol.[15] This supplier adds a small amount (<4%) of sunflower oil to increase solubility when blending.

Constituents/Impurities

Rosmarinus officinalis L. is composed of an array of constituents, primarily phenolic acids, flavonoids, monoterpenes, diterpenes, diterpenoids, and triterpenes. Structures for some of the principal components according to chemical family are depicted in Figures 1-5.

A detailed list of chemical constituents by plant part is presented in Table 3, and a more focused listing of constituents of *Rosmarinus officinalis* is provided in Table 4. Table 5 provides composition data on three rosmarinus officinalis (rosemary) leaf extracts, based on certificates of analysis provided by suppliers of rosmarinus officinalis (rosemary) leaf extract; these certificates report a phenolic diterpenes content of 14 or 25%.[16-19]

According to the European Cosmetic Regulations, specific allergen compounds are subject to declaration on the label if the concentration of this substance exceeds 0.001% in leave-on and 0.01% in rinse-off products. One supplier reported, separately from the certificate of analysis, the following concentrations of allergen compounds in a rosmarinus officinalis (rosemary) leaf extract that needed to be declared: <0.1% linalool and <0.2% d-limonene.[20]

The principal antioxidative components of rosmarinus officinalis (rosemary) leaf extract are the phenolic diterpenes carnosol and carnosic acid.[8] The amount of carnosol and carnosic acid present in the extract varies with the method of extraction, with levels as low as 5-7% carnosol plus carnosic acid found in rosemary extract prepared from a partially deodorized ethanol extract of rosemary to as high as 30% carnosol plus carnosic acid in an extract prepared with supercritical carbon dioxide.[2,7]

Carnosol and carnosic acid are not the only constituents that vary with extraction method. Table 6 provides a sample of the differences in constituent profiles in rosemary leaves based on extraction method. Some of the studies summarized in this report provided information on the amount of constituents present in the test article; when this information was available, it is included.

In addition to extraction method, the actual amount of constituents present also varies according to the stage of development, variety of plant, season harvested, and origin of the leaves.[2,8,21,22] Water and light conditions also affect the amount of the constituents found in rosemary plants; for example, highly oxidized diterpenes increase in rosemary plants exposed to drought and high light stress.[23] Although it is generally accepted that the geographical region and stage of growth affects plant composition, some researchers reported that, within one country, the chemical composition of rosemary essential oil (plant parts not specified) did not vary with geographical region or harvest time.[24]

Food-grade rosmarinus officinalis (rosemary) leaf extract has acceptance criteria of not more than 3 mg/kg arsenic and 2 mg/kg lead, and not more than 8.0% loss on drying.[7] Food-grade rosemary leaf oil is to have not less than 8.0% borneol and not less than 1.5% esters, calculated as bornyl acetate.[13]

Table 7 provides toxicity and other information on some constituents of *Rosmarinus officinalis* (rosemary)-derived ingredients. Because formulations may contain more than one botanical ingredient, caution was urged to avoid reaching levels of toxicity for constituents. Industry should use good manufacturing practices to limit impurities.

USE

Cosmetic

The *Rosmarinus officinalis* (rosemary)-derived ingredients included in this safety assessment have a variety of functions in cosmetics. Most of the ingredients function as a skin conditioning agent and/or as a fragrance ingredient; rosmarinus officinalis (rosemary) leaf powder is reported to function only as a flavoring agent.[1] A listing of all the reported functions for each ingredient is provided in Table 1.

The Food and Drug Administration (FDA) collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). VCRP data obtained from the FDA[25] and data received in response to a survey of the maximum reported use concentration by category conducted by the Personal Care Products Council (Council)[26,27] in 2013 indicate that nine of the ten ingredients included in this safety assessment are currently used in cosmetic formulations. Rosmarinus officinalis (rosemary) leaf extract has the greatest number of uses, 689, followed by rosmarinus officinalis (rosemary) leaf oil, 516. According to the results of the concentration of use survey, most cosmetic formulations contain very low concentrations of the *Rosmarinus officinalis* (rosemary)-derived ingredients, often much less than 0.1%. However, rosmarinus officinalis (rosemary) leaf extract is reported to be used at up to 10% in body and hand products and 3% in eye shadow formulations and bath soaps and detergents. Rosmarinus officinalis (rosemary) flower/leaf/stem water is the only ingredient not reported to be used.

Frequency and concentration of use data categorized by exposure and duration of use are provided in Table 8. In some cases, reports of uses were received in the VCRP, but concentration of use data are not available. For example, rosmarinus officinalis (rosemary) flower extract is reported to be used in 36 cosmetic formulations, but no use concentration data were reported. Additionally, for rosmarinus officinalis (rosemary) flower/leaf/stem extract, no reported uses were received in the VCRP, but a use concentration was provided in the industry survey; it should be presumed there is at least one use in a deodorant formulation, the category for which the concentration of use was reported

Products containing rosmarinus officinalis (rosemary)-derived ingredients may be applied to baby skin (e.g., 0.012% rosmarinus officinalis (rosemary) leaf extract in baby lotion, oils and creams), used in products that could be incidentally ingested (e.g., 0.012% rosmarinus officinalis (rosemary) leaf in lipstick formulations), or used near the eye area or mucous membranes (e.g., up to 3% rosmarinus officinalis (rosemary) leaf extract in eye shadow formulations and in bath soaps and detergents).[26] Additionally, *Rosmarinus officinalis* (rosemary)-derived ingredients are used in cosmetic sprays and powders; for example, rosmarinus officinalis (rosemary) leaf extract is used in other fragrance preparations at up to 0.5% and rosmarinus officinalis (rosemary) extract is used in face powders at up to 0.05%. These products could possibly be inhaled. In practice, 95 to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm.[28-31] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[28,31] Rosmarinus officinalis (rosemary) leaf extract is used in aerosol deodorants at concentrations up to 0.012%. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[28] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

All of the ingredients named in this safety assessment are listed in the European Union inventory of cosmetic ingredients.[32]

Non-Cosmetic

Rosmarinus officinalis L. is GRAS as a spice and other natural seasoning and flavoring when the intended use is for human consumption (21CFR182.10) and for animal drugs, feed, and related products (21CFR582.10). It is also GRAS as an essential oil, oleoresin (solvent-free), and natural extractive (including distillates) for human consumption (21CFR182.20) and for animal drugs, feed, and related products (21CFR582.20). Rosemary oil can be used in the formulation of denatured alcohol and rum (27CFR21.65).

In *The Official Journal of the European Union*, extracts of rosemary contain several anti-oxidant compounds, and although the European Food Safety Authority (EFSA) was not able to establish an acceptable daily intake due to insufficient toxicological data, the EFSA considered the margin of safety was high enough to conclude that dietary exposure was not a concern.[33] Extracts of rosemary are allowed in various food products at amounts of 30-1000 mg/kg, expressed as the sum of carnosol and carnosic acid.

Rosemary leaves are used as a seasoning in cooking.[34] Rosmarinus officinalis (rosemary) leaf oil is used as a condiment and flavoring agent in food; as an antioxidant in edible oils, meats, and other fat-containing foods; and as a dietary supplement. Rosemary oil is reported to have antimicrobial activities.[4]

Rosemary is reported to have use as an anti-inflammatory, antioxidant, and anti-microbial agent.[21,35-37] Rosemary has traditional or folk medicine uses, some with reported side effects.[2,5,6] Rosemary has been used as an antispasmodic in renal colic and dysmenorrhea, and it has been used for relieving respiratory disorders. The essential oil is used internally as a carminative and as an appetite stimulant; however, large amount of the oil are reported to cause gastroenteritis and nephritis.

The essential oil is added to bath water as a circulation stimulant. As the oil or as an ointment, external application use is as an analgesic liniment for rheumatism. Rosemary is used as a poultice for poorly healing wounds and in the treatment of eczema. It is used in lotions to treat baldness,[14] and the leaves and branches have been used for treating headaches.[4]

TOXICOKINETICS

Penetration Enhancement

The effect of rosemary oil on the permeation of aminophylline was determined in human skin *in vivo* using attenuated total refection Fourier transform infrared (ATR-FTIR) spectroscopy.[38] Rosemary oil did enhance the permeation of aminophylline; however, the increase in permeation was less than that observed with 50% ethanol.

TOXICOLOGICAL STUDIES

Single Dose (Acute) Toxicity

Single-dose toxicity studies are summarized in Table 9.[8,22,39-41] The acute toxicity of *Rosmarinus officinalis* (rosemary)-derived ingredients is not very remarkable. The dermal LD_{50} of rosmarinus officinalis (rosemary) leaf oil is > 10 ml/kg.[41] The oral LD_{50} of rosmarinus officinalis (rosemary) leaves is >2 g/kg,[22] of rosmarinus officinalis (rosemary) leaf extract is >8.5 g/kg,[8] and of rosmarinus officinalis (rosemary) leaf oil is 5.5 g/kg bw.[40]

Repeated Dose Toxicity

Repeated-dose toxicity studies are summarized in Table 10.[8,40] A number of oral repeated-dose toxicity studies were performed in mice and in rats with rosmarinus officinalis (rosemary) leaves extracted in a number of solvents. Doses as high as 14.1 g/kg bw rosmarinus officinalis (rosemary) leaf extract were tested (5 days by gavage), and studies were performed for up to 3 mos (dietary). Increases in absolute and relative liver-to-body weights were observed in many of the studies, independent of the extraction method; these changes were shown to be reversible, and no other signs of toxicity were observed. Oral administration of rosmarinus officinalis (rosemary) leaf oil also affected liver weights.

Ocular Irritation

Rosemary oil is reported to be a moderate ocular irritant.[21] (Details not provided.)

Anti-Inflammatory Effects

Rosmarinus Officinalis (Rosemary) Leaf Extract

Rosmarinus officinalis (rosemary) leaf extract has been shown to inhibit formaldehyde-induced plantar edema and 12-tetradecanoylphorbol 13-acetate (TPA)-induced and arachidonic acid-induced ear edema.[42,43]

In the formaldehyde-induced plantar edema study, groups of six male Balb/C mice were given an injection of 20 µl of 3% formaldehyde into the sub-plantar region of both hind paws.[42] After 2 h, one hind paw was treated with 10 µl of 12 mg/ml of an ethanol extract of *Rosmarinus officinalis* (rosemary) leaves topically, as an injection, or both. The mice were killed after 24 h. Topical administration reduced edema by 80%, the injection reduced it by 22%, and the combined application reduced edema by 24%.

The TPA-induced ear edema study was conducted in groups of 10 male Balb/c mice.[42] The effect of pretreatment with 10-1000 µg/cm^2 of an ethanol extract of *Rosmarinus officinalis* (rosemary) leaves at 30 min prior to induction of inflammation with 25ng/cm^2 TPA was evaluated. The mice were killed after 4 h. Doses of 100, 250, 500, and 1000 µg/cm^2 of the extract resulted in a statistically significant reduction of inflammation by 38, 79, 84, and 99%, respectively.

In a TPA-induced mouse ear edema study conducted in groups of six to 10 female CD-1 mice, a single dose of 20 µl acetone, 0.5 nmol TPA, or TPA and 0.04, 0.12, or 0.36 mg of a methanol extract of *Rosmarinus officinalis* (rosemary) leaves in 20 µl acetone was applied to one ear of each mouse.[43] The mice were killed after 5 h, and rosmarinus officinalis (rosemary) leaf extract inhibited TPA-induced inflammation by 17, 75, and 92% respectively. The extract also inhibited TPA-induced erythema.

In the arachidonic acid-induced mouse ear edema study, 0.02, 0.09, or 0.45 mg of a methanol extract of *Rosmarinus officinalis* (rosemary) leaves in 20 µl acetone was applied to groups of 10 female CD-1 mice at 30 min prior to treatment with 0.3 mg arachidonic acid in 20 µl acetone. Inflammation was inhibited by 12, 28, and 54%, respectively.[43] The mice were killed after 1 h.

Effect on Epidermal Hyperplasia

The dorsal skin of three to four CD-1 mice per groups was treated with either 200 µl acetone, 1 nmol TPA, or 1 nmol TPA and 3.6 mg rosmarinus officinalis (rosemary) leaf extract in 200 µl acetone twice a day for 4 days.[43] Topical application of the extract with TPA inhibited a TPA-induced increase in the number of epidermal cell layers and epidermal thickness.

Immunologic Effects

An aq. extract of up to 2.5 mg/ml *Rosmarinus officinalis* (rosemary) leaves was found to inhibit ultraviolet (UV)-induced up-regulation of matrix metalloproteinase-1 (MMP-1) gene transcription in dermal human fibroblasts; the release of cytokines interleukin (IL)-1α and IL-6 was prevented by the extract.[44]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Non-Human

Rosmarinus Officinalis (Rosemary) Leaf Extract

Oral administration of rosmarinus officinalis (rosemary) leaf extract adversely affected fertility in male rats.[45] Groups of 10 male Sprague Dawley rats were fed a diet with 0, 250 or 500 mg/kg bw/day of an ethanol extract of *Rosmarinus officinalis* (rosemary) leaves in distilled water. After 53 days of dosing, each male rat was mated with two untreated female rats for 10 days; the female rats had been given a subcutaneous (s.c.) dose of 5.0 mg estradiol benzoate 54 h before and 0.5 mg progesterone 6 h before being placed with the males. The males were dosed during, and killed after, the 10-day mating period, and the reproductive organs were examined. The females were killed 1 wk after the mating period, and the reproductive tract of each female was examined to determine pregnancy and the number of implantation sites, viable fetuses, and fetal resorptions.

The body weights of male rats of the test groups were similar to those of controls. The absolute and relative organ to body weights of the testes, epididymides, seminal vesicles, ventral prostates, and vas deferens of the high dose animals were statistically significantly reduced compared to the controls. The sperm motility in cauda epididymides, sperm density, seminiferous tubule diameter, Leydig cell nuclear diameter, and epithelial height in epididymides and seminal vesicles were also statistically significantly reduced in the animals dosed with 500 mg/kg bw/day rosmarinus officinalis (rosemary) leaf extract. Also in the high-dose group rats, germinal cells (i.e., spermatogonia, primary and secondary spermatocytes, and spermatids) and interstitial cells (i.e., fibroblasts and immature and mature Leydig cells) were statistically significantly decreased, and degenerating cells were statistically significantly increased. Clinical chemistry parameters were also evaluated; testosterone, follicle-stimulating hormone, and luteinizing hormone levels were statistically significantly decreased in high-dose male rats. Exposure to 500 mg/kg bw rosmarinus officinalis (rosemary) leaf extract reduced fertility; the number of pregnant females was decreased in this group, there was a statistically significant decrease in the number of implantations and viable fetuses, and the total number of resorptions was statistically significantly increased. The same trends were generally found in the rats of the low-dose groups, but the changes did not reach statistical significance.

Rosmarinus Officinalis (Rosemary) Flower/Leaf/Stem Extract

A group of 12 gravid female Wistar rats was dosed by gavage with 26 mg/day of a 30% aq. extract of rosmarinus officinalis (rosemary) flower/leaf/stem extract (13 mg/ml solids) on days 1-6 of gestation (preimplantation), and a group of 14 gravid rats was dosed with the extract on days 6-15 of gestation (organogenesis).[46] Negative control groups of 12 or 11 gravid rats were given saline by gavage on days 1-6 or 6-15 of gestation, respectively. All dams were killed on day 21 of gestation. No signs of maternal toxicity were observed, and maternal weight gains were similar for treated and control groups.

