Article

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

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Abstract

The Cosmetic Ingredient Review Expert Panel (Panel) assessed the safety of 10 *Rosmarinus officinalis* (rosemary)-derived ingredients and concluded these ingredients are safe as used in cosmetics when formulated to be nonsensitizing. The R officinalis-derived 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 final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should continue to use good manufacturing practices to limit impurities that could be present in botanical ingredients.

Keywords

safety, cosmetics, rosemary, *Rosmarinus officinalis*

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 waters derived from a defined part of the *R officinalis* (rosemary) plant.

R 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 use as a fragrance ingredient.[1] Two of the ingredients, that is, rosmarinus officinalis (rosemary) flower extract and rosmarinus officinalis (rosemary) leaf extract, are reported to function as antioxidants. However, rosmarinus officinalis (rosemary) leaf powder is reported to function only as a flavoring agent.

Chemistry

Definition

The definition of each *R officinalis* (rosemary)-derived ingredient indicates what part(s) of the plant from which the ingredient is obtained (Table 1). In some cases, the definition also gives insight as to the method of manufacture.

General Characterization

The *R officinalis* L. plant, from the botanical family Lamiaceae, is a scented, evergreen shrub with a very pungent odor

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Table 1. Definitions and Reported Functions.

Ingredient (CAS number)	Definition[1]	Reported function(s)[1]
Rosmarinus officinalis (rosemary) extract (84604-14-8)	The extract of the whole plant *R officinalis*	Skin conditioning agent—misc
R officinalis (rosemary) flower extract	The extract of the flowers of *R officinalis*	Antioxidant; deodorant agents; skin conditioning agents—misc
R officinalis (rosemary) flower/leaf/stem extract	The extract of the flowers, leaves, and stems of *R officinalis*	Fragrance ingredients; skin conditioning agents—misc
R officinalis (rosemary) flower/leaf/stem water	The aqueous solution of the steam distillates obtained from the flowers, leaves, and stems of *R officinalis*	Fragrance ingredient
R officinalis (rosemary) leaf	The leaf of *R officinalis*	Skin-conditioning agents—misc
R officinalis (rosemary) leaf extract (84604-14-8)	The extract of the leaves of *R officinalis*	Antimicrobial agents; antioxidant; fragrance ingredients; skin conditioning agents—mis; skin conditioning agents—occlusive
R officinalis (rosemary) leaf oil (8000-25-7)	The essential oil obtained from the flowering tops and leaves of *R officinalis*	Fragrance ingredients; skin conditioning agents—misc
R officinalis (rosemary) leaf powder	The powder derived from the dried, ground leaves of *R officinalis*	Flavoring agents
R officinalis (rosemary) leaf water	An aqueous solution of the steam distillate obtained from the leaves of *R officinalis*	Fragrance ingredient
R officinalis (rosemary) water	An aqueous solution of the steam distillate obtained from *R officinalis*	Fragrance ingredient

Abbreviation: misc, miscellaneous.

that is native to the Mediterranean region and Portugal; the odor is sometimes defined as camphor-like.[2,3] Rosemary has a spicy, harsh, bitter, aromatic taste. Bluish labiate flowers grow on the upper green part of the branches. Rosemary oil is produced mostly in Spain, France, and Tunisia.[4]

R officinalis L. is generally recognized as safe (GRAS) in foods as a spice and as a 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

R 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 *R officinalis*. Food-grade acetone, ethanol, hexane, or a combination of hexane and ethanol (in a 2-step process) are used as extraction solvents; the ethanol extract is sometimes deodorized or partially deodorized ethanol.[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.

An additional method of manufacturing the cosmetic ingredients 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").[9]

Both rosmarinus officinalis (rosemary) leaf extracts and rosmarinus officinalis (rosemary) leaf oil can be produced by supercritical fluid extraction with natural CO_2 and a small amount of ethanol as a solvent.[10-13] One supplier of the leaf extract reported that the essential oil is removed by multistep separation,[12] and a supplier of the leaf oil adds a small amount ($<4\%$) of sunflower oil to increase solubility when blending.[13]

Food-grade *R officinalis* (rosemary) leaf oil is the volatile oil obtained by steam distillation from the fresh flowering tops or dried crushed aerial parts of *R officinalis* L.[7] The oil from *R officinalis* can also be obtained by hydrodistillation of dried crushed aerial parts.[14] Essential oils prepared by a steam distillation process yields 2 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 the term water in the name. Therefore, rosmarinus officinalis (rosemary) leaf water is the water-soluble fraction of the steam distillation of *R officinalis* (rosemary) leaves.

Constituents/Impurities

R 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 to 5.

Table 2. Chemical and Physical Properties.

Property	Description	Reference
Rosmarinus officinalis (rosemary) leaf		
Odor	Strongly aromatic	36
R 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)	10,11
Solubility	Insoluble in water	7
Refractive index	1.4710-1.4740	16
Density	0.9165-0.9220	16
R officinalis (rosemary) leaf oil		
Physical state and appearance	Colorless or pale yellow liquid with characteristic odor and a warm, camphoraceous taste	7,35
	Colorless, pale yellow, or pale green liquid with a camphorous odor	73
Solubility	Almost insoluble in water	35
	Soluble in most vegetable oils; insoluble in alcohol and in propylene glycol	7
Density (d^{25}_{25})	0.894-0.912	35
	0.907-0.920	73
Index of refraction (n_D^{20})	1.464-1.476	35
R officinalis (rosemary) leaf powder		
Physical state and appearance	Grayish green to yellowish green powder	36

A detailed list of chemical constituents by plant part is presented in Table 3, and a more focused listing of constituents of *R officinalis* is provided in Table 4. Table 5 provides composition data on 3 *R 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%.[15-18]

According to the European Cosmetic Regulations, certain fragrance allergen compounds are subject to declaration on the label if the concentration of a specified allergen exceeds 0.001% in leave-on and 0.01% in rinse-off products.[19] One supplier declared the following concentrations of allergen compounds in a rosmarinus officinalis (rosemary) leaf extract: <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% to 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 safety assessment provided information on the amount of constituents present in the test article; when this information was available, it is included.

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] High-performance liquid chromatography analysis of dimethyl sulfoxide extracts of rosemary leaves indicated the highest accumulation rate of the phenolic diterpenes carnosic acid, carnosol, and 12-*O*-methylcarnosic acid, of rosmarinic acid, and of the flavones genkwanin and isocutellarein 7-*O*-glucoside was found in the young stages of plant development.[23] The diterpenes and rosmarinic acid, but not the flavones, were found in the flower, stem, and root extracts at lower concentrations than in the leaves during the early stages of plant growth, but the concentration of each, except for 12-*O*-methylcarnosic acid, tended to increase during flowering. Rosmarinic acid concentrations in the leaves also decreased once flowering started, while the level in the flowers was slightly increased during flowering. The flavones acted similarly to carnosic acid.

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.[24] 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.[25]

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.[7]

Table 7 provides toxicity and other information on some constituents of *R officinalis* (rosemary)-derived ingredients.