In the rats dosed on days 1-6 of gestation, a non-statistically significant increase in preimplantation loss was observed. No changes in post-implantation loss were seen as compared to controls, and no other reproductive parameters were affected. In the group treated on days 6-15 of gestation, a non-statistically significant increase in post-implantation loss rate (2.54%) was reported; analysis of the resorptions found that they occurred during the early post-implantation period. No other changes in reproductive parameters were observed when compared to the negative control group. Developmental effects were not observed in either group.

Human

According to the *PDR for Herbal Medicines*, rosemary preparations should not be used as a drug during pregnancy; very large quantities of the leaves reportedly can be misused as an abortifacient.[5] According to *Herbal Drugs and Phytopharmaceuticals*, toxic side effects may occur with components of the essential oil.[47] (Details were not provided.)

Effects on Estrogenic Activity

Non-Human

Rosmarinus Officinalis (Rosemary) Leaf Extract

Groups of seven or eight 6-wk old ovariectomized CD-1 mice were fed a diet containing 2% of a methanol extract of *Rosmarinus officinalis* (rosemary) leaves or the basal diet.[48] After 3 wks, the animals were given an i.p. injection of 0, 45, or 100 ng/mouse estradiol or estrone in 50 µl corn oil, once daily for 3 days. Eighteen houra after the last injection, the animals were killed and the uterus was removed. In the mice fed the basal diet, estradiol and estrone increased the uterine wet weight in a dose-dependent manner. Rosemary inhibited 35-50% of the uterine response; this was statistically significant.

Human

Rosmarinus Officinalis (Rosemary) Leaf Extract

In a study investigating the effects of a botanical supplement on sex steroid hormones and metabolic markers in premenopausal women, a few changes were found, however, the changes were not very remarkable.[49] A group of 15 premenopausal women were asked to take a supplement containing 100 mg *Rosmarinus officinalis* (rosemary) leaf 5:1 extract; 100 mg *Curcurma longa* (turmeric) root extract standardized to 95% curcumin; 100 mg *Cyanara scolymus* (artichoke) leaf 6:1 extract; 100 mg *Silybum marinum* (milk thistle) seed extracted standardized to 80% silybin, silichristin, silidianin, and silymarin; 100 mg *Taraxacum officinalis* (dandelion) root 4:1 extract; and 50 mg *Schidandra chinensis* (berry) 20:1 extract. Four capsules were to be taken twice a day with meals. Rice powder placebo capsules were given to a group of 15 premenopausal women using the same dosing regimen. Blood and urine samples were collected during the early-follicular and mid-luteal phases of study menstrual cycles 1 and 5.

On average, test subjects took 6.3 capsules/day, and controls took 7.1 capsules/day. Compared to the placebo group, the following changes from Cycle 1 to Cycle 5 in early-follicular phase serum hormone concentrations were statistically significant or borderline significant: decreases in serum dehydroepiandrosterone (-13.2%, p= 0.02); dehydroepiandrosterone sulfate (-14.6%, p=0.07); androstenedione (-8.6%, p=0.05); and estrone sulfate (-12.0%, p=0.08). No other statistically significant changes or trends were observed for other serum sex steroid hormones, serum metabolic markers, or urinary estrogen metabolites at either phase.

GENOTOXICITY

Genotoxicity studies are summarized in Table 11.[8,14,40,50-54] Rosmarinus officinalis (rosemary) leaf extract was not genotoxic when tested *in vitro* in an Ames test, in a chromosomal aberration assay in human lymphocytes, or in a gene-locus mutation assay in human lymphocytes, and it was not genotoxic when tested *in vivo* in a chromosomal aberration assay or micronucleus test.[8] Various extraction solvents were used. Rosmarinus officinalis (rosemary) leaf oil was not mutagenic *in vitro* in an Ames test.[51] *In vivo*, however, oils that were extracted by hydrodistillation did induce statistically significant increases in chromosomal aberrations without gaps in a chromosomal aberration assay at 2000 mg/kg bw, increases in micronucleated polychromatic erythrocytes (MNPCEs) in several micronucleus tests at 1000 and 2000 mg/kg bw, and increases in DNA damage in a comet assay at ≥300 mg/kg bw;[14] no genotoxic effects were seen in a micronucleus test at 1500 mg/kg bw/day with leaves extracted using absolute ethanol.[40] A mixture containing 19% *Rosmarinus officinalis* (rosemary) leaves, 71.5% St. John's Wort, and 9.5% spirulina induced statistically significant increases in MNPCEs at 760 and 1520 mg/kg bw/day in a micronucleus test; in frequency of aneuploidy, percent polyploidy, and total percent aberrations with 760 and 1520 mg/kg bw/day in a chromosomal aberration assay; and in frequency of banana-shaped, swollen achrosome, and triangular head sperm abnormalities and percent total spermatozoa abnormalities at 1520 mg/kg bw/day in a spermatozoa abnormality assay.[50] *In vitro*, rosmarinus officinalis (rosemary) leaf extract was shown to have anti-mutagenic potential.[54] *In vivo*, in micronucleus assays, rosmarinus officinalis (rosemary) leaf extract did not decrease the number of MNPCEs induced by a genotoxic agent.[40]

CARCINOGENICITY

Anti-Tumor Activity

Anti-tumor activity studies are summarized in Table 12. Topical application of methanol and double distilled water extracts of *Rosmarinus officinalis* (rosemary) leaves statistically significantly decreased skin tumors in mice; in these studies, 7,12-dimethylbenz[a]anthracene (DMBA) or benzo[a]pyrene (B(a)P)was used for initiation and TPA or croton oil was used for promotion. Dietary administration of rosmarinus officinalis (rosemary) leaf extract decreased the incidence of palpable mammary tumors in rats caused by DMBA.

IRRITATION AND SENSITIZATION

Skin Irritation/Sensitization

Non-Human

Rosmarinus Officinalis (Rosemary) Leaf Oil

An ointment containing 4.4% rosmarinus officinalis (rosemary) leaf oil (and other essential oils) was not irritating to rat skin.[55] The ointment was applied to the shaved skin of Lewis rats twice daily, for 14 days, at concentrations up to 40%. No gross or microscopic lesions were reported in the skin.

Rosmarinus officinalis (rosemary) leaf oil, applied undiluted to intact and abraded rabbit skin under occlusion, was moderately irritating.[41] No details were provided.

Human

Rosmarinus Officinalis (Rosemary) Leaf

The irritation potential of *Rosmarinus officinalis* (rosemary) leaves, tested undiluted with sufficient petrolatum for binding, was evaluated in a patch test in 234 patients with contact dermatitis or eczema.[56] Of the 234 subjects tested, 21 had +/- reactions, 18 had a + reaction, and 5 had a ++ reaction. No subjects had a +++ reaction.

Rosmarinus Officinalis (Rosemary) Leaf Extract

The dermal irritation potential of *Rosmarinus officinalis* (rosemary) leaves extracted with supercritical CO_2, as a concrete (insoluble waxes) extracted in hexane, and as an absolute (soluble in hexane) and a concrete (insoluble waxes) extracted in hexane, was evaluated in epicutaneous tests.[12] Each test substance was applied undiluted in petrolatum on three sites using Finn chambers. The absolute was tested in 25 subjects, and the other two extracts were tested in 20 subjects. The super-critical CO_2 extract of *Rosmarinus officinalis* (rosemary) leaves produced 1/20 positive reactions and the absolute produced 2/25 positive reactions; both were considered weak irritants. The concrete did not induce any irritation reactions.

A cream containing 0.2% rosmarinus officinalis (rosemary) leaf extract was not an irritant in a 24 h single insult occlusive patch test.[57] The test material was applied undiluted in 20 subjects. No reactions were observed, and the primary irritation index was 0.00.

Summary data submitted to the CIR reported that a hair spray containing 0.0013% rosmarinus officinalis (rosemary) leaf extract was not an irritant or sensitizer in a modified Draize human repeated insult patch test (HRIPT) in 102 subjects.[58] During induction, occlusive patches were applied for 24 h, and the sites were scored prior to the application of the next patch. Patches were applied three times per week for 3 wks. The material was allowed to volatilize and tested neat for 30 min prior to application. After a 2-wk non-treatment period, challenge patches were applied to a previously untreated site; the test sites were scored 24 and 72 h after application. Transient, barely perceptible to mild responses were observed in some subjects, but was not considered related to skin irritation or an allergic reaction.

A sunscreen cream containing 0.2% rosmarinus officinalis (rosemary) leaf extract was not a contact-sensitizer in a maximization study in 27 subjects.[59] During induction, an occlusive patch containing 0.1 ml of 0.25% aq. sodium lauryl sulfate (SLS) was applied to the upper outer arm, volar forearm, or back of each subject for 24 h. The SLS patch was removed and an occlusive patch with 0.1 ml undiluted test material then applied for 48 or 72 h; the patch was then removed and the test site examined. A total of five SLS/test material patches were applied during induction. After a 10-day non-treatment period, an occlusive patch with 0.1 ml of a 5% aq. SLS solution was applied to a previously untreated site for 1 h; this patch was removed and an occlusive patch containing 0.1 ml undiluted test material was then applied for 48 h. The challenge site was graded 1 and 24 h after patch removal. No reactions were observed at either reading.

Rosmarinus Officinalis (Rosemary) Leaf Oil

Rosmarinus officinalis (rosemary) leaf oil, tested at a concentration of 10% in petrolatum, was not an irritant in a 48-h closed patch test (number of subjects not specified), and it was not a sensitizer in a maximization study in 25 subjects.[41] No other details were provided.

A leave-on massage oil containing 1.5% rosmarinus officinalis (rosemary) leaf oil did not induce allergic contact dermatitis in an HRIPT in 104 subjects.[60] An occlusive patch containing 50 µl of undiluted test material was applied for 48 h; the patches were then removed and a new patch applied. Nine induction patches were applied. Patches of 0.5% SLS were used as a positive control, and deionized water as a negative control. Challenge was performed 12-14 days after induction at the original test site and a previously untested site for 48 h. These sites were scored at 48 and 96 h. No reactions to the formulation containing 1.5% rosmarinus officinalis (rosemary) leaf oil were observed during induction or at challenge.

Phototoxicity

Rosmarinus Officinalis (Rosemary) Leaf Extract

The phototoxicity of rosmarinus officinalis (rosemary) leaf extract, extracted with supercritical CO_2, as a concrete extracted in hexane, and as an absolute and a concrete extracted in hexane, was evaluated as a part of the epicutaneous irritation test described above.[12] Photopatch tests were performed on two of the three test sites; one site was irradiated with 10 J/cm^2 UVA and the second site with 75% of the minimal erythema dose of UVB. The test sites were scored after 48 and 72 h, and were compared to the non-irradiated site. None of the extracts were phototoxic.

Case Reports

Several cases of allergic reactions to *Rosmarinus officinalis* (rosemary) have been reported, and are summarized in Table 13.[61-69] In some of the studies, follow-up patch testing included photopatch tests; generally, reactions were stronger in the photopatch tests, compared to standard testing.[65,66] Some of the follow-up patch testing included carnosol; testing with carnosol resulted in positive reactions.[62,66]

SUMMARY

This report addresses the safety of 10 *Rosmarinus officinalis* (rosemary)-derived ingredients as used in cosmetics. Most of the ingredients included in this review are extracts, essential oils, powders, or solutions derived from a defined part of the *Rosmarinus officinalis* (rosemary) plant. The *Rosmarinus officinalis* (rosemary)-derived ingredients are reported to have a number of functions, and the most common functions in cosmetics are as a skin conditioning agent or as a fragrance ingredient. According to VCRP data obtained from the FDA, rosmarinus officinalis (rosemary) leaf extract has the most uses, 689, followed by rosmarinus officinalis (rosemary) leaf oil, which has 516 uses. Most of the reported use concentrations for *Rosmarinus officinalis* (rosemary)-derived ingredients are well below 0.1%. However, rosmarinus officinalis (rosemary) leaf extract has higher concentrations of use reported, specifically, use at up to 10% in body and hand products and 3% in eye shadow formulations and bath soaps and detergents. Rosmarinus officinalis (rosemary) flower/leaf/stem water is the only ingredient not reported to be used.

Rosmarinus officinalis (rosemary) extract is prepared by extraction from the leaves of *Rosmarinus officinalis* with acetone, ethanol, hexane, a combination of hexane and ethanol (in a two-step process), or supercritical CO_2; it can also be prepared from a deodorized or partially deodorized ethanol extract of rosemary. Additional methods include extraction with absolute ethanol (resulting in an absolute) or a collection of the insoluble waxes (resulting in a concrete).

Rosmarinus officinalis L. is composed of an array of constituents, primarily phenolic acids, flavonoids, monoterpenes, diterpenes, diterpenoids, and triterpenes. The principal antioxidative components of rosmarinus officinalis (rosemary) leaf extract are the phenolic diterpenes carnosol and carnosic acid. The actual amount of constituents present varies according to the stage of development, variety of plant, season harvested, origin of the leaves, and extraction method.

Rosemary oil increased the permeation of aminophylline through human skin, but the increase was not as great as that seen with 50% ethanol.

The acute toxicity of *Rosmarinus officinalis* (rosemary)-derived ingredients is not very remarkable. The dermal LD_{50} of rosmarinus officinalis (rosemary) leaf oil is > 10 ml/kg. The oral LD_{50} of rosmarinus officinalis (rosemary) leaves is >2 g/kg, of rosmarinus officinalis (rosemary) leaf extract is >8.5 g/kg, and of rosmarinus officinalis (rosemary) leaf oil is 5.5 g/kg bw.

A number of oral repeated-dose toxicity studies were performed in mice and in rats with *Rosmarinus officinalis* (rosemary) leaves extracted in a various solvents. Doses as high as 14.1 g/kg bw rosmarinus officinalis (rosemary) leaf extract were tested (5 days by gavage), and studies were performed for up to 3 mos (dietary). Increases in absolute and relative liver-to-body weights were observed in many of the studies, independent of the extraction method; these changes were shown to be reversible, and no other signs of toxicity were observed. Oral administration of rosmarinus officinalis (rosemary) leaf oil also affected liver weights.

Rosmarinus officinalis (rosemary) leaf extract has been shown to have anti-inflammatory activity. Rosmarinus officinalis (rosemary) leaf extract inhibited a TPA-induced increase in the number of epidermal cell layers and epidermal thickness in mouse skin.

A high dose (500 mg/kg/day) of *Rosmarinus officinalis* (rosemary) leave extract was a reproductive toxicant in a dietary study in rats. In a study in gravid female Wistar rats, no statistically significant changes were observed after oral dosing with 26 mg/day of a 30% aq. rosmarinus officinalis (rosemary) flower/leaf/stem extract during preimplantation or during organogenesis. In a dietary study in ovariectomized CD-1 mice, 2% of a methanol extract of *Rosmarinus officinalis* (rosemary) leaves inhibited the uterine response in a statistically significant manner.