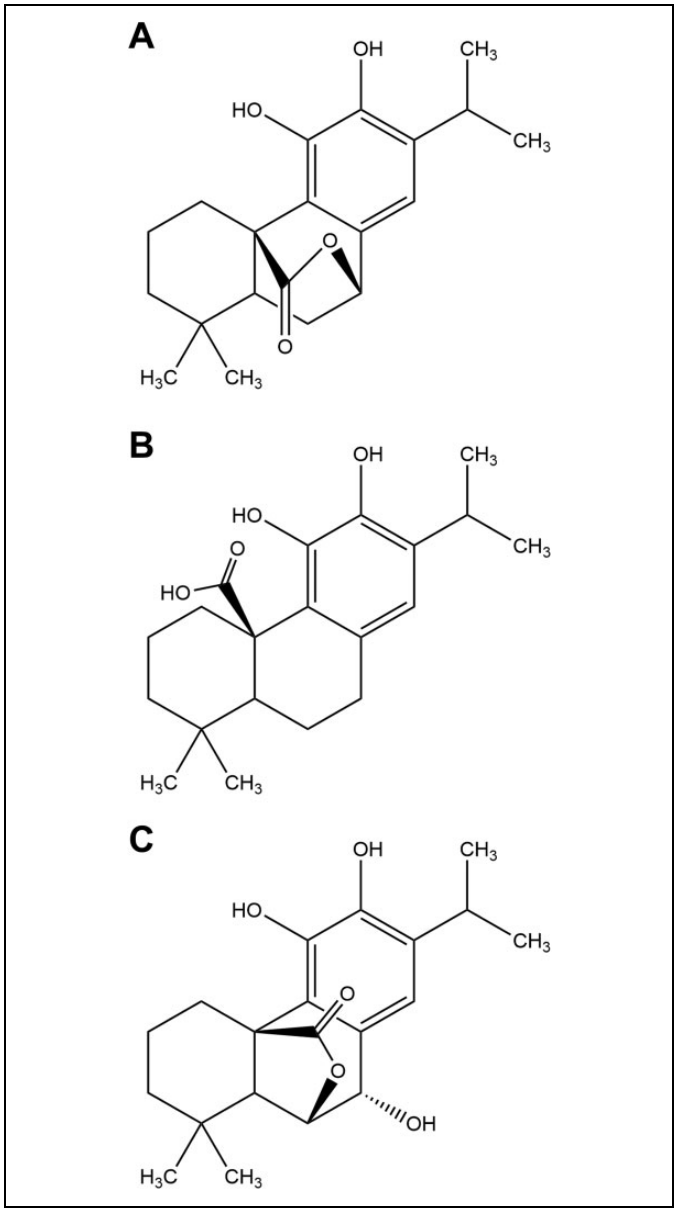

Figure 1. Principal diterpenes. A, Carnosol. B, Carnosic acid. C, Rosmanol.

Use

Cosmetic

The *R officinalis* (rosemary)-derived ingredients included in this safety assessment have a variety of functions in cosmetics (Table 1). 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]

The US 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). The VCRP data obtained from the FDA in 2014,[26] and data received in response to a survey of the maximum reported use concentration by category conducted by the Personal Care Products Council (Council)[27,28] in 2013, indicating that 9 of the 10 ingredients included in this safety assessment are currently used in cosmetic formulations (Table 8). Rosmarinus officinalis (rosemary) leaf extract has the greatest number of uses, 729, followed by rosmarinus officinalis (rosemary) leaf oil, 474 uses, and rosmarinus officinalis (rosemary) extract, 404 uses. According to the results of the concentration of use survey, most cosmetic formulations contain very low concentrations of the *R officinalis* (rosemary)-derived ingredients, often much less than 0.1%. However, *R 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.

In some cases, reports of uses were received in the VCRP, but concentration of use data were not provided. For example, rosmarinus officinalis (rosemary) flower extract is reported to be used in 32 cosmetic formulations, but no use concentration data were reported. In other cases, no uses were reported in the VCRP, but concentration of use data were received from industry; *R officinalis* (rosemary) flower/leaf/stem extract had no reported uses in the VCRP, but a use concentration in a deodorant was provided in the industry survey. Therefore, it should be presumed there is at least one use in a deodorant formulation.

Products containing *R officinalis* (rosemary)-derived ingredients may be applied to baby skin (eg, 0.012% *R officinalis* [rosemary] leaf extract in baby lotion, oils, and creams), used in products that could be incidentally ingested (eg, 0.012% rosmarinus officinalis [rosemary] leaf in lipstick formulations), or used near the eye area (eg, up to 3% rosmarinus officinalis [rosemary] leaf extract in eye shadow formulations) or mucous membranes (eg, up to 3% rosmarinus officinalis [rosemary] leaf extract in bath soaps and detergents).[27] Additionally, *R officinalis* (rosemary)-derived ingredients are used in cosmetic sprays and powders; for example, rosmarinus officinalis (rosemary) leaf extract is reported to be 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.[29-32] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[29,32]

Rosmarinus officinalis (rosemary) 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.[29] 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.

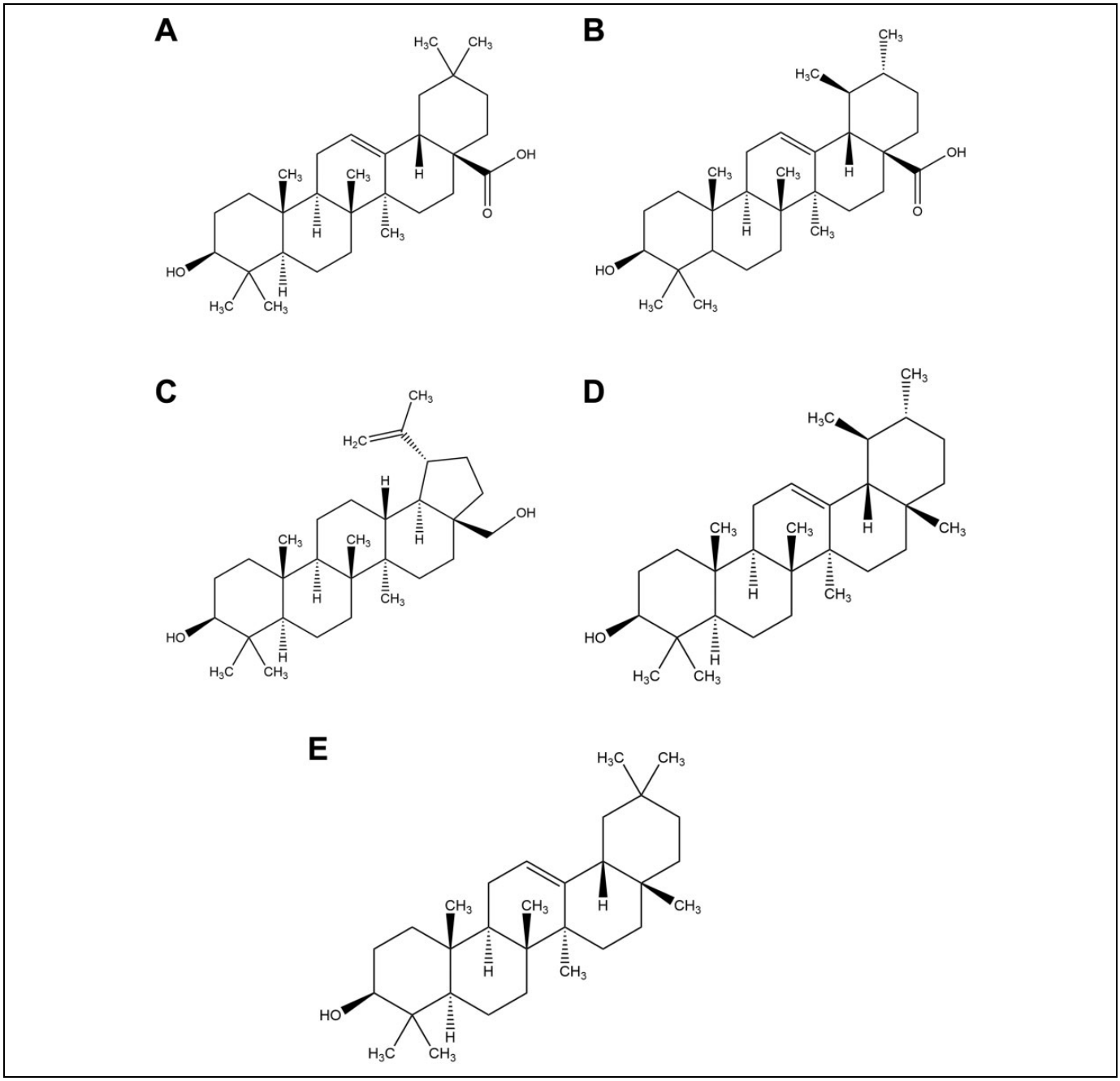

Figure 2. Principal triterpenes. A, Oleanolic acid. B, Ursolic acid. C, Betulin. D, α-Amyrin. E, β-Amyrin.

All of the ingredients named in this safety assessment are listed in the European Union inventory of cosmetic ingredients.[33]

Noncosmetic

Rosmarinus officinalis L. is GRAS as a spice and as a 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).