In a clinical study investigating the effects on sex steroid hormones and metabolic markers of a botanical supplement containing 100 mg *Rosmarinus officinalis* (rosemary) leaf 5:1 extract (and other botanical ingredients) in premenopausal women, a few changes were found. Overall, the changes were not remarkable.

Rosmarinus officinalis (rosemary) leaf extract was not genotoxic when tested *in vitro* in an Ames test, in a chromosomal aberration assay in human lymphocytes, or in a gene-locus mutation assay in human lymphocytes, and it was not genotoxic when tested *in vivo* in a chromosomal aberration assay or micronucleus test. Various extraction solvents were used. Rosmarinus officinalis (rosemary) leaf oil was not mutagenic *in vitro* in an Ames test. However, *in vivo*, oils that were extracted by hydrodistillation did induce statistically significant increases in chromosomal aberrations without gaps in a chromosomal aberration assay at 2000 mg/kg bw, increases in MNPCEs in several micronucleus tests at 1000 and 2000 mg/kg bw, and increases in DNA damage in a comet assay at ≥300 mg/kg bw; no genotoxic effects were seen in a micronucleus test at 1500 mg/kg bw/day with an oil that was extracted using absolute ethanol. A mixture containing 19% rosmarinus officinalis (rosemary) leaves, 71.5% St. John's Wort, and 9.5% spirulina induced statistically significant increases in MNPCEs at 760 and 1520 mg/kg bw/day in a micronucleus test; in frequency of aneuploidy, percent polyploidy, and total percent aberrations with 760 and 1520 mg/kg bw/day in a chromosomal aberration assay; and in frequency of banana-shaped, swollen achrosome, and triangular head sperm abnormalities and percent total spermatozoa abnormalities at 1520 mg/kg bw/day in a spermatozoa abnormality assay. *In vitro*, rosmarinus officinalis (rosemary) leaf extract was shown to have anti-mutagenic

potential. *In vivo* in micronucleus assays, rosmarinus officinalis (rosemary) leaf extract did not decrease the number of MNPCEs induced by a genotoxic agent.

Topical application of methanol and double distilled water extracts of rosmarinus officinalis (rosemary) leaves statistically significantly decreased skin tumors in mice; in these studies, DMBA or benzo[a]pyrene was used for initiation and TPA or croton oil was used for promotion. Dietary administration of rosmarinus officinalis (rosemary) leaf extract decreased the incidence of palpable mammary tumors in rats caused by DMBA.

An ointment containing 4.4% rosmarinus officinalis (rosemary) leaf oil (and other essential oils), applied at concentrations up to 40%, was not irritating to rat skin. However, in a rabbit study, occlusive application to intact and abraded skin produced moderate irritation.

In clinical testing, *Rosmarinus officinalis* (rosemary) leaves produced irritation (scores of +/-, +, or ++) in 44/234 patients with contact dermatitis or eczema. A supercritical extract and the absolute of *Rosmarinus officinalis* (rosemary) leaves were considered weak irritants in a small study with test populations of 20-25 subjects; the extracts were not phototoxic. Formulations containing up to 0.2% rosmarinus officinalis (rosemary) leaf extract were not irritants or sensitizers. Rosmarinus officinalis (rosemary) leaf oil, 10% in petrolatum, was not an irritant in a 48-h closed patch test, or a sensitizer in a maximization study; a formulation containing 1.5% rosmarinus officinalis (rosemary) leaf oil was not an irritant or a sensitizer in an HRIPT.

Several cases of allergic reactions to *Rosmarinus officinalis* (rosemary) have been reported. In some of the studies, follow-up patch testing included photopatch tests; generally, reactions were stronger in the photopatch tests, compared to standard testing. Some also evaluated the effect of carnosol; testing with carnosol resulted in positive reactions.

DISCUSSION

Upon initial review of the safety assessment of *Rosmarinus officinalis* (rosemary)-derived ingredients, the Panel issued an Insufficient Data Announcement requesting the following:

1. Dermal sensitization data for 10% rosmarinus officinalis (rosemary) leaf extract (i.e., a human repeated-insult patch test in a sufficient number of subjects at concentration of use);
2. Chemical characterization of the flower, if available;
3. Additional information on the deodorizing process performed during preparation of some of the ingredients, including information on what by-products may form; and
4. Information as to why the *PDR of Herbal Medicines* states that rosemary preparations should not be used during pregnancy.

The majority of these data were not received. *Rosmarinus officinalis* is GRAS as a spice, and although that alleviates the concern of oral toxicity with cosmetic use, dermal irritation and sensitization data are necessary to determine safety. Because the Panel did not receive dermal sensitization data of rosmarinus officinalis (rosemary) leaf extract at the highest reported use concentration (i.e., 10%.), the Panel set a use concentration in leave-on products at the highest concentration for which test data were available (i.e., 0.2%).

The rosemary plant itself is well-defined in the published literature, but the chemical characterization of the flower was not well-defined. Because information on the chemical characterization of the flower was not provided, the Panel found the available data insufficient for determining that safety of rosmarinus officinalis (rosemary) flower extract for use in cosmetics.

Additional information on the deodorizing process that is part of the preparation of some of the ingredients also was not received. After further discussion, the Panel stated that the deodorizing process is part of the preparation of food-grade rosmarinus officinalis (rosemary) extract. Therefore, the Panel was not concerned with obtaining additional information on this process or the by-products that might form.

The Panel did note that because botanical ingredients, derived from natural plant sources, are complex mixtures, there is concern that multiple botanical ingredients may each contribute to the final concentration of a single constituent. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse effects. Specific examples of constituents that could possibly induce sensitization or adverse effects are caffeic acid, thujone, and terpenes, especially linalool, linolyl acetate, limonene, and methyleugenol.

The Expert Panel expressed concern about pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

One study evaluated the irritation potential of *Rosmarinus officinalis* (rosemary) leaves, tested undiluted, in patients with contact dermatitis or eczema. The Panel stated that observations of irritation following the application of undiluted leaves to eczematous skin were not pertinent to cosmetic use.

The Panel discussed the positive results observed in a reproductive and development toxicity study in rats fed 500 mg/kg/day as well as the caution in the *PDR for Herbal Medicines* stating that rosemary preparations should not be used as a drug during pregnancy. The effects in the rat study were observed at exposure concentrations that would be well above those used in cosmetic products and the *PDR* refers to the use of rosemary as a drug in preparations at very high concentrations. Because these effects were observed only at very high concentrations, reproductive and developmental toxicity is not a concern with cosmetic use of Rosmarinus officinalis (rosemary)-derived ingredients.

Finally, the Panel discussed the issue of incidental inhalation exposure to *Rosmarinus officinalis* (rosemary)-derived ingredients. The Panel stated that although there were no inhalation data available, the *Rosmarinus officinalis* (rosemary)-derived ingredients are used at very low concentrations in products that could incidentally be inhaled, e.g., rosmarinus officinalis (rosemary) leaf extract is used in other fragrance preparations at up to 0.5% and rosmarinus officinalis (rosemary) extract is used in face powders at up to 0.05%. The Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that the following eight Rosmarinus officinalis (rosemary)-derived ingredients are safe in the present practices of use and concentration in cosmetics described in this safety assessment:

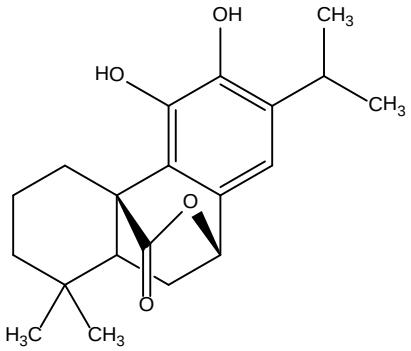
Rosmarinus Officinalis (Rosemary) Extract
Rosmarinus Officinalis (Rosemary) Flower/Leaf Stem Extract
Rosmarinus Officinalis (Rosemary) Flower/Leaf/Stem Water*
Rosmarinus Officinalis (Rosemary) Leaf
Rosmarinus Officinalis (Rosemary) Leaf Oil
Rosmarinus Officinalis (Rosemary) Leaf Powder
Rosmarinus Officinalis (Rosemary) Leaf Water
Rosmarinus Officinalis (Rosemary) Water

*Not reported to be in current use. If this ingredient was to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.

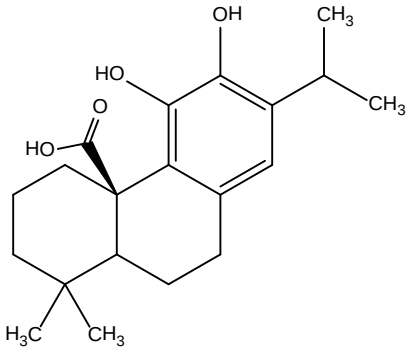
The Panel also concluded that Rosmarinus Officinalis (Rosemary) Leaf Extract is safe at ≤0.2% in leave-on products and safe as used in rinse-off products, and that the data are insufficient to support the safety of Rosmarinus Officinalis (Rosemary) Flower Extract as used in cosmetics.

FIGURES

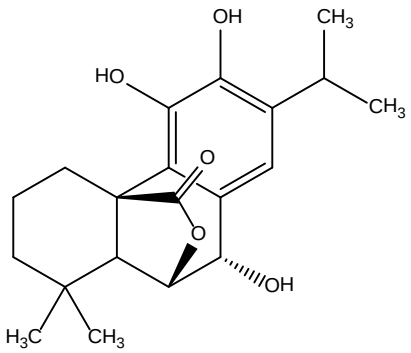
Figure 1. Principal diterpenes



1a. Carnosol

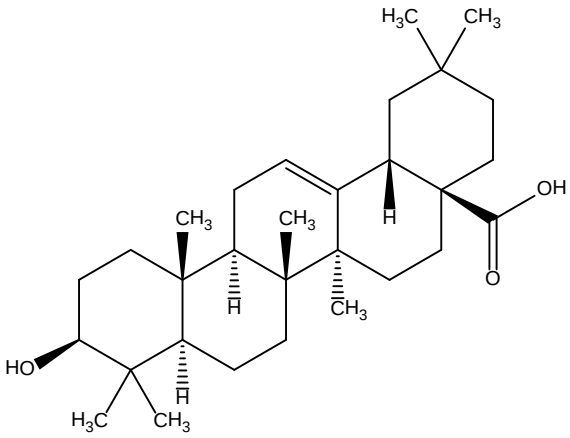


1b. Carnosic acid

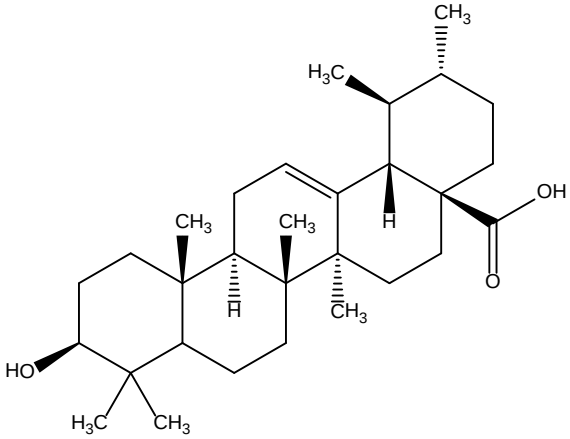


1c. Rosmanol

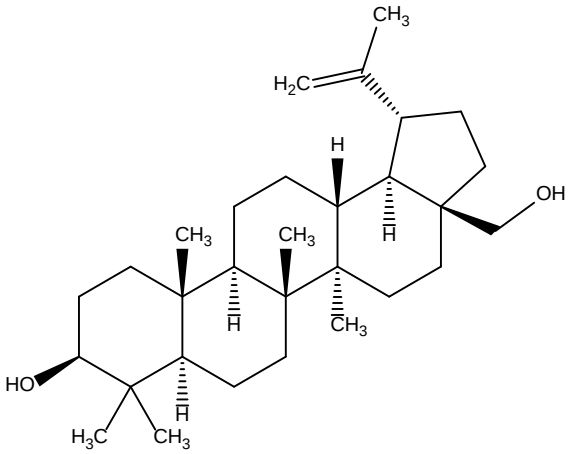
Figure 2. Principal triterpenes



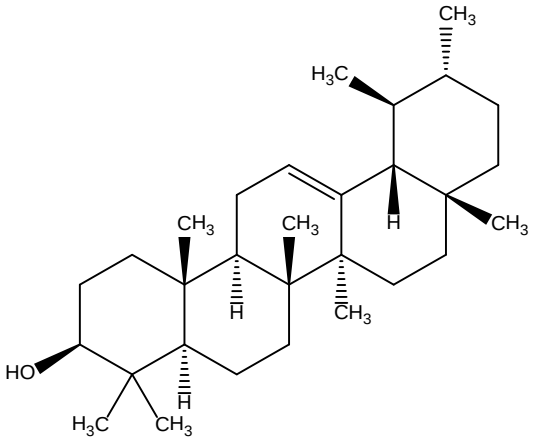
2a. Oleanolic acid



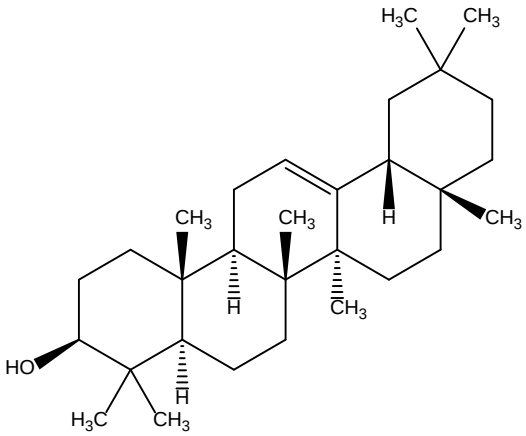
2b. Ursolic acid



2c. Betulin

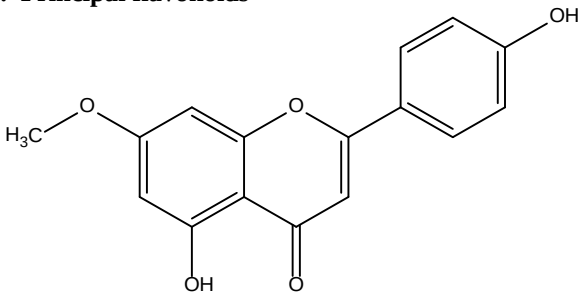


2d. α-Amyrin

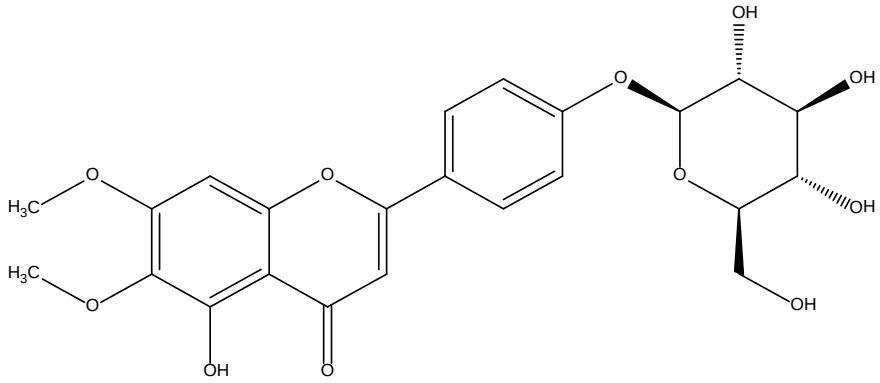


2e. β-Amyrin

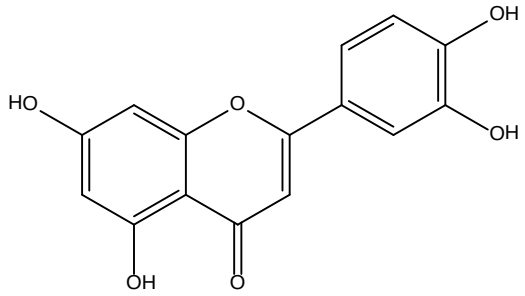
Figure 3. Principal flavonoids



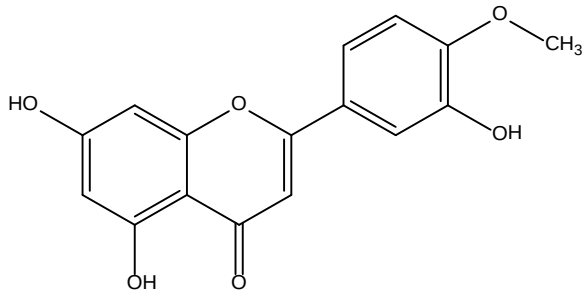
3a. Genkwanin



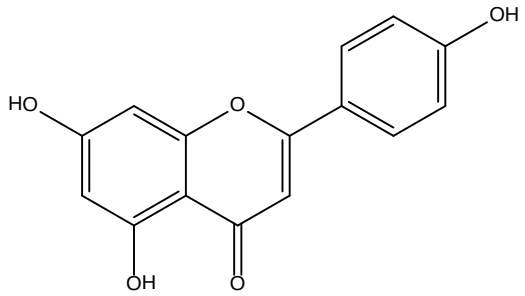
3b. Cirsimarin



3c. Luteolin

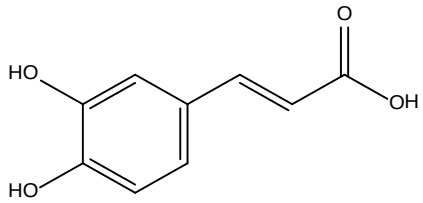


3d. Diosmetin

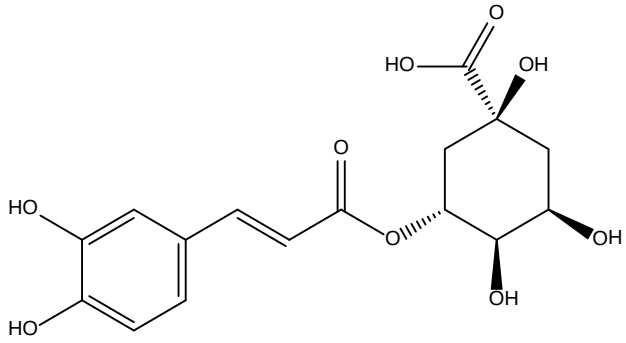


3e. Apigenin

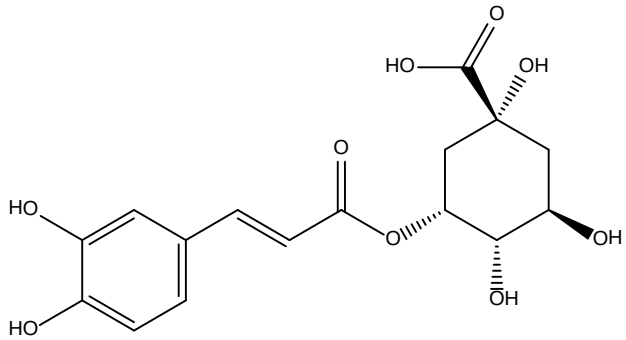
Figure 4. Phenolic acids



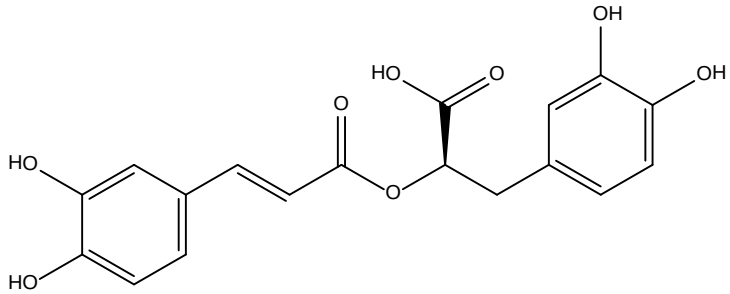
4a. Caffeic acid



4b. Chlorogenic acid

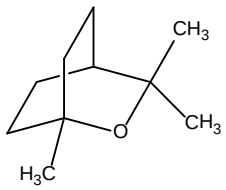


4c. Neochlorogenic acid

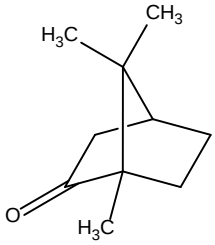


4d. Labiatic acid

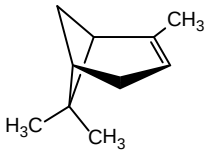
Figure 5. Principal Volatiles



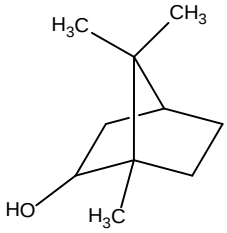
5a. 1,8-Cineole



5b. Camphor



5c. α-Pinene



5d. Borneol

TABLES

Table 1. Definitions and reported functions

Ingredient (CAS No.)	Definition[1]	Reported Function(s)[1]
Rosmarinus Officinalis (Rosemary) Extract (84604-14-8)	the extract of the whole plant *Rosmarinus officinalis*	skin-conditioning agent – misc
Rosmarinus Officinalis (Rosemary) Flower Extract	the extract of the flowers of *Rosmarinus officinalis*	antioxidant; deodorant agents; skin-conditioning agents – misc
Rosmarinus Officinalis (Rosemary) Flower/Leaf/Stem Extract	the extract of the flowers, leaves and stems of *Rosmarinus officinalis*	fragrance ingredients; skin-conditioning agents - misc
Rosmarinus Officinalis (Rosemary) Flower/Leaf/Stem Water	the aqueous solution of the steam distillates obtained from the flowers, leaves and stems of *Rosmarinus officinalis*	fragrance ingredient
Rosmarinus Officinalis (Rosemary) Leaf	the leaf of *Rosmarinus officinalis*	skin-conditioning agents – misc
Rosmarinus Officinalis (Rosemary) Leaf Extract (84604-14-8)	the extract of the leaves of *Rosmarinus officinalis*	antimicrobial agents; antioxidant; fragrance ingredients; skin-conditioning agents - miscellaneous; skin-conditioning agents – occlusive
Rosmarinus Officinalis (Rosemary) Leaf Oil (8000-25-7)	the essential oil obtained from the flowering tops and leaves of *Rosmarinus officinalis*	fragrance ingredients; skin-conditioning agents – misc
Rosmarinus Officinalis (Rosemary) Leaf Powder	the powder derived from the dried, ground leaves of *Rosmarinus officinalis*	flavoring agents
Rosmarinus Officinalis (Rosemary) Leaf Water	an aqueous solution of the steam distillate obtained from the leaves of *Rosmarinus officinalis*	fragrance ingredient
Rosmarinus Officinalis (Rosemary) Water	an aqueous solution of the steam distillate obtained from *Rosmarinus officinalis*	fragrance ingredient

Table 2. Chemical and physical properties

Property	Description	Reference
	Rosmarinus Officinalis (Rosemary) Leaf	
odor	strongly aromatic	35
	Rosmarinus Officinalis (Rosemary) Leaf Extract	
physical state and appearance	powder or liquid	7
	colorless, volatile oil	8
	dark brown viscous liquid with a characteristic smell and taste (as the extract (and) Helianthus Annuus Seed Oil)	9,10
solubility	insoluble in water	7
refractive index	1.4710 - 1.4740	17
density	0.9165 - 0.9220	17
	Rosmarinus Officinalis (Rosemary) Leaf Oil	
physical state and appearance	colorless or pale yellow liquid with characteristic odor and a warm, camphoraceous taste	13,34
	colorless, pale yellow, or pale green liquid with a camphorous odor	70
solubility	almost insoluble in water	34
	soluble in most vegetable oils; insoluble in alcohol and in propylene glycol	13
density (d^{25}_{25})	0.894-0.912	34
	0.907-0.920	70
index of refraction (n_D^{20})	1.464-1.476	34
	Rosmarinus Officinalis (Rosemary) Leaf Powder	
physical state and appearance	greyish-green to yellowish-green powder	35

Table 3. Chemical constituents by plant part (ppm) [71]

Constituent*	Plant	Leaf	Flower	Shoot	Resin, Exudate, Sap	Essential Oil	Tissue Culture
carbohydrates	640,600-704,660	-	-	-	-	-	-
fiber	165,420-206,338	-	-	-	-	-	-
fat	134,020-187,418	-	-	-	-	-	-
water	77,900-108,300	-	-	-	-	-	-
ash	61,900-75,570	-	-	-	-	-	-
protein	40,700-62,568	-	-	-	-	-	-
ursolic acid	28,000-41,000	-	-	20	-	-	-
rosmarinic acid	25,000	3500	-	13,500	-	-	38,957
EO	3300-25,000	-	-	-	-	-	-
calcium	10,919-16,150	-	-	-	-	-	-
potassium	8842-11,284	-	-	-	-	-	-
oleanolic acid	10,500	-	-	20	-	-	-
carnosol	-	530-9803					
cineole	168-9728	-	-	-	-	-	-
1,8-cineole	8125	-	-	-	-	-	-
camphor	60-5800	-	-	-	-	-	-
myrcene	25-5605	-	-	-	-	-	-
bornyl acetate	5054	-	-	-	-	-	-
α –pinene	235-4750	-	-	-	-	-	-
borneol	12-4237	-	-	-	-	-	-
magnesium	2142-2483	-	-	-	-	-	-
rosmaric acid	3000-3500	-	-	-	-	-	-
camphene	23-2350	-	-	-	-	-	-
β-caryophyllene	12-2075	-	-	70-2075			
toluene	436-2071	-	-	-	-	-	-
limonene	1950	-	-	-	-	-	-
α –terpineol	24-1555	-	-	-	-	-	-
β-pinene	17-1425	-	-	-	-	-	-
phosphorus	490-1000	-	-	-	-	-	-
p-cymene	25-950	-	-	-	-	-	-
carvone	16-760	-	-	-	-	-	-
α-humulene	-	-	-	725			
salicylates	-	70-680	-	-	-	-	-
ascorbic acid	612-673	-	-	-	-	-	-
α-amorphene	70-665	-	-	-	-	-	-
γ-muurolene	70-665	1	-	-	-	-	-
phytosterols	580-640	-	-	-	-	-	-
sodium	462-592	-	-	-	-	-	-
linalool	585	-	-	-	-	-	-
α –terpinene	4-555	-	-	-	-	-	-
terpinen-4-ol	4-521	-	-	-	-	-	-
α –thujene	1-475	-	-	-	-	-	-
δ-terpineol	7-418	-	-	-	-	-	-
iron	220-400	-	-	-	-	-	-
α –thujone	84-399	-	-	-	-	-	-
(E)-β-ocimene	-	-	-	380			
verbenone	10-375	-	-	-	-	-	-
geraniol	50-370	-	-	-	-	-	-
3-hexanone	74-351	-	-	-	-	-	-
terpinolene	12-350	-	-	-	-	-	-
caryophyllene	16-340	-	-	-	-	-	-
δ-3-carene	330	-	-	-	-	-	-
fenchone	250	-	-	-	-	-	-
β-thujone	11-209	-	-	-	-	-	-
β-elemene	-	-	-	3-200			
sabinene	190	-	-	-	-	-	-
mesityl alcohol	40-190	-	-	-	-	-	-
linalool acetate	32-152	-	-	-	-	-	-
α –phellandrene	133	-	-	-	-	-	-
α- fenchyl alcohol	28-133	-	-	-	-	-	-
p-menth-3-en-1-ol	28-133	-	-	-	-	-	-
3,5,5-trimethylhexan-1-ol	28-133	-	-	-	-	-	-
trans-ocimene	4-130	-	-	-	-	-	-
cis-pinan-3-one	-	17-110	-	-	-	-	-
4-terpinenyl-acetate	-	12-110	-	-	-	-	-
safrole	32-95	-	-	-	-	-	-
cis-β-terpineol	20-95	-	-	-	-	-	-

Table 3. Chemical constituents by plant part (ppm) [71]

Constituent*	Plant	Leaf	Flower	Shoot	Resin, Exudate, Sap	Essential Oil	Tissue Culture
α- fenchyl acetate	20-95	-	-	-	-	-	-
longifolene	20-95	-	-	-	-	-	-
isoborneol	7-95	-	-	-	-	-	-
rosmanol	-	92	-	-	-	-	-
(+)-limonene	16-76	-	-	-	-	-	-
δ-cadinene	75	-	-	-	-	-	-
caryophyllene oxide	75	-	-	-	-	-	-
(Z)-β-ocimene	-	-	-	75	-	-	-
trans-pinocarveol	-	32-42	-	-	-	-	-
3-octanone	20-40	-	-	-	-	-	-
boron	22-39	-	-	-	-	-	-
zinc	30-38	-	-	-	-	-	-
AR-curcumene	8-38	-	-	-	-	-	-
methyl heptenone	8-38	-	-	-	-	-	-
myrtenol	8-38	-	-	-	-	-	-
lavandulol	7-34	-	-	-	-	-	-
trans-β-terpineol	7-34	-	-	-	-	-	-
trans-myrtenol	-	32	-	-	-	-	-
benzyl alcohol	7-32	-	-	-	-	-	-
elemol	7-32	-	-	-	-	-	-
γ-eudesmol	7-32	-	-	-	-	-	-
rosmadial	-	30	-	-	-	-	-
α-amyrenone	-	-	-	30	-	-	-
β-amyrenone	-	-	-	30	-	-	-
epirosmanol	-	26	-	-	-	-	-
β-carotene	19-21	-	-	-	-	-	-
rofficerone	-	-	-	20	-	-	-
trans-sabinene hydrate	19	-	-	-	-	-	-
manganese	18-19	-	-	-	-	-	-
cis-α-bisabolene	4-19	-	-	-	-	-	-
isopinocarveol	4-19	-	-	-	-	-	-
isopulegol	4-19	-	-	-	-	-	-
3-octanol	4-19	-	-	-	-	-	-
dimethyl styrene	1-19	-	-	-	-	-	-
7-methoxy-rosmanol	-	-	-	18			
isorosmanol	-	-	17	-	-	-	-
cis-myrtenol	-	11-17	-	-	-	-	-
cisimaritrin	-	-	-	16	-	-	-
α-amyrin	NS	-	-	13	-	-	-
β-amyrin	NS	-	-	13	-	-	-
botulin	-	-	-	12.1	-	-	-
α –muurolene	NS	2-12	-	-	-	-	-
3-o-acetyloleanolic acid	-	-	-	11	-	-	-
3-o-acetylursolic acid	-	-	-	11	-	-	-
niacin	10-11	-	-	-	-	-	-
peperitenone	-	4-8	-	-	-	-	-
eugenol methyl ether	-	5-7	-	-	-	-	-
copper	5-6	-	-	-	-	-	-
thiamin	5-6	-	-	-	-	-	-
carvacrol	NS	5-6	-	-	-	-	-
α -terpinenyl acetate	-	5-6	-	-	-	-	-
allo-aromadendrene	-	4-5	-	-	-	-	-
neo-thujol	-	1.5-5	-	-	-	-	-
calamenene	1-5	-	-	-	-	-	-
trans-carveol	1-5	-	-	-	-	-	-
p-cymen-8-ol	1-5	-	-	-	-	-	-
nopol	1-5	-	-	-	-	-	-
γ-candinene	NS	1-5	-	-	-	-	-
α-copaene	-	2-4	-	-	NS	-	-
epi- α -bisabolol	-	3	-	-	-	-	-
sabinyl acetate	-	1.5	-	-	-	-	-
β-gurjunene	-	0.5	-	-	-	-	-
cis-sabinene hydrate	NS	0.4	-	-	-	-	-
β-phellandrene	trace	-	-	-	-	-	-
tricyclene	trace	-	-	-	-	-	-
α-fenchol	-	trace	-	-	-	-	-
p-menth-cis-en-1-ol	-	trace	-	-	-	-	-