According to *The Official Journal of the European Union*, extracts of rosemary contain several antioxidant 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.[34] Extracts of rosemary are allowed in various

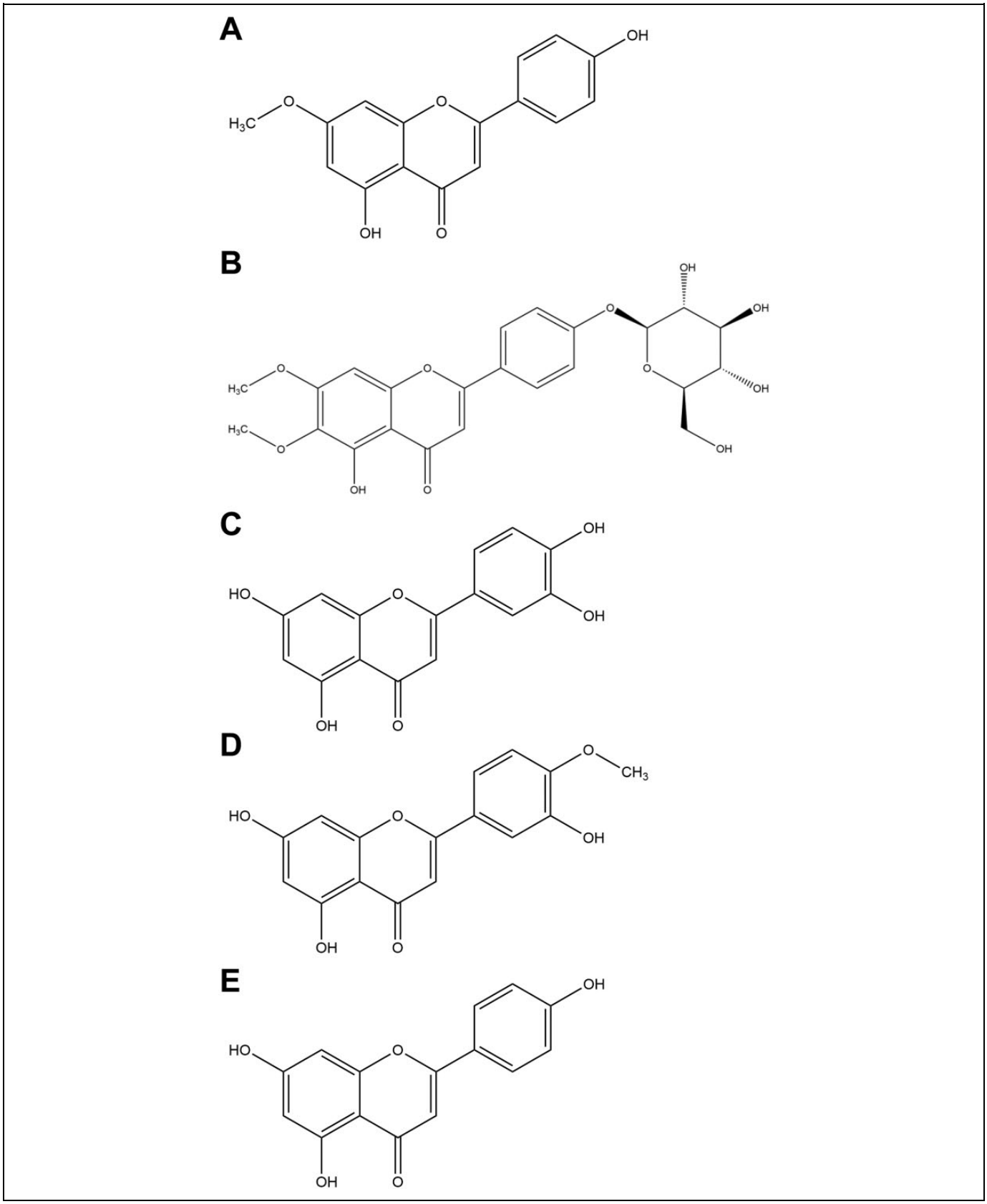

Figure 3. Principal flavonoids. A, Genkwanin. B, Cirsimarin. C, Luteolin. D, Diosmetin. E, Apigenin.

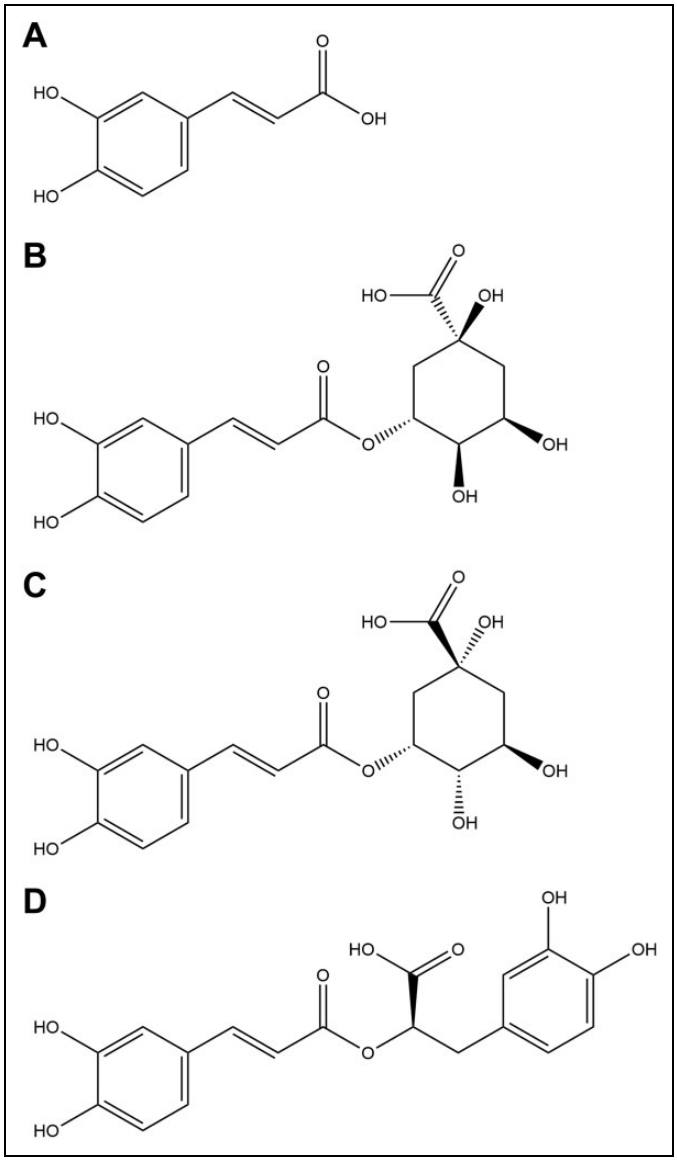

Figure 4. Phenolic acids. A, Caffeic acid. B, Chlorogenic acid. C, Neochlorogenic acid. D, Labiatic acid.

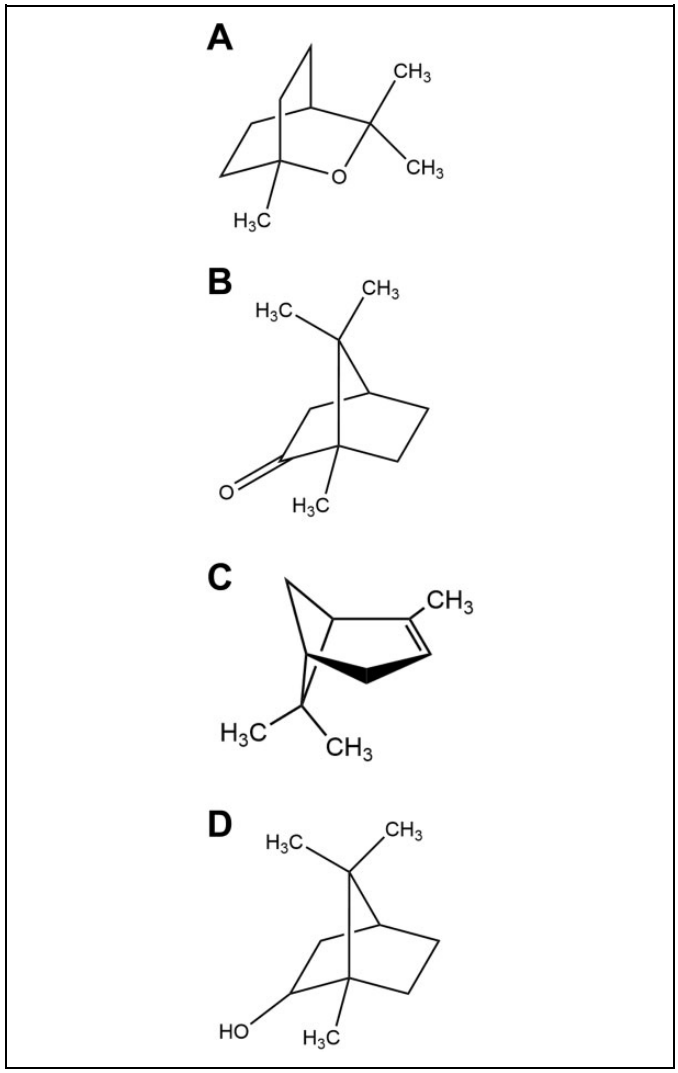

Figure 5. Principal volatiles. A, 1,8-Cineole. B, Camphor. C, α-Pinene. D, Borneol.

food products at amounts of 30 to 1,000 mg/kg, expressed as the sum of carnosol and carnosic acid.