Table 3. Chemical constituents by plant part (ppm) [71]

Constituent*	Plant	Leaf	Flower	Shoot	Resin, Exudate, Sap	Essential Oil	Tissue Culture
p-menth-trans-en-1-ol	-	trace	-	-	-	-	-
trans-anethole	NS	-	-	-	-	-	-
apigen-7-glucoside	NS	-	-	-	-	-	-
betulin	NS	-	-	-	-	-	-
bornylene	NS	-	-	-	-	-	-
cadalene	NS	-	-	-	-	-	-
caffeic acid	NS	-	-	-	-	-	-
calacorene	NS	-	-	-	-	-	-
carnosic acid	NS	-	-	-	-	-	-
chlorogenic acid	NS	-	-	-	-	-	-
cirsilion	NS	-	-	-	-	-	-
cubenene	NS	-	-	-	-	-	-
diosmetin	NS	-	-	-	-	-	-
epi- α -amyrin	NS	-	-	-	-	-	-
eriodictiol	NS	-	-	-	-	-	-
ethanol	NS	-	-	-	-	-	-
α-fenchene	NS	-	-	-	-	-	-
β-fenchene	NS	-	-	-	-	-	-
genkwanin-4'-methyl ether	NS	-	-	-	-	-	-
glycolic acid	NS	-	-	-	-	-	-
genkwanin	NS	-	-	-	-	-	-
hesperidin	NS	-	-	-	-	-	-
hispidulin	NS	-	-	-	-	-	-
hispiduloside	NS	-	-	-	-	-	-
humulene epoxide I	NS	-	-	-	-	-	-
humulene epoxide II	NS	-	-	-	-	-	-
5-hydroxy-4',7-dimethoxyflavone	NS	-	-	-	-	-	-
hydroxybenzoic acid-4- β -D-glucoside	NS	-	-	-	-	-	-
4-hydroxybenzoyl glucoside	NS	-	-	-	-	-	-
α-hydroxyhydrocaffeic acid	NS	-	-	-	-	-	-
2- β -hydroxyoleanolic acid	NS	-	-	-	-	-	-
3-β-hydroxyurea-12,20(30)-dien-17-on acid	NS	-	-	-	-	-	-
19- α -hydroxyursolic acid	NS	-	-	-	-	-	-
isobornyl acetate	NS	-	-	-	-	-	-
isobutyl acetate	NS	-	-	-	-	-	-
isorosmaricine	NS	-	-	-	-	-	-
labiatic acid	NS	-	-	-	-	-	-
ledene	NS	-	-	-	-	-	-
luteolin	NS	NS	-	-	-	-	-
luteolin-7-glucoside	NS	-	-	-	-	-	-
6-methoxy-genkwanin	NS	-	-	-	-	-	-
6-methoxy-luteolin	NS	-	-	-	-	-	-
6-methoxy-luteolin-7-glucoside	NS	-	-	-	-	-	-
6-methoxyluteolin-7-methyl ether	NS	-	-	-	-	-	-
methyl ether	NS	-	-	-	-	-	-
methyl eugenol	NS	-	-	-	-	-	-
N-methyl rosmaricine	NS	-	-	-	-	-	-
neo-chlorogenic acid	NS	-	-	-	-	-	-
nepetin	NS	-	-	-	-	-	-
nepetrin	NS	-	-	-	-	-	-
1-octen-3-ol	NS	-	-	-	-	-	-
picrosalvin	NS	-	-	-	-	-	-
rosmadiol	NS	-	-	-	-	-	-
rosmaricine	NS	-	-	-	-	-	-
rosmaridiphenol	NS	-	-	-	-	-	-
rosmarinol	NS	-	-	-	-	-	-
rosmariquinone	NS	-	-	-	-	-	-
salvigenin	NS	-	-	-	-	-	-
santene	NS	-	-	-	-	-	-
salicylic-acid-2-β-D-glucoside	NS	-	-	-	-	-	-
α –selinene	NS	-	-	-	-	-	-
sinensetin	NS	-	-	-	-	-	-
β-sitosterol	NS	-	-	-	-	-	-

Table 3. Chemical constituents by plant part (ppm) [71]

Constituent*	Plant	Leaf	Flower	Shoot	Resin, Exudate, Sap	Essential Oil	Tissue Culture
squalene	NS	-	-	-	-	-	-
syringic-acid-4-β-D-glucoside	NS	-	-	-	-	-	-
tannin	NS	-	-	-	-	-	-
thymol	NS	-	-	-	-	-	-
trimethylalkane	NS	-	-	-	-	-	-
o-o-N-trimethylrosmaricine	NS	-	-	-	-	-	-
vanillic-acid-4-β-D-glucoside	NS	-	-	-	-	-	-
verbenol	NS	-	-	-	-	-	-
betulinic acid	-	NS	-	-	-	-	-
δ-4-carene	-	NS	-	-	-	-	-
diosmin	-	NS	-	-	-	-	-
7-ethoxy-rosmanol	-	NS	-	-	-	-	-
luteolin-3'-o-(3"-o-acetyl)- β -D-glucuronide	-	NS	-	-	-	-	-
luteolin-3'-o-(4"-o-acetyl)- β -D-glucuronide	-	NS	-	-	-	-	-
luteolin-3'-o- β -D-glucuronide	-	NS	-	-	-	-	-
monomethyl alkane	-	NS	-	-	-	-	-
pristane	-	NS	-	-	-	-	-
protocatechuic-acid-4-β-D-glucoside	-	NS	-	-	-	-	-
pectin	-	-	-	NS	-	-	-
acetic acid	-	-	-	-	NS	-	-
butan-2-ol	-	-	-	-	NS	-	-
caproic acid	-	-	-	-	NS	-	-
deca-trans-2,trans-4-dien-1-al	-	-	-	-	NS	-	-
hept-trans-2-en-1-al	-	-	-	-	NS	-	-
heptan-1-al	-	-	-	-	NS	-	-
heptan-2-ol	-	-	-	-	NS	-	-
heptanoic acid	-	-	-	-	NS	-	-
hexan-1-al	-	-	-	-	NS	-	-
hexan-1-ol	-	-	-	-	NS	-	-
3-methyl-butan-1-ol	-	-	-	-	NS	-	-
β-ocimene	-	-	-	-	NS	-	-
octan-1-ol	-	-	-	-	NS	-	-
octane-2,3-dione	-	-	-	-	NS	-	-
octanoic acid	-	-	-	-	NS	-	-
pentan-1-al	-	-	-	-	NS	-	-
pentan-1-ol	-	-	-	-	NS	-	-
pentan-2-ol	-	-	-	-	NS	-	-
zingiberene	-	-	-	-	NS	-	-
dipentene	-	-	-	-	-	NS	-

*constituents reported in ppm
NS – amount not specified
" – " means not reported

Table 4. Constituent data by plant part

	Reference
Plant part not specified	
- volatile oil (0.5-2.5%): 1,8-cineole (20-50%); camphor (10-25%); α-pinene (up to 25%); other monoterpenes (including borneol and limonene)	[2,4,5]
- rosmarinic acid	
- diterpene bitter substances: carnosol; carnosolic acid (picrosalvin); isorosmanol; rosmanol; rosmadiol; rosmaridiphenol rosmariquinone	
- triterpene acids: ursolic acid; oleanolic acids; rosmanol; 7-ethoxyrosmanol; betulic acid; carnosol; traces of 19 α -hydroxyursolic, 2 β -hydroxyoleanolic, and 3 β -hydroxyurea-12,20(30)-dien-17-oic acids	
- triterpene alcohols: α-amyrin; β-amyrin; betulin	
- flavonoids: luteolin; genkwanin (7-*O*-methlylapigenin); diosmetin; diosmin; genkwanin-4'-methyl ether; 6-methoxygenkwanin; 6-methyoxyluteolin; 6-methoxyluteolin-7-glucoside; 6-methoxyluteolin-7-methylether; hispidulin; apigenin	
- corresponding glycosides	
Leaf	
- volatile oil (1.0-2.5%): 1,8-cineole (15-55%); camphor (5-25%); α-pinene (9-26%); camphene (2.5-12%); β-pinene (2-9%); borneol (1.5-6%); limonene (1.5-5%); bornyl acetate (1-5%); isobutyl acetate; β-caryophyllene; p-cymene; linalool; myrcene; α-terpineol (12-24%); verbenol	[5,22,34,35,72]
- diterpenes (up to 4.6%): carnosic acid; carnosol; isorosmanol; rosmadiol; rosmaridiphenol; rosmanol; rosmariquinone; triacetylrosmanol; dimethylrosmanol	
- triterpenes: oleanolic acid (10%); ursolic acid (2-5%); α-amyrin; β-amyrin; epi-α-amyrin; 19-α-ursolic acid; 2-β-hydroxy oleanolic acid; betulin	
- phenolic acids (2-3%): rosmarinic acid (3.5%); chlorogenic acid; neo-chlorogenic acid; caffeic acid; labiatic acid	
- flavonoids: genkwanin; cirsimarin; diosmetin; apigenin; luteolin; nepetin; nepitrin; diosmin; hesperidin; homoplantiginin; phegopolin	
- alkaloids: rosmaricin; isorosmaricine	
- tannins	
- saponins	
- glycolic acid and glyceric acid	
- vitamin C; vitamin P	
- choline	
Leaf Oil	
- α-pinene (8-25%), β-pinene (7.6%); eucalyptol (20-50%), camphor (10-27.6%), borneol (20%), 1,8-cineole (15.8%); β-myrcene (10%); camphene (5.2-5.8%), limonene (5.9%); *p*-cymene (4.8%); β-caryophyllene (3.1%); verbenone (2.6%); linalool	[34,39,40,70,73]
- From one sample (concentration in the oil):	[40]
- monoterpenoid esters (24.76%): bornyl acetate (20.86%); linolyl acetate (2.90%); terpinyl acetate (1.0%)	
- monoterpenoid alcohols (23.78%): borneol (8.25%); linalool (5%); isoborneol (4.13%); γ-terpineol (2.94%); α-terpineol (1.9%); terpinene 4-ol (1.43%); carveol (0.13%)	
- monoterpenoid ketones (18.67%): L-camphor (14.06%); verbenone (2.56%); carvone (1.9%); α -thujone (0.15%)	
- monoterpenoid ethers (10.86%): methyl eugenol (5.46%); 1,8-cineole (5.05%); linalool oxide (0.35%)	
- sesquiterpenes (8.96%): β -caryophellene (4.31%); caryophellene oxide (3.19%); spathulenol (1.27%); α -copene (0.19%)	
- phenols (4.06%): thymole (3.06%); carvacrol (0.91%); methyl chavicol (0.19%)	
- monoterpenes (3.4%): p-cymene (1.15%); α -pinene (0.95%); camphene (0.81%); myrcene (0.22%); limonene (0.15%)	
Seed	
- 560.5 µg/g α -tocotrienol; 300.3 µg/g β-tocotrienol; 109.4 µg/g γ -tocotrienol	[74]
Essential Oil	
- mainly monoterpenes: α -pinene (20.1-21.7%), β -pinene; camphene; limonene; 1,8-cineole (23.5-26.5%); eucalyptol (4.5%); and borneol	[4,75,76]
- camphor (7.2%); berbonone (7.6%); linalool; verbenol; terpineol; 3-octanone; isobornyl acetate	

Table 5. Rosmarinus Officinalis (Rosemary) Leaf Extracts (CO_2 extract) – Certificates of Analysis

Analytical Detail	Specifications (%)	Results (%)
Rosmarinus Officinalis (Rosemary) Extract (CO_2) [17]		
Essential Oil Content	78-88	78
Volatile components:		
α -pinene	8-12	11.4
camphene	n.s.	4.0
β -pinene	n.s.	3.7
myrcene	n.s.	2.7
p-cymene	n.s.	1.2
limonene	2-4	2.4
1,8-cineole	>40	41.3
linalool	n.s.	0.83
camphor	6-13	13.0
borneol	n.s.	3.8
α -terpineol	n.s.	3.9
verbenone	n.s.	0.45
bornyl acetate	n.s.	0.94
carophyllene	3-10	4.7
Rosmarinus Officinalis (Rosemary) Leaf Extract (CO_2; 14% diterpene phenols) (and) Helianthus Annuus Seed Oil [18]		
Essential Oil Content	<2	1.9
Phenolic diterpenes:		
rosmanol	n.s.	0.07
7-methyl-rosmanol	n.s.	0.09
carnosol	n.s.	1.2
carnosolic acid	n.s.	10.5
12-methyl-carnosolic acid	n.s.	2.4
sum of phenolic diterpenes	13-15	14.3
Reference antioxidant compounds (carnesol + carnosic acid, calculated as carnosic acid)	n.s.	9.5
Ursolic Acid	n.s,	0.43
Oleanolic Acid	n.s.	0.62
residual ethanol	<2	0.71
water content	<1	0.30
Rosmarinus Officinalis (Rosemary) Leaf Extract (CO_2; 25% diterpene phenols) (and) Helianthus Annuus Seed Oil [19]		
Essential Oil Content	<4	3.0
Phenolic diterpenes:		
rosmanol	n.s.	0.13
7-methyl-rosmanol	n.s.	0.18
carnosol	n.s.	1.4
carnosolic acid	n.s.	18.7
12-methyl-carnosolic acid	n.s.	4.5
sum of phenolic diterpenes	24-26	24.9
Ursolic Acid	n.s.	0.29
Oleanolic Acid	n.s.	0.51
residual ethanol	<2	0.39
water content	<1	0.91
Rosmarinus Officinalis (Rosemary) Leaf Extract (CO_2; 25% diterpene phenols) (and) Helianthus Annuus Seed Oil [16]		
Essential Oil Content	<4	1.7
Phenolic diterpenes:		
rosmanol	n.s.	0.t3
7-methyl-rosmanol	n.s.	0.32
carnosol	n.s	2.9
carnosic acid	> t6	20.6
12-methyl-carnosic acid	n.s.	1.0
sum of phenolic diterpenes	24-26	25.0
Ursolic Acid	n.s.	0.42
Oleanolic Acid	n.s.	0.52
residual ethanol	<2	0.33
water content	<1	0.15

n.s. – not specified

Table 6. Differences in constituent profiles in Rosmarinus officinalis (rosemary) Leaf Extract based on extraction method *[8]