Rosemary leaves are used as a seasoning in cooking.[35] 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. Also, rosemary oil is reported to have antimicrobial activities.[4]

Anti-inflammatory, antioxidant, and antimicrobial uses have been reported for rosemary.[21,36-38] Rosemary has traditional or folk medicine uses, some with negative 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. Additional folk medicine practices include use in lotions to treat baldness[14] and use of the leaves and branches in 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 reflection Fourier transform infrared spectroscopy.[39] Rosemary oil did enhance the permeation of aminophylline; however, the increase in permeation was less than that observed with 50% ethanol.

Table 3. Chemical Constituents by Plant Part (ppm).[74,a]

Constituent[b]	Plant	Leaf	Flower	Shoot	Resin, exudate, sap	Essential Oil
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	3,500	–	13,500	–	–
EO	3,300-25,000	–	–	–	–	–
calcium	10,919-16,150	–	–	–	–	–
potassium	8,842-11,284	–	–	–	–	–
oleanolic acid	10,500	–	–	20	–	–
carnosol	–	530-9,803				
cineole	168-9,728	–	–	–	–	–
1,8-cineole	8,125	–	–	–	–	–
camphor	60-5,800	–	–	–	–	–
myrcene	25-5,605	–	–	–	–	–
bornyl acetate	5,054	–	–	–	–	–
α-pinene	235-4,750	–	–	–	–	–
borneol	12-4,237	–	–	–	–	–
magnesium	2,142-2,483	–	–	–	–	–
rosmaric acid	3,000-3,500	–	–	–	–	–
camphene	23-2,350	–	–	–	–	–
β-caryophyllene	12-2,075	–	–	70-2075		
toluene	436-2,071	–	–	–	–	–
limonene	1,950	–	–	–	–	–
α-terpineol	24-1,555	–	–	–	–	–
β-pinene	17-1,425	–	–	–	–	–
phosphorus	490-1,000	–	–	–	–	–
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	–	–	–	–	–

(continued)

Table 3. (continued)

Constituent[b]	Plant	Leaf	Flower	Shoot	Resin, exudate, sap	Essential Oil
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	–	–	–	–	–
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	–	–	–	–
cis-imaritrin	–	–	–	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	–	–	–	–	–

(continued)

Table 3. (continued)

Constituent[b]	Plant	Leaf	Flower	Shoot	Resin, exudate, sap	Essential Oil
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	–	–	–	–
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-4́-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	–	–	–	–	–

(continued)

Table 3. (continued)

Constituent[b]	Plant	Leaf	Flower	Shoot	Resin, exudate, sap	Essential Oil
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	–	–	–	–	–
neochlorogenic 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	–	–	–	–	–
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	–

(continued)

Table 3. (continued)

Constituent[b]	Plant	Leaf	Flower	Shoot	Resin, exudate, sap	Essential Oil
pentan-1-ol	–	–	–	–	NS	–
pentan-2-ol	–	–	–	–	NS	–
zingiberene	–	–	–	–	NS	–
dipentene	–	–	–	–	–	NS

Abbreviations: EO, essential oil; NS, amount not specified.
[a]– indicates not reported.
[b]Constituents reported in ppm.

Toxicological Studies

Single-Dose (Acute) Toxicity

The acute toxicity of *R officinalis* (rosemary)-derived ingredients is not very remarkable (Table 9).[8,22,40-42] The dermal the median lethal dose (LD_{50}) of rosmarinus officinalis (rosemary) leaf oil is >10 mL/kg.[42] 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 body weight (bw).[41]

Repeated-Dose Toxicity

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 (Table 10). Doses as high as 14.1 g/kg bw rosmarinus officinalis (rosemary) leaf extract were tested (5 days by gavage), and some studies were performed for up to 3 months (dietary) with doses of up to 400 mg/kg bw/d.[8] 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 with carbon tetrachloride, but not without, resulted in an increase in liver weights.[41]

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.[43,44]

In the formaldehyde-induced plantar edema study, groups of 6 male Balb/C mice were given an injection of 20 µL of 3% formaldehyde into the subplantar region of both hind paws.[43] After 2 hours, one hind paw was treated with 10 µL of 12 mg/mL of an ethanol extract of *R officinalis* (rosemary) leaves topically, as an injection, or both. The mice were killed after 24 hours. Topical administration of the extract reduced edema by 80%, 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.[43] The effect of pretreatment with 10 to 1,000 µg/cm^2 of an ethanol extract of *R officinalis* (rosemary) leaves at 30 minutes prior to induction of inflammation with 25 ng/cm^2 TPA was evaluated. The mice were killed after 4 hours. Doses of 100, 250, 500, and 1,000 µg/cm^2 of the extract statistically significantly reduced inflammation by 38%, 79%, 84%, and 99%, respectively.

In a TPA-induced mouse ear edema study conducted in groups of 6 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 *R officinalis* (rosemary) leaves in 20 µL acetone was applied to one ear of each mouse.[44] The mice were killed after 5 hours, 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, and 0.45 mg of a methanol extract of *R officinalis* (rosemary) leaves in 20 µL acetone was applied to groups of 10 female CD-1 mice at 30 minutes prior to treatment with 0.3 mg arachidonic acid in 20 µL acetone.[44] The mice were killed after 1 hour. Inflammation was inhibited by 12%, 28%, and 54%, respectively.

Effect on Epidermal Hyperplasia

Two hundred microliter acetone, 1 nmol TPA, or 1 nmol TPA and 3.6 mg rosmarinus officinalis (rosemary) leaf extract in 200 µL acetone were applied twice a day for 4 days to the dorsal skin of mice.[44] Three or 4 CD-1 mice were used per group. 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 aqueous (aq) extract of up to 2.5 mg/mL *R officinalis* (rosemary) leaves was found to inhibit UV-induced upregulation of matrix metalloproteinase 1 gene transcription in dermal human fibroblasts.[45] The release of the cytokines interleukin (IL) 1α and IL-6 was prevented by the extract.

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 19α-hydroxyursolic, 2β-hydroxyoleanolic, and 3β-hydroxyurea-12,20(30)-dien-17-oic acids	
- Triterpene alcohols: α-amyrin, β-amyrin, betulin	
- Flavonoids: luteolin, genkwanin (7-*O*-methlylapigenin), diosmetin, diosmin, genkwanin-4́-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	5,22,35,36,75
- 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, neochlorogenic 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	35,40,41,73,76
- From one sample (concentration in the oil):	41
- Monoterpenoid esters (24.76%): bornyl acetate (20.86%), linalyl 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%)	
Flower	
- Carnosic acid, carnosol, 12-*O*-methylcarnosic acid, at levels that are less than that found in the leaves	23
- Highest levels of rosmarinic acid are found in the flower	
- The flavones genkawanin and isoscutellarein 7-*O*-glucoside were not found in a DMSO extract	
Seed	
- 560.5 μg/g α-tocotrienol; 300.3 μg/g β-tocotrienol; 109.4 μg/g γ-tocotrienol	77
Essential oil	
- Mainly monoterpenes: α-pinene (20.1%-21.7%), β-pinene, camphene, limonene, 1,8-cineole (23.5%-26.5%), eucalyptol (4.5%), borneol	4,78,79
- Camphor (7.2%), berbonone (7.6%), linalool, verbenol, terpineol, 3-octanone, isobornyl acetate	

Abbreviation: DMSO, dimethyl sulfoxide.

Table 5. *Rosmarinus officinalis* (Rosemary) Leaf Extracts (CO_2 Extract): Certificates of Analysis.

Analytical detail	Specifications (%)	Results (%)
R officinalis (rosemary) extract (CO_2)[16]		
Essential oil content	78-88	78
Volatile components:		
α-Pinene	8-12	11.4
Camphene	ns	4.0
β-Pinene	ns	3.7
Myrcene	ns	2.7
p-Cymene	ns	1.2
Limonene	2-4	2.4
1,8-Cineole	>40	41.3
Linalool	ns	0.83
Camphor	6-13	13.0
Borneol	ns	3.8
α-Terpineol	ns	3.9
Verbenone	ns	0.45
Bornyl acetate	ns	0.94
Carophyllene	3-10	4.7
R officinalis (rosemary) leaf extract (CO_2; 14% diterpene phenols) (and) *Helianthus annuus* seed oil[17]		
Essential oil content	<2	1.9
Phenolic diterpenes:		
Rosmanol	ns	0.07
7-Methyl-rosmanol	ns	0.09
Carnosol	ns	1.2
Carnosolic acid	ns	10.5
12-Methyl-carnosolic acid	ns	2.4
Sum of phenolic diterpenes	13-15	14.3
Reference antioxidant compounds (carnesol + carnosic acid, calculated as carnosic acid)	ns	9.5
Ursolic acid	ns	0.43
Oleanolic acid	ns	0.62
Residual ethanol	<2	0.71
Water content	<1	0.30
R officinalis (rosemary) leaf extract (CO_2; 25% diterpene phenols) (and) *H annuus* seed oil[18]		
Essential oil content	<4	3.0
Phenolic diterpenes:		
Rosmanol	ns	0.13
7-Methyl-rosmanol	ns	0.18
Carnosol	ns	1.4
Carnosolic acid	ns	18.7
12-Methyl-carnosolic acid	ns	4.5
Sum of phenolic diterpenes	24-26	24.9
Ursolic acid	ns	0.29
Oleanolic acid	ns	0.51
Residual ethanol	<2	0.39
Water content	<1	0.91
R officinalis (rosemary) leaf extract (CO_2; 25% diterpene phenols) (and) *H annuus* seed oil[12,15]		
Essential oil content	<4	1.7
Phenolic diterpenes:		
Rosmanol	ns	0.13
7-Methyl-rosmanol	ns	0.32
Carnosol	n.s	2.9
Carnosic acid	> 16	20.6
12-Methyl-carnosic acid	ns	1.0
Sum of phenolic diterpenes	24-26	25.0
Ursolic acid	ns	0.42
Oleanolic acid	ns	0.52
Residual ethanol	<2	0.33
Water content	<1	0.15

Abbreviation: ns, not specified.

Table 6. Differences in Constituent Profiles in *Rosmarinus officinalis* (Rosemary) Leaf Extract Based on Extraction Method.[a,8]

		Extraction Method				
Constituent (ppm)	Dried leaves	Supercritical CO_2	Acetone	Ethanol extract, partially deodorized	Ethanol extract, deodorized	Decolorized and deodorized using hexane and ethanol
Triterpenes						
Betulin	<4,760	6,000	5,600	8,450	9,460	6,790
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	1,700	1,320	53	30
Camphor	25,200	220	2,360	2,080	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

[a]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%[80] - Caffeic acid is reported to penetrate skin and have UV photoprotective activity[81] - 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 animals of caffeic acid; no data were available on the carcinogenicity in humans, and IARC concluded that caffeic acid is possibly carcinogenic to humans[82] - The carcinogenic potency of caffeic acid, estimated based on an average human intake of 1 mg/kg bw/d, was less than 1,000 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 weeks to 2 years 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 years, showed treatment-induced carcinomas of the forestomach, whereas 2 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[83]
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[81] - 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[83]
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[81]
Diterpenes	
Carnosic acid	- A known antioxidant[84]; in a toxicokinetic study in male Sprague Dawley rats, carnosic acid was absorbed into the bloodstream 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 route of elimination was the feces[84]; not mutagenic in an Ames test, with or without metabolic activation, at doses equivalent to the concentration present in up to 6,000 μg/plate of a decolorized and deodorized rosemary leaf extract[8]

(continued)

Table 7. (continued)

Component	Toxicity information
Carnosol	- Topical application of carnosol isolated from rosemary inhibited TPA-induced ear inflammation and tumor promotion in mice[44]; not mutagenic in an Ames test, with or without metabolic activation, at doses equivalent of the concentration present in up to 6,000 μg/plate of a decolorized and deodorized rosemary leaf extract[8]
Monoterpenes	- These chemicals may be skin sensitizers[81]
d-Limonene	- *d*-Limonene consumption has been estimated as 0.2 to 2 mg/kg bw/d; in men, oral intake induced transient proteinuria[82] - 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[85] - The few genotoxicity studies available indicated that *d*-limonene and its 1,2-epoxide metabolite are not genotoxic[85] - 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[85]
α-Pinene	- Negative in the Ames assay and a mouse micronucleus test[86]
1,8-Cineole	- Positive in a sister chromatid exchange assay; negative in a chromosomal aberration assay; negative in an Ames test[87]
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/d and the NOAEL for peri- and postnatal developmental toxicity was 0.25 g/kg bw/d; 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 SCE in cultured hepatic tumor cells; was not genotoxic in vivo in rat bone marrow cells[88]; up to 1.0 g/kg bw was administered in corn oil, by gavage, to rats and mice, and there was clear evidence of carcinogenic activity in male rats (increased incidences of renal tubule neoplasms) and male mice (increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma), and equivocal evidence in female rats (increased incidences of renal tubule adenoma) and female mice (marginally increased incidences of hepatocellular adenoma and carcinoma)[89]
Linalool	- Safe at up to 4.3% (20% in consumer fragrance); listed as a fragrance allergen by the European Commission[81] - International Fragrance Association (IFRA) stated pure linalool is not a sensitizer, but hydroperoxides and other oxidation products have shown sensitizing properties; one of the major oxidation products of linalool was isolated and identified as 7-hydroperoxy-3,7-dimethyl-octa-1,5-diene-3-ol; in sensitization studies in guinea pigs, linalool of high purity gave no reactions, while linalool that had been oxidized for 10 weeks sensitized the animals; it was concluded that autoxidation of linalool is essential for its sensitizing potential[90]
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[91]
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[92] - IFRA stated available metabolic, biochemical, and toxicological data in laboratory species provide clear evidence of nonlinearity in the dose–response relationship with respect to metabolic activation and mechanisms associated with carcinogenic effects; consideration of these data indicates an NOEL in the rat in the dose range of 1 to 10 mg/kg bw/d; based on the lower end of the NOEL and applying a 1,000× safety factor for systemic effects a daily dose of 60 μg/d is supported; taking into account a dermal penetration factor of 40% leads to an acceptable dose of 150 μg/d[93]
Terpene alcohols	
α-Terpineol	- Oral LD_{50} in mice, 2,830 mg/kg; 1,000 mg/kg bw/d for 2 weeks 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 intraperitoneal injections; a dermal irritant in animals studies, but not a dermal irritant in a 4-hour clinical study; not a sensitizer in guinea pigs; in clinical patch tests, 5% in pet. had 1/1,606 positive and 11/1,606 questionable reactions in one study and 2/1,200 positive reactions in another[94]
Ursolic acid	- Topical application of ursolic acid isolated from rosemary inhibited TPA-induced ear inflammation and tumor promotion in mice[44]
Triterpene alcohols	- Hepatoprotective and anticarcinogenic activity has been suggested for lupeol; no toxicity data were available; triterpene alcohols were considered to have intermediate risk[81]

Abbreviations: IARC, International Agency for Research on Cancer; MMC, mitomycin-C; NOAEL, no-observable-adverse-effect level; TPA, 12-tetradecanoylphorbol-13-acetate; UV, ultraviolet; wt, weight.

Table 8. Frequency and Concentration of Use According to Duration and Type of Exposure.