Constituent (ppm)	dried leaves	Extraction Method				
		supercritical CO_2	acetone	ethanol extract, partially deodorized	ethanol extract, deodorized	decolorized and deodorized using hexane and ethanol
Triterpenes						
betulin	<4760	6000	5600	8450	9460	6790
amyrin	<500	34	200	160	230	360
oleanic+ursolic acid	148,100	48,500	100,500	119,800	164,500	60,000
Flavonoids						
genkwanin	2.9	0.65	1.60	2.30	3.66	2.1
Volatiles						
1,8-cineole	56,100	80	1700	1320	53	30
camphor	25,200	220	2360	2080	120	20
borneol	10,000	90	960	840	40	10
Heavy Metals						
lead	2.90	0.09	0.03	0.13	0.15	0.18
arsenic	1.14	<0.034	0.05	0.25	0.25	0.32

* standardized to 10% carnosic acid + carnosol content

Table 7. Toxicity information on constituents of Rosmarinus officinalis (rosemary)

Component	Toxicity information
Phenol Acids	
Caffeic Acid	- in a MMC-induced SCE assay in human lymphocytes, 100 µM caffeic acid enhanced MMC-induced SCEs by 55%; 100 µM caffeic acid alone enhanced MMC-induced SCEs by 26%[77] - caffeic acid is reported to penetrate skin and have UV photoprotective activity[78] - humans and animals metabolize caffeic acid to the same metabolites and hydrolyze chlorogenic acid to caffeic acid; IARC concluded that there is sufficient evidence for carcinogenicity in animal; no data were available on the carcinogenicity in humans, and IARC concluded that caffeic acid is possibly carcinogenic to humans[79] - the carcinogenic potency of caffeic acid, estimated based on an average human intake of 1 mg/kg bw/day, was less than 1000 cancer cases per 1,000,000 individuals; in rats 1 or 2% (10,000 or 20,000 ppm) caffeic acid in the diet for 51 wks to 2 yrs induced papillomas of the forestomach and renal adenomas; one study, in which rats were exposed to 2% (20,000 ppm) caffeic acid in the diet for 2 yrs, showed treatment-induced carcinomas of the forestomach, whereas two studies with shorter exposure durations showed no such effect; caffeic acid was shown to exert strong promotion activity for forestomach carcinogenesis; chronic exposure to caffeic acid in the diet induced hyperplasia of the forestomach (mice, rats, and hamsters), hyperplasia of the kidney (mice and rats), and increased liver and kidney wts (rats); few toxic effects resulted from acute exposure; subchronic dietary exposures did not induce clinical symptoms of toxicity, however, hyperplasia of the forestomach was observed; some genotoxic effects seen in vitro but not in vivo[80]
Chlorogenic Acid	-an antioxidant that inhibited tumor promotion by phorbol esters in mice; some controversy exists over allergic reactions in green coffee beans, but it was accepted that chlorogenic acid was not the allergen[78] -in mice, 2% (20,000 ppm) chlorogenic acid in the diet for 96 weeks induced papillomas and carcinomas of the forestomach, alveolar type II-cell tumors of the lung, and renal cell adenomas; few toxic effects resulted from acute exposure; subchronic dietary exposures did not induce clinical symptoms of toxicity, however, reduced kidney and adrenal wts and hyperplasia of the forestomach were observed; some genotoxic effects seen in vitro but not in vivo[80]
Flavonoids	epidemiological studies implicated high dietary intake levels of flavonoids in heart disease, but a study of cancer risk failed to find a link; some evidence of genotoxicity in bacterial assays, but a European Organization of Cosmetic Ingredients Industries and Services (UNITIS) report stated that flavonoids do not appear to be genotoxic to mammals in vivo; flavonoids are not considered allergens[78]
Diterpenes	
Carnosic Acid	- is a known antioxidant;[81] in a toxicokinetic study in male Sprague-Dawley rats, carnosic acid was absorbed into the blood stream after oral administration and was bioavailable, traces of the acid were found in the intestinal content, liver, and muscle tissue of the abdomen and legs, carnosic acid was present in its free form, and the main rout of elimination was the feces;[81] not mutagenic in an Ames test, with or without metabolic activation, at doses equivalent of the concentration present in up to 6000 µg/plate of a decolorized and deodorized rosemary leaf extract[8]
Carnosol	- topical application of carnosol isolated from rosemary inhibited TPA-induced ear inflammation and tumor promotion in mice;[43] not mutagenic in an Ames test, with or without metabolic activation, at doses equivalent of the concentration present in up to 6000 µg/plate of a decolorized and deodorized rosemary leaf extract[8]

Table 7. Toxicity information on constituents of Rosmarinus officinalis (rosemary)

Component	Toxicity information
Monoterpenes	these chemicals may be skin sensitizers[78]
d- Limonene	- d-limonene consumption has been estimated as 0.2 -2 mg/kg bw/day; in men, oral intake induced transient proteinuria[79] - developmental toxicity in the form of delayed prenatal growth has been observed in mice, rats and rabbits exposed to *d*-limonene during gestation, and skeletal anomalies have also been observed in the fetuses of exposed mice and rabbits;[82] - the few genotoxicity studies available indicated that *d*-limonene and its 1,2-epoxide metabolite are not genotoxic[82] - in a mouse study, administration by gavage did not result in any treatment-related tumors; in a rat study, administration by gavage significantly increased the combined incidence of renal tubular adenomas and carcinomas and induced renal tubular hyperplasia in male rats, but no increases were seen in female rats;[82] oral treatment with *d*-limonene after administration of N-nitrosoethylhydroxy-ethylamine enhanced the development of renal adenomas and renal tubular hyperplasia in male Fischer 344 rats but not in male NBR rats;[79] - IARC found there are sufficient evidence for carcinogenicity in animals, concluding that *d*-limonene produces renal tubular tumors in male rats by a non-DNA-reactive mechanism, through an α_{2u}-globulin-associated response, and therefore, the mechanism by which *d*-limonene increases the incidence of renal tubular tumors in male rats is not relevant to humans; no data were available on the carcinogenicity in humans, and IARC concluded that d-limonene is not classifiable as to its carcinogenicity in humans[82]
α-Pinene	negative in the Ames assay and a mouse micronucleus test[83]
1,8-Cineole	positive in a sister chromatid exchange assay; negative in a chromosomal aberration assay; negative in an Ames test[84]
β-Myrcene	has been reported to cause dermatitis and conjunctivitis in humans; in Wistar rats, the NOAEL for embryotoxicity was 0.5 g/kg bw/day and the NOAEL for peri- and post-natal developmental toxicity was 0.25 g/kg bw/day; was not genotoxic *in vitro* in SCE and chromosomal aberration assays in Chinese hamster cells or human lymphocytes, but it did induce a slight increase is SCEs in cultured hepatic tumor cells; was not genotoxic *in vivo* in rat bone marrow cells[85]
Linalool	safe at up to 4.3% (20% in consumer fragrance); listed as a fragrance allergen by the European Commission[78]
α,β-Thujone	α,β-thujone was not mutagenic in the Ames test; in the micronucleus test, negative in male and positive in female mice; β-thujone: *some evidence of carcinogenicity in male rats* – significant incidence of cancers of the preputial gland in male rats given 25 mg/kg by gavage, and an increase in adrenal gland tumors in male rats may have been due to β-thujone; no increase in in cancer incidence in female rats (dosed with up to 50 mg/kg by gavage) or male or female mice (dosed with up to 25 mg/kg by gavage); all rats dosed with 50 mg/kg and all female mice dosed with 25 mg/kg died[86]
Methyleugenol	- IARC concluded that there is sufficient evidence in experimental animals for carcinogenicity; no data were available on the carcinogenicity in humans, and IARC concluded that methyleugenol is possibly carcinogenic to humans[87]
Terpene Alcohols	
α-Terpineol	- oral LD50 in mice, 2830 mg/kg; 1000 mg/kg bw/day for 2 wks caused reduced body wt gains and an increase in serum cholesterol; not mutagenic in an Ames test or mouse lymphoma assay; did not induce pulmonary tumors in mice given i.p. injections; a derma irritant in animals studies, but not a dermal irritant in a 4-h clinical study; not a sensitizer in guinea pigs; in clinical patch tests, 5% in pet. had 1/1606 positive and 11/1606 questionable reactions in one study and 2/1200 positive reactions in another[88]
Ursolic acid	topical application of carnosol isolated from rosemary inhibited TPA-induced ear inflammation and tumor promotion in mice[43]
Triterpene Alcohols	hepatoprotective and anti-carcinogenic activity has been suggested for lupeol; no toxicity data were available; triterpene alcohols were considered to have intermediate risk[78]

Table 8. Frequency and concentration of use according to duration and type of exposure

	# of Uses[25]	*Max. Conc. of Use (%)*[26]	*# of Uses*[25]	*Max. Conc. of Use (%)*[26]	*# of Uses*[25]	*Max. Conc. of Use (%)*[26]
	Rosmarinus Officinalis (Rosemary) Extract		**Rosmarinus Officinalis (Rosemary) Flower Extract**		**Rosmarinus Officinalis (Rosemary) Flower/Leaf/Stem Extract**	
Totals*	**387**	**0.00004-0.16**	**36**	**NR**	**NR**	**0.0024**
Duration of Use						
Leave-On	*234*	*0.00096 – 0.051*	*11*	*NR*	*NR*	*0.0024*
Rinse Off	*150*	*0.00004 -0.16*	*25*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*3*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	18	0.01-0.05	2	NR	NR	NR
Incidental Ingestion	7	0.011	NR	NR	NR	NR
Incidental Inhalation-Spray	6[a]	0.00096-0.01[a]	1	NR	NR	NR
Incidental Inhalation-Powder	NR	0.05	NR	NR	NR	NR
Dermal Contact	265	0.00096-0.16	11	NR	NR	0.0024
Deodorant (underarm)	NR	not spray: 0.0098 aerosol: 0.0098-0.012	NR	NR	NR	0.0024
Hair - Non-Coloring	112	0.00004-0.003	25	NR	NR	NR
Hair-Coloring	1	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	27	0.0005-0.16	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 8. Frequency and concentration of use according to duration and type of exposure

	# of Uses[25]	*Max. Conc. of Use (%)*[26]	*# of Uses*[25]	*Max. Conc. of Use (%)*[26]	*# of Uses*[25]	*Max. Conc. of Use (%)*[26]
	Rosmarinus Officinalis (Rosemary) Leaf		**Rosmarinus Officinalis (Rosemary) Leaf Extract**		**Rosmarinus Officinalis (Rosemary) Leaf Oil**	
Totals*	**16**	**0.002**	**689**	**0.00001-10**	**516**	**0.00001-1.5**
Duration of Use						
Leave-On	*1*	*0.002*	*422*	*0.00001-10*	*342*	*0.0003-1.5*
Rinse Off	*14*	*NR*	*263*	*0.00001-3*	*149*	*0.00001-0.12*
Diluted for (Bath) Use	*1*	*NR*	*4*	*0.0002-0.04*	*25*	*0.5-0.97*
Exposure Type						
Eye Area	NR	NR	36	0.002-3	8	*NA*
Incidental Ingestion	NR	NR	25	0.00001-0.002	3	0.008
Incidental Inhalation-Spray	NR	NR	9[a]	0.001-0.5 aerosol: 0.0016 pump spray: 0.0001-0.005	32	0.011-1.5 aerosol: 0.007
Incidental Inhalation-Powder	NR	NR	8	0.0002	3	0.0003
Dermal Contact	4	NR	416	0.00001-10	425	0.0003-1.5
Deodorant (underarm)	NR	NR	NR	NR	1	NA
Hair - Non-Coloring	12	0.002	225	0.00001-0.5	87	0.00001-1.5
Hair-Coloring	NR	NR	22	0.04	1	NA
Nail	NR	NR	1	0.005-0.053	NR	NA
Mucous Membrane	1	NR	74	0.00001-3	66	0.002-0.97
Baby Products	NR	NR	7	0.012	4	NA
	Rosmarinus Officinalis (Rosemary) Leaf Powder		**Rosmarinus Officinalis (Rosemary) Leaf Water**		**Rosmarinus Officinalis (Rosemary) Water**	
Totals*	**1**	**0.05**	**22**	**0.000069-1**	**1**	---
Duration of Use						
Leave-On	*1*	*NR*	*7*	*0.000069-1*	*1*	*NR*
Rinse Off	*NR*	*0.05*	*15*	*0.00015-0.25*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	NR	0.000069-0.00016	NR	NR
Incidental Ingestion	NR	NR	NR	0.005	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR
Dermal Contact	1	NR	7	0.00009-0.36	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	0.05	15	0.00019-1	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	0.005	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
	Rosemary [#]					
Totals*	**12**	---				
Duration of Use						
Leave-On	*4*	---				
Rinse Off	*7*	---				
Diluted for (Bath) Use	*1*	---				
Exposure Type						
Eye Area	NR	---				
Incidental Ingestion	NR	---				
Incidental Inhalation-Spray	NR	---				
Incidental Inhalation-Powder	1	---				
Dermal Contact	8	---				
Deodorant (underarm)	NR	---				
Hair - Non-Coloring	4	---				
Hair-Coloring	NR	---				
Nail	NR	---				
Mucous Membrane	2	---				
Baby Products	NR	---				

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses
NR – not reported
[a] Includes suntan preparations, and it t is not known whether or not those product are sprays
[#]Plant part and method of extraction not known

Table 9. Single-dose toxicity studies

Test Article	Extraction Solvent/Method	Species	No./Group	Vehicle	Conc/Dose Range	LD_{50}/Results	Reference
DERMAL							
Rosmarinus Officinalis (Rosemary) Leaf Oil	-----	rabbits	not stated	not stated	not stated	>10 ml/kg	[41]
Rosmarinus Officinalis (Rosemary) Leaf Oil	-----	rabbits	not stated	not stated	not stated	>10 g/kg	[39]
ORAL							
Rosmarinus Officinalis (Rosemary) Leaves – 2 samples; one harvested in autumn (112.7, 477.8, 700.1 µg/mg extract carnosol, carnosic acid, total diterpenes, respectively) and one in spring (45.9, 245.9, 343.1 µg/mg extract carnosol, carnosic acid, total diterpenes, respectively)	supercritical CO_2	Wistar rats	6 M/6F	corn oil	2 g/kg bw [8,22,39-41](gavage)	>2 g/kg	[22]
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	ethanol extract, partially deodorized	mice	not stated	none stated	8.5 g/kg bw (males) 10 g/kg bw (females)	>8.5 g/kg bw (males) >10 g/kg bw (females)	[8]
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	ethanol extract, deodorized	mice	not stated	none stated	24 g/kg bw (males) 28.5 g/kg bw (females)	>24 g/kg bw (males) >28.5 g/kg bw (females)	[8]
Rosmarinus Officinalis (Rosemary) Leaf Oil (see Table 4 for composition)	hydrodistillation	Swiss albino rats	20/group	-----	2-9 g/kg bw (gavage)	LD_{50} = 5.50 g/kg bw LD_{10} = 1.10 g/kg bw LD_{100} = 9 g/kg bw	[40]
Rosmarinus Officinalis (Rosemary) Leaf Oil (see Table 5 for composition)	-----	rats	not stated	none stated	not stated	5 ml/kg bw	[41]