	Number of uses[26]	Max. conc. of use (%)[27]	Number of uses[26]	Max. conc. of use (%)[27]	Number of uses[26]	Max. conc. of use (%)[27]
	Rosmarinus officinalis (rosemary) extract		*R officinalis* (rosemary) flower extract		*R officinalis* (rosemary) flower/leaf/stem extract	
Totals[a]	404	0.00004-0.16	32	NR	NR	0.0024
Duration of use						
Leave-on	247	0.00096-0.051	14	NR	NR	0.0024
Rinse-off	154	0.00004-0.16	18	NR	NR	NR
Diluted for (bath) use	3	NR	NR	NR	NR	NR
Exposure type						
Eye area	20	0.01-0.05	2	NR	NR	NR
Incidental: ingestion	7	0.011	1	NR	NR	NR
Incidental: inhalation-spray	93[b]; 64[c]	0.00096-0.001[b]	1 2[b]; 1[c]	NR	NR	NR
Incidental: inhalation-powder	64[c]	0.05	1[c]	NR	NR	NR
Dermal contact	306	0.00096-0.16	6	NR	NR	0.0024
Deodorant (underarm)	NR	Not spray: 0.0098 Aerosol: 0.0098-0.012	NR	NR	NR	Not spray: 0.0024
Hair: noncoloring	116	0.00004-0.003	25	NR	NR	NR
Hair: coloring	1	NR	NR	NR	NR	NR
Nail	1	NR	NR	NR	NR	NR
Mucous membrane	26	0.0005-0.16	1	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR
	Number of uses[26]	Max. conc. of use (%)[27]	Number of uses[26]	Max. conc. of use (%)[27]	Number of uses[26]	Max. conc. of use (%)[27]
	R officinalis (rosemary) leaf		*R officinalis* (rosemary) leaf extract		*R officinalis* (rosemary) leaf oil	
Totals[a]	16	0.002	729	0.00001-10	474	0.00001-1.5
Duration of use						
Leave-on	1	0.002	473	0.00001-10	308	0.0003-1.5
Rinse-off	14	NR	254	0.00001-3	141	0.00001-0.12
Diluted for (bath) use	1	NR	2	0.0002-0.04	25	0.5-0.97
Exposure type						
Eye area	NR	NR	27	0.002-3	8	NR
Incidental: ingestion	NR	NR	27	0.00001-0.009	3	0.008
Incidental: inhalation-spray	1[b]	0.002[b]	8; 185[b]; 110[c]	0.001-0.5 Aerosol: 0.0016 Pump spray: 0.0001-0.005 0.00002-0.5[b]	25 90[b]; 106[c]	0.011-1.5 Aerosol: 0.007
Incidental: inhalation-powder	NR	NR	2 7[d]; 110[c]	0.0002	2 1[d]; 106[c]	0.0003
Dermal contact	4	NR	460	0.00001-10	381	0.0003-1.5
Deodorant (underarm)	NR	NR	1[b]	NR	NR	NR
Hair: noncoloring	12	0.002	219	0.00001-0.5	89	0.00001-1.5
Hair: coloring	NR	NR	22	0.04	1	NR
Nail	NR	NR	1	0.005-0.053	NR	NR
Mucous membrane	1	NR	71	0.00001-3	64	0.0002-0.97
Baby products	NR	NR	8	0.012	4	NR

(continued)

Table 8. (*Continued*)

	Number of uses[26]	Max. conc. of use (%)[27]	Number of uses[26]	Max. conc. of use (%)[27]	Number of uses[26]	Max. conc. of use (%)[27]
	R officinalis (rosemary) leaf powder		*R officinalis* (rosemary) leaf water		*R officinalis* (rosemary) water	
Totals[a]	2	0.05	25	0.000069-1	2	–
Duration of use						
Leave-on	1	NR	11	0.000069-1	2	NR
Rinse-off	1	0.05	14	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	1[c]	NR	4[b]; 4[c]	NR	1[b]; 1[c]	NR
Incidental: inhalation-powder	1[c]	NR	4[c]	NR	1[c]	NR
Dermal contact	2	NR	10	0.00009-0.36	2	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair: noncoloring	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[e]	
Totals[d]	**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	1; 2[c]	–
Incidental: inhalation-powder	1; 2[c]	–
Dermal contact	8	–
Deodorant (underarm)	NR	–
Hair: noncoloring	4	–
Hair: coloring	NR	–
Nail	NR	–
Mucous membrane	2	–
Baby products	NR	–

Abbreviation: NR, not reported.

[a]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[b]Includes products that can be sprays, but it is not known whether the reported uses are sprays.

[c]Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories.

[d]Includes products that can be powders, but it is not known whether the reported uses are powders.

[e]Plant part and type of preparation not known.

Table 9. Single-Dose Toxicity Studies.

Test article	Extraction solvent/ method	Species	Number/group	Vehicle	Concentration/dose range	LD_{50}/results	Reference
Dermal							
Rosmarinus officinalis (rosemary) leaf oil	–	Rabbits	Not stated	Not stated	Not stated	>10 mL/kg	42
R officinalis (rosemary) leaf oil	–	Rabbits	Not stated	Not stated	Not stated	>10 g/kg	40
Oral							
R 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	6M/6F	Corn oil	2 g/kg bw[8,22,40-42](gavage)	>2 g/kg	22
R 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
R 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
R officinalis (rosemary) leaf oil (see Table 4 for composition)	Hydrodistillation	Swiss albino rats	20/group	–	2 to 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	41
R officinalis (rosemary) leaf oil (see Table 5 for composition)	–	Rats	Not stated	None stated	Not stated	5 mL/kg bw	42

Abbreviations: F, female; M, male.

Table 10. Repeated-Dose Toxicity Studies

Test Article	Extraction solvent/method	Animals/group	Study duration	Vehicle	Dose/concentration parameters examined	Results[a]	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	4,300 mg/kg bw (males) 5,000 mg/kg bw (females) - Parameters included signs of toxicity, body wts, feed consumption, organ wts, gross lesions	- 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
R officinalis (rosemary) leaf extract (see Table 5 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) - Parameters as above	- No changes in body wts; liver wts of females were slightly increased; fatty livers were observed in test animals at necropsy.	8
R officinalis (rosemary) leaf extract (see Table 5 for composition)	Acetone	Rats; no./group not stated	14 days (diet)	–	Up to 3,800 mg/kg diet - Parameters included signs of toxicity, body wts, feed consumption, clinical chem	- No treatment-related signs of toxicity, mortality, or changes in body wts or feed consumption	8
R officinalis (rosemary) leaf extract (see Table 5 for composition)	Supercritical CO_2	Rats; no./group not stated	14 days (diet)	–	Up to 2,400 mg/kg diet - Parameters as above	- No treatment-related signs of toxicity, mortality, or changes in body wts or feed consumption	8
R officinalis (rosemary) leaf extract (see Table 5 for composition)	Acetone	20 rats/group	13 weeks (diet)	–	300, 600, 2,400, or 3,800 mg/kg diet - Parameters included signs of toxicity, body wts, feed consumption, gross and microscopic lesions, clinical chem, hematology, organ wts	- 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 3,800 mg/kg group - NOAEL was 3,800 mg/kg diet	8
R officinalis (rosemary) leaf extract (see Table 5 for composition)	Supercritical CO_2	20 rats/group	13 weeks (diet)	–	300, 600, or 2,400 mg/kg diet - Parameters as above	- 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 wts and feed consumption in the animals of the 2,400 mg/kg diet groups were attributed to a lack of palatability of the feed - Changes were more notable in females - NOAEL was 2,400 mg/kg diet (equiv to 180 and 200 mg/kg bw/d for males and females, respectively)	8
R 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 2,400 mg/kg diet (equiv to 0 or 195 mg/kg bw/d) - Parameters included signs of toxicity, body wts, feed consumption, gross and microscopic lesions, organ wts	- 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 the liver enzymes CYP1A, CYP2B, or CYP3A	8

(continued)

Table 10. (continued)

Test Article	Extraction solvent/method	Animals/group	Study duration	Vehicle	Dose/concentration parameters examined	Results[a]	Reference
R 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, 1,500, or 5,000 mg/ kg diet (equiv to 0, 40, 120, or 400 mg/kg bw/d) - Parameters included signs of toxicity, body wts, feed consumption, microscopic lesions, organ wts, hematology	- Body wts in the high-dose group were very slightly reduced, most likely as a result of decreased feed consumption - A dose–response relationship was observed for relative liver-to-body wt, in which a slight but stat sig increase was observed - No microscopic changes in the liver were reported	8
R officinalis (rosemary) leaf extract (see Table 5 for composition)	Ethanol extract, deodorized	Sprague Dawley rats; no./group not stated	90 days (diet)	–	0, 500, 1,500, or 5,000 mg/kg diet (equiv to 0, 40, 120, or 400 mg/kg bw/d) - Parameters as above	- 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
R officinalis (rosemary) leaf extract (see Table 5 for composition)	Hexane and ethanol (2-step extraction)	Sprague Dawley rats; no./group not stated	3 months (diet); 28-day interim group; 1-mntho recovery period	–	0, 1,000, 2,500, or 5,000 mg/kg diet (equiv to 0, 65, 164, or 320 mg/kg bw/d) - Parameters as above	- No signs of toxicity, no m, 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/d	8
R officinalis (rosemary) leaf extract (after the volatile oil [1.1%] was removed)	Absolute ethanol	Swiss albino mice; 6M/ group	3 weeks (gavage)	Olive oil	1,500 mg/kg extract Controls—olive oil	- No stat sig changes in relative liver, spleen, heart, or lung-to-body wt compared to controls; there were no stat sig changes in clinical chemistry parameters	41
			Single-dose CCl_4 (gavage), then 3 weeks extract (gavage)	Olive oil	3.3% CCl_4 (100 mg/kg bw) 1,500 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	

(continued)

Table 10. (continued)

Test Article	Extraction solvent/method	Animals/group	Study duration	Vehicle	Dose/concentration parameters examined	Results[a]	Reference
R officinalis (rosemary) leaf oil (see Table 4 for composition)	Hydrodistillation	Swiss albino mice; 6M/ group	3 weeks (gavage)	–	1,100 mg/kg bw controls—olive oil	- No stat sig changes in relative liver, spleen, heart, or lung-to-body wt compared to controls; there were no stat sig changes in clinical chemistry parameters	41
			Single-dose CCl_4 (gavage), then 3 weeks oil (gavage)	Olive oil (for CCl_4)	3.3% CCl_4 (100 mg/kg bw) 1,100 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; F, female; m, male; NOAEL, no-observable adverse effect level; stat sig, statistically significant; wt, weight.
[a]If not described in the results, details on histopathology or organ weights were not provided.

Reproductive and Developmental Toxicity

Nonhuman

Rosmarinus Officinalis (Rosemary) Leaf Extract. Oral administration of high doses of rosmarinus officinalis (rosemary) leaf extract adversely affected fertility in male rats.[46] Groups of 10 male Sprague Dawley rats were fed a diet with 0, 250, or 500 mg/kg bw/d of an ethanol extract of *R officinalis* (rosemary) leaves in distilled water. After 53 days of dosing, each male rat was mated with 2 untreated female rats for 10 days; the female rats had been given a subcutaneous dose of 5.0 mg estradiol benzoate 54 hours and 0.5 mg progesterone at 54 and 6 hours, respectively, prior to 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 week 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.

Body weights of the male rats of the test groups were similar to those of the control group. However, the high-dose group exhibited statistically significantly reduced absolute weights and organ to body weight ratios of testes and male accessory sex organs, diameters of seminiferous tubules and Leydig cell nuclei, height of epithelia of the epididymes and seminal vesicles, germinal and interstitial cell counts, levels of sex hormones, and sperm density and motility when compared to the controls. The numbers of interstitial degenerating cells were statistically significantly increased in the high-dose group. Exposure of the males to the high dose resulted in a reduced number of pregnant females, implantations and viable fetuses, and an increased number of resorptions. Results from the low-dose groups suggested dose–response trends in these parameters, although statistically significant differences were observed only with the high-dose group.

Rosmarinus Officinalis (Rosemary) Flower/Leaf/Stem Extract. A group of 12 gravid female Wistar rats were dosed by gavage with 26 mg/d of a 30% aq extract of rosmarinus officinal (rosemary) flower/leaf/stem extract (13 mg/mL solids) on days 1 to 6 of gestation (preimplantation), and a group of 14 gravid rats were dosed with the extract on days 6 to 15 of gestation (organogenesis).[47] Negative control groups of 12 or 11 gravid rats were given saline by gavage on days 1 to 6 or 6 to 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 to 6 of gestation, a nonstatistically significant increase in preimplantation loss was observed. No changes in postimplantation loss were seen as compared to controls, and no other reproductive parameters were affected. In the group treated on days 6 to 15 of gestation, a nonstatistically significant increase in postimplantation loss rate (2.54%) was reported; analysis of the resorptions found that they occurred during the early postimplantation 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.[48] (Details were not provided.)

Effects on Estrogenic Activity

Nonhuman

Rosmarinus Officinalis (Rosemary) Leaf Extract. Groups of seven or eight 6-week-old ovariectomized CD-1 mice were fed either a diet containing 2% of a methanol extract of *R officinalis* (rosemary) leaves or the basal diet.[49] After 3 weeks, the animals were given an intraperitoneal injection of 0, 45, or 100 ng/mouse estradiol or estrone in 50 μL corn oil, once daily for 3 days. Eighteen hours 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% to 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.[50] A group of 15 premenopausal women were asked to take a supplement containing 100 mg *R officinalis* (rosemary) leaf 5:1 extract; 100 mg *Curcurma longa* (turmeric) root extract standardized to 95% curcumin; 100 mg *Cynara scolymus* (artichoke) leaf 6:1 extract; 100 mg *Silybum marinum* (milk thistle) seed extracted; 100 mg *Taraxacum officinalis* (dandelion) root 4:1 extract; and 50 mg *Schisandra 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 midluteal phases of study menstrual cycles 1 and 5.

On average, test participants 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%, $P = .02$), dehydroepiandrosterone sulfate (14.6%, $P = .07$), androstenedione (−8.6%, $P = .05$), and estrone sulfate (−12.0%, $P = .08$). 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

In vitro, rosemary extract (solvent not specified)[51] and rosmarinus officinalis (rosemary) leaf oil[52] were not mutagenic in an Ames test, and rosmarinus officinalis (rosemary) leaf extract was not genotoxic in an Ames test, a chromosomal aberration assay in human lymphocytes, or a gene locus mutation assay in human lymphocytes[8] (Table 11). In in vivo studies in mice and rats, oils that were extracted by hydrodistillation induced statistically significant increases in chromosomal aberrations without gaps in a chromosomal aberration assay at 2,000 mg/kg bw, increases in micronucleated polychromatic erythrocytes (MNPCEs) in several micronucleus tests at 1,000 and 2,000 mg/kg bw, and increases in DNA damage in a comet assay at $\geq$300 mg/kg bw[14,41]; however, no genotoxic effects were seen in mice in a micronucleus test at 1,500 mg/kg bw/d with leaves extracted with absolute ethanol.[41] A hydroalcoholic extract of rosemary was not genotoxic in a chromosomal aberration assay or a micronucleus test in rats.[53] A mixture containing 19% *R officinalis* (rosemary) leaves, 71.5% St John's wort, and 9.5% spirulina induced in mice statistically significant increases in MNPCEs at 760 and 1,520 mg/kg bw/d in a micronucleus test; in frequency of aneuploidy, percent polyploidy, and total percent aberrations with 760 and 1,520 mg/kg bw/d in a chromosomal aberration assay; and in frequency of banana-shaped, swollen acrosome, and triangular head sperm abnormalities and percent total spermatozoa abnormalities at 1,520 mg/kg bw/d in a spermatozoa abnormality assay.[54]

Rosmarinus officinalis (rosemary) leaf extract was shown to have antimutagenic potential, in vitro, in an Ames test with *Salmonella typhimurium* and in Comet assays in a human hepatoma cell line.[51] In vivo, in micronucleus assays, rosmarinus officinalis (rosemary) leaf extract did not decrease the number of MNPCEs induced in mice by a genotoxic agent.[41]

Carcinogenicity

Effects on Tumor Promotion

Topical application of methanol and double distilled water extracts of *R 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[44] or croton oil[55,56] was used for promotion (Table 12). Dietary administration of 1.0% *R officinalis* (rosemary) leaf extract decreased the incidence of palpable mammary tumors in rats caused by DMBA.[57]

Irritation and Sensitization

Skin Irritation/Sensitization

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 (Table 13).[58] However, in a rabbit study, occlusive application to intact and abraded skin produced moderate irritation.[42]

In clinical testing, *R officinalis* (rosemary) leaves produced irritation (scores of +/−, +, or ++) in 44 of 234 patients with contact dermatitis or eczema (Table 13).[59] A supercritical extract and the absolute of *R officinalis* (rosemary) leaves were considered weak irritants in a small study with test populations of 20 to 25 patients; the extracts were not phototoxic.[9] Formulations containing up to 0.2% *R officinalis* (rosemary) leaf extract were not irritants or sensitizers.[60-62] Rosmarinus officinalis (rosemary) leaf oil, 10% in petrolatum, was not an irritant in a 48-hour closed patch test or a sensitizer in a maximization study[42]; a formulation containing 1.5% rosmarinus officinalis (rosemary) leaf oil was not an irritant or a sensitizer in a human repeated insult patch test (HRIPT).[63]

Phototoxicity

Rosmarinus Officinalis (Rosemary) Leaf Extract. The phototoxicity of *R officinalis* (rosemary) leaf extract extracted with supercritical CO_2, as a concrete (insoluble wax) extracted in hexane, or as a concrete extracted in hexane, was evaluated.[9] Photopatch tests were performed on 2 of 3 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 hours and were compared to the nonirradiated site. None of the extracts were phototoxic.