Table 10. Repeated-Dose Toxicity Studies

Test Article	Extraction Solvent/Method	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
				ORAL			
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	ethanol extract, partially deodorized	mice; no./group not stated	5 days (gavage)	none stated	4300 mg/kg bw (males) 5000 mg/kg bw (females)	- no mortality - body wt increased slightly in males, but no changes were seen in females; "marked increase" in fatty liver was observed in males after repeated administration	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table for composition)	ethanol extract, deodorized	mice; no./group not stated	5 days (gavage)	none stated	11,800 mg/kg bw (males) 14,100 mg/kg bw (females)	- no changes in body wts; liver wts of females were slightly increased; fatty livers were observed in test animals at necropsy.	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	acetone	rats; no./group not stated	14 day (diet)	-----	up to 3800 mg/kg diet	- no treatment-related signs of toxicity, mortality, or changes in body wts or feed consumption	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	supercritical CO_2	rats; no./group not stated	14 days (diet)	-----	up to 2400 mg/kg diet	- no treatment-related signs of toxicity, mortality, or changes in body wts or feed consumption	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	acetone	20 rats/group	13 wks (diet)	-----	300, 600, 2400, or 3800 mg/kg diet	- variations in clinical chemistry parameters at times were stat sig, but the researchers stated that because the changes were inconsistent, they were not considered dose-related - stat. sig, decrease in alkaline phosphate in the 3800 mg/kg group - NOAEL was 3800 mg/kg diet	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	supercritical CO_2	20 rats/group	13 wks (diet)	-----	300, 600, or 2400 mg/kg diet	- variations in clinical chemistry parameters at times were stat sig; the researchers stated that because the changes were inconsistent, they were not considered dose-related - a marginal reduction in body weights and feed consumption in the animals of the 2400 mg/kg diet groups were attributed to a lack of palatability of the feed - changes were more notable in females - NOAEL was 2400 mg/kg diet (equiv. to 180 and 200 mg/kg bw/day for males and females, respectively)	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	supercritical CO_2	female rats; no./ group not stated	91 days (diet); 28-day recovery period	-----	0 or 2400 mg/kg diet (equiv. to 0 or 195 mg/kg bw/day)	- slight increase in liver wts after 91-days of dosing, but not in those killed after the 28-day recovery period - an increase in microsomal protein concentration observed after 91 days of dosing was also reversible - no notable effects on the activity of selected enzymes	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	ethanol extract, partially deodorized	Sprague-Dawley rats; no./group not stated	90 days (diet)	-----	0, 500, 1500,or 5000 mg/ kg diet (equiv. to 0, 40, 120, or 400 mg/kg bw/day)	- a dose-response relationship was observed for relative liver-to-body wt; extracts; a slight but stat sig increase was observed - no microscopic changes in the liver were reported	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	ethanol extract, deodorized	Sprague-Dawley rats; no./group not stated	90 days (diet)	-----	0, 500, 1500,or 5000 mg/ kg diet (equiv. to 0, 40, 120, or 400 mg/kg bw/day)	- a dose-response relationship was observed for relative liver-to-body wt; extracts; a slight but stat sig increase was observed - no microscopic changes in the liver were reported	8

Table 10. Repeated-Dose Toxicity Studies

Test Article	Extraction Solvent/Method	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
Rosmarinus Officinalis (Rosemary) Leaf Extract (see Table 5 for composition)	hexane and ethanol (2-step extraction)	Sprague-Dawley rats; no./group not stated	3 mos (diet); 28-day interim group; 1-mo recovery period	-----	0, 1000, 2500, or 5000 mg/ kg diet (equiv. to 0, 65, 164, or 320 mg/kg bw/day)	- no signs of toxicity, no mortality and no gross lesions at necropsy - reversible dose-dependent increases in absolute liver wts and relative liver-to-body wts; stat sig in the high dose group only - treatment-related increase in bile duct hyperplasia at the interim necropsy; the incidence was decreased at the end of dosing and not seen after recovery - in females, a decrease in pancreas wt was observed at the interim necropsy - no stat sig changes in hematology parameters, and no microscopic changes - the NOAEL was at least 320 mg/kg bw/day	8
Rosmarinus Officinalis (Rosemary) Leaf Extract (after the volatile oil [1.1%] was removed)	absolute ethanol	Swiss albino mice; 6M/group	3 wks (gavage)	olive oil	1500 mg/kg extract controls – olive oil	no stat sig changes in relative liver, spleen, heart, or lung wt to body wt compared to controls; there were no stat sig changes in clinical chemistry parameters	40
			single dose CCl_4 (gavage), then 3 wks extract (gavage)	olive oil	3.3% CCl_4 (100 mg/kg bw) 1500 mg/kg extract	- with CCl_4 only, stat sig increases in relative liver to body wt (18%) and spleen to body wt (45.6%) compared to olive oil controls; CCl_4 affected all measured clinical chemistry parameters - with the extract, the increase in relative spleen to body wt was stat sig, but not as great as with CCl_4 alone (34.9%); there was no stat sig increase in relative liver to body wt; many of the changes in clinical chemistry values were reduced or were non-stat sig	
Rosmarinus Officinalis (Rosemary) Leaf Oil (see Table 4 for composition)	hydrodistillation	Swiss albino mice; 6M/group	3 wks (gavage)	-----	1100 mg/kg bw controls – olive oil	no stat sig changes in relative liver, spleen, heart, or lung wt to body wt compared to controls; there were no stat sig changes in clinical chemistry parameters	40
			single dose CCl_4 (gavage), then 3 wks oil (gavage)	olive oil (for CCl_4)	3.3% CCl_4 (100 mg/kg bw) 1100 mg/kg extract	- (effects of CCl_4 only are described above) - with the oil, the increases in relative liver to body wt (9.8%) and spleen to body wt (38.8%) were stat sig, but not as great as with CCl_4 alone; many of the changes in clinical chemistry values were reduced but were still stat sig	

Abbreviations: CCl_4: - carbon tetrachloride; conc – concentration; equiv. – equivalent; NOAEL – no-observable adverse effect level; stat sig – statistically significant

Table 11. Genotoxicity studies

Test Article	Extraction Solvent/Method	Conc./Vehicle	Procedure	Test System	Results	Reference
			IN VITRO			
Rosemary Extract (not defined; water-soluble; contained 17% rosmarinic acid)	-----	50, 100, or 200 µg/plate	Ames test, with and without metabolic activation	*S. typhimurium* TA98	not mutagenic	54
as above	-----	50 µg/ml (highest non-cytotoxic dose)	comet assay	human hepatoma cell line (HepG2)	not genotoxic	54
Rosemary Extract (not defined; oil-soluble; contained 50.27% carnosic acid and 5.65% carnosol)	-----	50, 100, or 200 µg/plate	Ames test, with and without metabolic activation	*S. typhimurium* TA98	not mutagenic	54
as above	-----	5 µg/ml (highest non-cytotoxic dose)	comet assay	human hepatoma cell line (HepG2)	not genotoxic	54
Rosmarinus Officinalis (Rosemary) Leaf Extract	supercritical CO_2	up to 5000 µg/plate	bacterial assay, with and without metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA102	not mutagenic - in TA102 only, toxicity at the highest dose with metabolic activation	8
Rosmarinus Officinalis (Rosemary) Leaf Extract	ethanol extract,, partially deodorized	up to 20,000 µg/plate	bacterial assay, with and without metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA102	not mutagenic - some bactericidal effects in all strains; effects were reduced with metabolic activation	8
Rosmarinus Officinalis (Rosemary) Leaf Extract	ethanol extract, deodorized	up to 20,000 µg/plate	bacterial assay, with and without metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA102	not mutagenic - some bactericidal effects in all strains; effects were reduced with metabolic activation	8
Rosmarinus Officinalis (Rosemary) Leaf Extract	hexane and ethanol (2-step extraction)	up to 6000 µg/plate	Ames test, with and without metabolic activation	*S. typhimurium* TA97, TA98, TA100, TA102	-mutagenic in TA102 in one set of trials; not reproducible with less cytotoxic conc -not mutagenic in the other strains - without metabolic activation: bactericidal for all strains at 3000-6000 µg/plate; bactericidal to TA102 at almost all dose levels -with metabolic activation, bactericidal only at the highest dose level, if at all	8
Rosmarinus Officinalis (Rosemary) Leaf Extract	ethanol extract, partially deodorized	up to 100 mg/ml	chromosomal aberration assay, with and without metabolic activation	human lymphocytes	not genotoxic	8
Rosmarinus Officinalis (Rosemary) Leaf Extract	hexane and ethanol (2-step extraction)	not clearly specified but at least up to 50 µg/ml without and 35 µg/ml with metabolic activation	gene-locus mutation assay, with and without metabolic activation	thymidine kinase (tk) and hgprt loci of a human lymphoblastoid cell line (TK6)	-not genotoxic without metabolic activation at up to 50 µg/ml - 35 µg/ml increased mutations in the tk, but not the hgprt, locus with activation; the increase was stat sig when compared to solvent control, but not when compared to untreated cells; determined to be not mutagenic under the conditions used because of a lack of a dose-dependent increase in mutation frequency and a lack of a stat sig increase of mutation frequency compared to controls	8
Rosmarinus Officinalis (Rosemary) Leaf Oil	-----	not stated	Ames test	not stated	negative	51

Table 11. Genotoxicity studies

Test Article	Extraction Solvent/Method	Conc./Vehicle	Procedure	Test System	Results	Reference
			IN VIVO			
Rosmarinus Officinalis	hydro-alcoholic	6.43, 100, and 200 mg/kg bw	chromosomal aberration assay	Wistar rats; 6/group	not genotoxic	52
Rosmarinus Officinalis	hydro-alcoholic	6.43, 100, and 200 mg/kg bw	micronucleus assay	Wistar rats; 6/group	not genotoxic	52
Rosmarinus Officinalis (Rosemary) Leaf Extract (after the volatile oil [1.1%] was removed)	absolute ethanol	1500 mg/kg bw/day in olive oil	micronucleus test; dosed by gavage for 7 days; negative controls were given olive oil; positive controls were given a single i.p. dose of 100 mg/kg bw CPA; bone marrow cells collected 24 h after dosing	Swiss albino mice	not genotoxic; no stat sig change in the number of MNPCE or NCE or in PCE/NCE	40
Rosmarinus Officinalis (Rosemary) Leaf Oil (see Table 4 for composition)	hydrodistillation	1100 mg/kg bw/day	same protocol	Swiss albino mice	no stat sig change in no. of MNPCE no. of NCE was stat sig decreased ($p<0.05$) PCE/NCE was stat sig increased ($p<0.01$)	40
Rosmarinus Officinalis (Rosemary) Leaf Oil	hydrodistillation	300, 1000, or 2000 mg/kg bw (by gavage)	chromosome aberration assay; single 0.5 ml dose; negative controls were given distilled water; positive controls were dosed with 50 mg CPA/kg; bone marrow cells collected 24 h after dosing	Wistar rats; 3M/3F per group	- chromosomal aberrations without gaps were stat sig increased at 2000 mg/kg bw - mitotic index was stat sig increased with 300 mg/kg, but not with other doses or the positive control	14
Rosmarinus Officinalis (Rosemary) Leaf Oil	hydrodistillation	300, 1000, or 2000 mg/kg bw (by gavage)	micronucleus test; single 0.5 ml dose; negative controls were given distilled water; positive controls were dosed with 50 mg CPA/kg; bone marrow cells collected 24 h after dosing	Swiss mice; 3M/3F per group	- stat sig increase in MNPCEs with 1000 and 2000 mg/kg bw - PCE/NCE was not stat sig different from controls	14
Rosmarinus Officinalis (Rosemary) Leaf Oil	hydrodistillation	300, 1000, or 2000 mg/kg bw (by gavage)	micronucleus test; protocol as above; bone marrow cells collected 24 h after dosing	Wistar rats; 3M/3F per group	stat sig increase in MNPCEs with 2000 mg/kg bw	14
Rosmarinus Officinalis (Rosemary) Leaf Oil	hydrodistillation	300, 1000, or 2000 mg/kg bw (by gavage)	comet assay; single 0.5 ml dose; negative controls were given distilled water; positive controls were dosed with 50 mg cyclophosphamide/kg; liver and peripheral blood cells collected 24 h after dosing	Swiss mice; 3M/3F per group	all 3 doses induced stat sig increases in DNA damage in peripheral blood cells and liver cells; most of the damaged cells showed minor damage, very few had a large amount of damage	14
mixture containing 19% Rosmarinus officinalis (rosemary) leaves, 71..5% St. John's Wort; 9.5% spirulina	-----	0, 380, 760, or 1520 mg/ kg bw/day in water (gavage)	micronucleus test; mice were dosed for 7 days; femoral bone marrow cells were used	male Swiss albino mice; 30/group	- stat. sig. increase in MNPCEs with 760 and 1520 mg/kg bw/day - PCE/NCE was not stat sig different from controls	50
mixture defined above	-----	0, 380, 760, or 1520 mg/ kg bw/day in water (gavage)	chromosomal aberration assay; mice were dosed for 7 days and killed 19 days after last dose	male Swiss albino mice; 30/group	- stat sig increased in frequency of aneuploidy with 760 and 1520 mg/kg bw/day - % polyploids and total % aberrations were stat sig increased at these doses	50

Table 11. Genotoxicity studies

Test Article	Extraction Solvent/Method	Conc./Vehicle	Procedure	Test System	Results	Reference
mixture defined above	-----	0, 380, 760, or 1520 mg/ kg bw/day in water (gavage)	assay for spermatozoa abnormality; mice were dosed for 7 days and killed 5 wks after last dose	male Swiss albino mice; 30/group	- stat sig increase in frequency of banana-shaped, swollen achrosome, and triangular head sperm abnormalities with 1520 mg/kg bw/day - % total spermatozoa abnormalities stat sig increased with 1520 mg/kg bw/day	[50]
ANTI-MUTAGENIC EFFECTS						
			IN VITRO			
Rosemary Extract (not defined; contained 8.8-10.6% carnosic acid and 1.2-1.4% carnosol) + tBOOH	-----	≤0.8 mg/ml in medium-chain triglycerides; only the carnosic acid and carnosol were soluble	Ames test; 0.5 ml rosemary extract was incubated with 0.5 ml tBOOH	*S. typhimurium* TA102	stat sig reduced tBOOH-induced mutagenicity	[53]
Rosemary Extract (not defined; water-soluble; contained 17% rosmarinic acid) + IQ	-----	50, 100, or 200 µg/ plate extract 10 ng/plate IQ	Ames test, with metabolic activation	*S. typhimurium* TA98	a stat sig reduction in IQ-induced genotoxicity was observed only at the highest dose	[54]
as above + NQNO	-----	0, 50, 100, or 200 µg/ plate extract 500 ng/plate NQNO	Ames test, without metabolic activation	*S. typhimurium* TA98	no stat sig effect on NQNO-induced genotoxicity	[54]
as above + tBOOH	-----	0, 0.05, 0.5, 5, or 50 µg/ml extract; 0.05 mM tBOOH	Comet assay; pretreatment with extract for 21 h, followed by 20 min exposure to tBOOH	human hepatoma cell line (HepG2)	stat sig reduction in tBOOH-induced DNA damage at all doses; the reduction was not dose-dependent – 0.05 µg/ml caused a greater reduction than 0.5 µg/ml	[54]
as above + tBOOH	-----	0, 0.05, 0.5, 5, or 50 µg/ml extract; 0.05 mM tBOOH	Comet assay; co-treatment with extract and tBOOH for 20 min	human hepatoma cell line (HepG2)	no stat sig effect on tBOOH-induced DNA damage	[54]
as above + tBOOH	-----	0, 0.05, 0.5, 5, or 50 µg/ml extract; 0.05 mM tBOOH	Comet assay; pretreatment with extract for 21 h, followed by co-treatment with extract and tBOOH for 20 min	human hepatoma cell line (HepG2)	stat sig reduction in tBOOH-induced DNA damage at all except the lowest dose	[54]
as above + BaP	-----	0, 0.05, 0.5, 5, or 50 µg/ml extract; 40 µM BaP	by co-treatment with extract and BaP for 21 h	human hepatoma cell line (HepG2)	stat sig reduction in BaP-induced DNA damage only at the highest dose	[54]
as above + PhIP	-----	0, 0.05, 0.5, 5, or 50 µg/ml extract; 80 µM PhIP	Comet assay; by co-treatment with extract and PhIP for 21 h	human hepatoma cell line (HepG2)	stat sig reduction in PhIP-induced DNA damage only at the highest dose	[54]
Rosemary Extract (not defined; oil-soluble; contained 50.27% carnosic acid and 5.65% carnosol) + IQ	-----	50, 100, or 200 µg/ plate extract 10 ng/plate IQ	Ames test, with metabolic activation	*S. typhimurium* TA98	suppressed IQ-induced mutations in a stat sig, dose-dependent, manner	[54]
as above + NQNO	-----	50, 100, or 200 µg/ plate extract 500 ng/plate NQNO	Ames test, without metabolic activation	*S. typhimurium* TA98	suppressed NQNO-induced mutations in a stat sig, dose-dependent, manner	[54]
as above + tBOOH	-----	0, 0.05, 0.5, or 5 µg/ml extract; 0.05 mM tBOOH	comet assay; pretreatment with extract for 21 h, followed by 20 min exposure to tBOOH	human hepatoma cell line (HepG2)	stat sig reduction in tBOOH-induced DNA damage at all doses	[54]

Table 11. Genotoxicity studies

Test Article	Extraction Solvent/Method	Conc./Vehicle	Procedure	Test System	Results	Reference
as above + tBOOH	-----	0, 0.05, 0.5, or 5 µg/ml extract; 0.05 mM tBOOH	comet assay; co-treatment with extract and tBOOH for 20 min	human hepatoma cell line (HepG2)	no stat sig effect on tBOOH-induced DNA damage	[54]
as above + tBOOH	-----	0, 0.05, 0.5, or 5 µg/ml extract; 0.05 mM tBOOH	comet assay; pretreatment with extract for 21 h, followed by co-treatment with extract and tBOOH for 20 min	human hepatoma cell line (HepG2)	stat sig reduction in tBOOH-induced DNA damage at all doses; the reduction was not dose-dependent`	[54]
as above + BaP	-----	0, 0.05, 0.5, or 5 µg/ml extract; 40 µM BaP	by co-treatment with extract and BaP for 21 h	human hepatoma cell line (HepG2)	stat sig reduction in BaP-induced DNA damage at the two highest doses	[54]
as above + PhIP	-----	0, 0.05, 0.5, or 5 µg/ml extract; 80 µM PhIP	by co-treatment with extract and PhIP for 21 h	human hepatoma cell line (HepG2)	stat sig reduction in PhIP-induced DNA damage at the two highest doses	[54]
			IN VIVO			
Rosmarinus Officinalis (Rosemary) Leaf Extract (after the volatile oil [1.1%] was removed) + CPA	absolute ethanol	1500 mg/kg bw/day in olive oil	micronucleus test; dosed by gavage with the extract for 7 days, then given a single i.p. dose of 100 mg/kg bw CPA; bone marrow cells collected 24 h after dosing; olive oil was used as a negative control	Swiss albino mice	stat sig increase in the number of MNPCE and NCE compared to olive oil only; no stat sig change in PCE/NCE	[40]
Rosmarinus Officinalis (Rosemary) Leaf Oil (contained 20.86% bornyl acetate; 16.24% L-camphor, and 8.25% borneol) + CPA	hydrodistillation	1100 mg/kg bw/day	micronucleus test; dosed by gavage with the oil for 7 days, then given a single i.p. dose of 100 mg/kg bw CPA; bone marrow cells collected 24 h after dosing; olive oil was used as a negative control	Swiss albino mice	stat sig increase in the number of MNPCE and NCE, and a stat sig decrease in PCE/NCE, compared to olive oil only	[40]

Abbreviations: BaP – benzo(*a*)pyrene; conc – concentration; CPA - cyclophosphamide: IQ – 2-amino-3-methyl-3H-imidao[4,5-F]quinoline; MMS – methyl methanesulfonate; MNPCE – micronucleated polychromatic erythrocytes; NCE – normochromatic erythrocytes; NQNO – 4-nitroquinoline-N-oxide; PCE/NCE – ratio of polychromatic erythrocytes to normochromatic erythrocytes; PhIP – 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; stat sig – statistically significant; tBOOH - t-butyl hydroperoxide

Table 12. Anti-tumor activity

Test Article	Extraction Solvent/Method	Dose/Exposure Route	Species No./Group	Tumor Type	Carcinogenicity Model	Results	Reference
Rosmarinus Officinalis (Rosemary) Leaf Extract (contained 16.5-19.2% urosolic acid; 3.8-4.6% carnosol; 0.1-0.5% carnosic acid; trace-0.1% miltirone)	methanol	1.2 or 3.6 mg; dermal	CD-1 mice; 30F/grp	skin	- initiation: topical treatment with 200 nmol DMBA in 200 µl acetone - promotion: after 1 wk, topical treatment with 200 µl acetone (controls), 5 nmol TPA in 200 µl acetone (carc grp), or 5 nmol TPA and extract in 200 µl acetone (RE grp), 2x/wk, for 20 wks	1.2 mg: decreased tumor/mouse by 48, 27, and 28% after 7, 11, and 15 wks TPA promotion 3.6 mg: decreased tumor/mouse by 84, 37, and 48% after 7, 11, and 15 wks TPA promotion	[43]
as above	methanol	1.2 or 3.6 mg; 5 min prior to B(a)P; dermal	CD-1 mice; 30F/grp	skin	- initiation: topical treatment with 200 µl acetone (controls) or with extract in 200 µl acetone (RE grp) 5 min prior to each 20 nmol application of B(a)P or 2 nmol DMBA, 1x/wk, for 10 wks - promotion: after 1 wk, promotion with 15 nmol TPA in 200 µl acetone, 2x/wk, for 20 wks	1.2 mg: decreased tumor/mouse by 15, 42, and 54% after 9, 13, or 21 wks TPA promotion 3.6 mg: decreased tumor/mouse by 62, 63, and 64% after 9, 13, or 21 wks TPA promotion	[43]
as above	methanol	3.6 mg; dermal	CD-1 mice; 30F/grp	skin	- initiation: topical treatment with 200 µl acetone (controls) or 3.6 mg extract in 200 µl acetone (RE grp) at 120, 60, and 5 min before topical application of 200 nmol B(a)P in 200 µl acetone - promotion: after 1 wk, 15 nmol in 200 µl acetone, 2x/wk, for 20 wks	decreased tumor/mouse by 83, 81, and 58% after 9, 13, or 21 wks TPA promotion	[43]
Rosmarinus Officinalis (Rosemary) Leaf Extract	DDW	500 mg/kg bw; gavage	Swiss albino mice; 12M/grp	skin	DMBA-initiated and croton oil-promoted skin tumorigenesis Grp 1: controls – topical treatment with 100 µl acetone; DDW by gavage for 15 wks Grp 2: 500 mg/kg bw/day RE in 100 µl DDW for 15 wks Grp 3: single topical dose 100 µg DMBA in 100 µl acetone; 2 wks later, 1% croton oil in acetone, 3 x/wk; also, 100 µl by gavage for 15 wks Grp 4: single topical dose 100 µg DMBA in 100 µl acetone; 500 mg/kg bw RE by gavage 7 days before, during, and 7 days after DMBA; 2 wks after DMBA, 1% croton oil in acetone, 3x/wk Grp 5: single topical dose 100 µg DMBA in 100 µl acetone; after 2 wks, 500 mg/kg bw RE extract by gavage for 15 days and 1% croton oil in acetone 3x/wk Grp 6: single topical dose 100 µg DMBA in 100 µl acetone; 500 mg/kg bw RE by gavage 7 days before DMBA until study end; 2 wks after DMBA, 1% croton oil in acetone, 3x/wk	-a stat sig decrease in tumor number, diameter, and weight and a stat sig increase in the avg. latency period was observed in grps given RE compared to Grp 3 (the carcinogen-control grp) - blood serum and liver lipid peroxidation level was stat sig decreased in all RE grps compared to grp 3 - Grp 6 had the greatest changes for all the above parameters - no tumors were found in animals given RE only - RE had no effect on body weight gains	[89]
Rosmarinus Officinalis (Rosemary) Leaf Extract	DDW	1000 mg/kg bw in DDW; gavage	Swiss albino mice; 12M/grp	skin	DMBA-initiated and croton oil-promoted skin tumorigenesis -same protocol as above (Grps 1-6), except 1000 mg/kg bw RE was used	- stat sig decrease in tumor burden and tumor yield, and a stat sig increase in avg. latency period, in grps given RE compared to Grp 3 (the carcinogen-control grp); tumor incidence was decreased - blood serum lipid peroxidation level was stat sig decreased in all RE grps, and the liver glutathione levels stat sig increased, compared to grp 3 - RE did not cause any adverse effects; no tumors were seen in the RE-only grp.	[90]

Table 12. Anti-tumor activity

Test Article	Extraction Solvent/Method	Dose/Exposure Route	Species No./Group	Tumor Type	Carcinogenicity Model	Results	Reference
Rosmarinus Officinalis (Rosemary) Extract	not specified	1.0%, in diet	Sprague-Dawley rats; 20F/grp	mammary	- rats were fed untreated or RE-supplemented diet throughout the study (16 wks post-DMBA) - after 27 days of the test diet, each rat was dosed with 30.9 mg/kg bw DMBA in corn oil by gavage	- the incidence of palpable mammary tumors was less in the RE-fed rats than the controls; at study termination, the tumor incidence was 47% less; this difference was stat sig - the difference in tumors per tumor-bearing rat was not stat sig btwn the two grps - at study termination, 94% and 90% of tumor-bearing rats of the control and RE groups, respectively, possessed mammary adenocarcinomas - RE had no effect on body wt	[91]

Abbreviations: B(a)P – benzo[a]pyrene; DDW – double-distilled water; DMBA – 7,12-dimethylbenz[a]anthracene; grp – group; GR – glutathione reductase; GSH – reduced glutathione; GST – glutathione-s-transferase; RE – Rosmarinus officinalis (rosemary) leaf extract; stat sig – statistically significant; TPA – 12-*O*-tetradecanoylphorbol-13-acetate

Table 13. Case reports with *Rosmarinus officinalis* (rosemary)

Mode of Contact	Indication	Patch Testing	Reference
cosmetics and cleansing gel containing 0.1% Rosmarinus officinalis (rosemary) leaf extract	itchy erythema of the face; red papules around the eyes and on the nose and cheeks	patch test with cosmetics and 1% aq. cleansing gel gave positive result (+) to gel only on D3 - patch tested gel ingredients, only positive reaction (+) was to 0.1% aq. Rosmarinus officinalis (rosemary) leaf extract on D3	[61]
occupational exposure to a Rosmarinus officinalis (rosemary) leaf extract	severe hand, forearm, and face dermatitis	patch tested with 5 and 10% extract in petrolatum; + reaction to 5 and 10% on D2 and D5; 1 control was negative - patch tested with carnosol in ethanol; ?+ reaction to 0.1% at 3 and D7, + reaction to 1% on D3 and D7; controls were negative to 0.1 (n=110) and 1% (n=116) carnosol	[62]
occupational use of essential aromatherapy oils (5 cases)	hand eczema in all; other involvement seen	- patch testing with the European baseline series, fragrance series, and 2% of each essential oil in petrolatum; ++ reaction to rosemary oil in 2 subjects, + in one, among other positive reactions	[63]
history of eating foods spiced with rosemary	severe cheilitis	patch tested with 41 antigens, 21 flavoring agents and dyes, and medications; ++ on D2 and + on D5 to rosemary (also + to nickel on D2 and D5; + to wood tars on D2)	[64]
picked rosemary leaves	developed hand, forearm, and face dermatitis within hours	prick-by-prick testing was negative at 15 min and positive (++) at D2 - patch testing gave positive reactions with rosemary (++) and thyme (+) on D2 and D4 - a photopatch test (10 J/cm) with rosemary and thyme showed stronger reactions (+++ and ++, respectively, on D4) - 5 controls were negative	[65]
walked near, and touched, odorous plants	cutaneous lesions on the hand and face; developed edema and eczematous lesions on her hands, eyelids, and face	patch and photopatch test with 1% rosemary extract was positive (+++) - patch and photopatch test with rosemary leaves was positive; more intense with photopatch (++/+++) - hydrophilic and lipophilic rosemary extracts 10%, patch and photopatch tests were positive - patch test with 0.1% carnosol in alcohol was positive - patch test with sage and oregano were negative -5 controls were negative with all	[66]
rosemary leaf plasters applied to knee	after 3 days, acute dermatitis in the application area	positive (++ on D2; +++ on D4) reactions in a patch test with rosemary leaves, but not thyme, origanum, or mint - 10 controls did not react to rosemary leaves	[67]
applied a poultice containing rosemary and thyme	after 24 h, acute, cutaneous, eczematous lesion on right thigh, with vesicles and blisters	positive patch test results with the poultice (++ on D2 and D4); rosemary (++ on D2 and D4); thyme (- on D2, ++ on D4); and colophony (+ on D2 and D4); negative results with arnica, chamomile, and horsetails - 12 controls were negative with rosemary and thyme	[68]
rosemary alcohol applied to chest	swelling of face, chest, and dorsal aspect of arms, followed by peeling	positive reactions were found in patch test with fresh Rosmarinus officinalis (rosemary) leaves (+++ on D2, D3, D4), dry rosemary leaves (+ reaction on D2, D3, D3), dry leaves wetted with water (+ reaction on D2, D3, D3), the flower (++ reaction on D2, D3, D3), and rosemary alcohol ((+ reaction on D2, D3, D3) - negative reactions to 50% aq. rosemary alcohol - positive reactions were also found with sage and lavender	[69]

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