Case Reports

Several cases of allergic reactions to *R officinalis* (rosemary) have been reported (Table 14).[64-72] In some of the studies, follow-up patch testing included photopatch tests; generally, reactions were stronger in the photopatch tests when compared to standard testing.[68,69] Some of the follow-up patch testing included carnosol; testing with 0.1 and 1.0% carnosol resulted in positive reactions.[65,69]

Summary

This report addresses the safety of 10 *R officinalis* (rosemary)-derived ingredients as used in cosmetics. Most of the ingredients included in this review are extracts, essential oils, powders, or waters derived from a defined part of the *R officinalis* (rosemary) plant. The *R officinalis* (rosemary)-derived ingredients are reported to have a number of functions in cosmetics, and the most common functions 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, 729, followed by rosmarinus officinalis (rosemary) leaf oil, 474 uses, and rosmarinus officinalis (rosemary) extract, 404 uses. Most of the reported use concentrations for *R 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.

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	*Salmonella typhimurium* TA98	Not mutagenic	51
As above	–	50 μg/mL (highest noncytotoxic dose)	Comet assay	Human hepatoma cell line (HepG2)	Not genotoxic	51
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	51
As above	–	5 μg/mL (highest noncytotoxic dose)	Comet assay	Human hepatoma cell line (HepG2)	Not genotoxic	51
Rosmarinus officinalis (rosemary) leaf extract	Supercritical CO_2	Up to 5,000 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 6,000 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 3,000 to 6,000 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

(continued)

Table 11. (continued)

Test article	Extraction solvent/method	Conc./vehicle	Procedure	Test system	Results	Reference
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 in mutation frequency compared to controls	8
Rosmarinus officinalis (rosemary) leaf oil	–	Not stated	Ames test	Not stated	Negative	52
In vivo						
Rosmarinus officinalis	Hydroalcoholic	6.43, 100, and 200 mg/kg bw	Chromosomal aberration assay	Wistar rats; 6/group	Not genotoxic	53
Rosmarinus officinalis	Hydroalcoholic	6.43, 100, and 200 mg/kg bw	Micronucleus assay	Wistar rats; 6/group	Not genotoxic	53
Rosmarinus officinalis (rosemary) leaf extract (after the volatile oil [1.1%] was removed)	Absolute ethanol	1,500 mg/kg bw/d in olive oil	Micronucleus test; dosed by gavage for 7 days; negative controls were given olive oil; positive controls were given a single ip dose of 100 mg/kg bw CPA; bone marrow cells collected 24 hours after dosing	Swiss albino mice	Not genotoxic; no stat sig change in the number of MNPCE or NCE or in PCE/NCE	41
Rosmarinus officinalis (rosemary) leaf oil (see Table 4 for composition)	Hydrodistillation	1,100 mg/kg bw/d	Same protocol	Swiss albino mice	No stat sig change in no. of MNPCE Number of NCE was stat sig decreased ($P < .05$) PCE/NCE was stat sig increased ($P < .01$)	41
Rosmarinus officinalis (rosemary) leaf oil	Hydrodistillation	300, 1,000, or 2,000 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 hours after dosing	Wistar rats; 3M/3F per group	- Chromosomal aberrations without gaps were stat sig increased at 2,000 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, 1,000, or 2,000 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 hours after dosing	Swiss mice; 3M/3F per group	- Stat sig increase in MNPCEs with 1,000 and 2,000 mg/kg bw - PCE/NCE was not stat sig different from controls	14
Rosmarinus officinalis (rosemary) leaf oil	Hydrodistillation	300, 1,000, or 2,000 mg/kg bw (by gavage)	Micronucleus test; protocol as above; bone marrow cells collected 24 hours after dosing	Wistar rats; 3M/3F per group	Stat sig increase in MNPCEs with 2,000 mg/kg bw	14

(continued)

Table 11. (continued)

Test article	Extraction solvent/method	Conc./vehicle	Procedure	Test system	Results	Reference
Rosmarinus officinalis (rosemary) leaf oil	Hydrodistillation	300, 1,000, or 2,000 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 CPA/kg; liver and peripheral blood cells collected 24 hours 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 1,520 mg/kg bw/d 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 1,520 mg/kg bw/d - PCE/NCE was not stat sig different from controls	54
Mixture defined above	–	0, 380, 760, or 1,520 mg/kg bw/d 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 1,520 mg/kg bw/d - Percent polyploids and total percent aberrations were stat sig increased at these doses	54
Mixture defined above	–	0, 380, 760, or 1,520 mg/kg bw/d in water (gavage)	Assay for spermatozoa abnormality; mice were dosed for 7 days and killed 5 weeks after last dose	Male Swiss albino mice; 30/group	- Stat sig increase in frequency of banana-shaped, swollen acrosome, and triangular head sperm abnormalities with 1,520 mg/kg bw/d - Percent total spermatozoa abnormalities stat sig increased with 1,520 mg/kg bw/d	54
Antimutagenic 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	tat sig reduced tBOOH-induced mutagenicity	95
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	51
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	51
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 hours, followed by 20 minutes 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	51
As above + tBOOH	–	0, 0.05, 0.5, 5, or 50 μg/mL extract; 0.05 mM tBOOH	Comet assay; cotreatment with extract and tBOOH for 20 minutes	Human hepatoma cell line (HepG2)	No stat sig effect on tBOOH-induced DNA damage	51

(continued)

Table 11. (continued)

Test article	Extraction solvent/method	Conc./vehicle	Procedure	Test system	Results	Reference
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 hours, followed by cotreatment with extract and tBOOH for 20 minutes	Human hepatoma cell line (HepG2)	Stat sig reduction in tBOOH-induced DNA damage at all except the lowest dose	51
As above + B(a)P	–	0, 0.05, 0.5, 5, or 50 μg/mL extract; 40 μM B(a)P	By cotreatment with extract and B(a)P for 21 hours	Human hepatoma cell line (HepG2)	Stat sig reduction in B(a)P-induced DNA damage only at the highest dose	51
As above + PhIP	–	0, 0.05, 0.5, 5, or 50 μg/mL extract; 80 μM PhIP	Comet assay; by cotreatment with extract and PhIP for 21 hours	Human hepatoma cell line (HepG2)	Stat sig reduction in PhIP-induced DNA damage only at the highest dose	51
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	51
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	51
As above + tBOOH	–	0, 0.05, 0.5, or 5 μg/mL extract; 0.05 mM tBOOH	Comet assay; pretreatment with extract for 21 hours, followed by 20 minutes exposure to tBOOH	Human hepatoma cell line (HepG2)	Stat sig reduction in tBOOH-induced DNA damage at all doses	51
As above + tBOOH	–	0, 0.05, 0.5, or 5 μg/mL extract; 0.05 mM tBOOH	Comet assay; cotreatment with extract and tBOOH for 20 minutes	Human hepatoma cell line (HepG2)	No stat sig effect on tBOOH-induced DNA damage	51
As above + tBOOH	–	0, 0.05, 0.5, or 5 μg/mL extract; 0.05 mM tBOOH	Comet assay; pretreatment with extract for 21 hours, followed by cotreatment with extract and tBOOH for 20 minutes	Human hepatoma cell line (HepG2)	Stat sig reduction in tBOOH-induced DNA damage at all doses; the reduction was not dose dependent'	51
As above + B(a)P	–	0, 0.05, 0.5, or 5 μg/mL extract; 40 μM B(a)P	By cotreatment with extract and B(a)P for 21 hours	Human hepatoma cell line (HepG2)	Stat sig reduction in B(a)P-induced DNA damage at the 2 highest doses	51
As above + PhIP	–	0, 0.05, 0.5, or 5 μg/mL extract; 80 μM PhIP	By cotreatment with extract and PhIP for 21 hours	Human hepatoma cell line (HepG2)	Stat sig reduction in PhIP-induced DNA damage at the 2 highest doses	51
In vivo						
Rosmarinus officinalis (rosemary) leaf extract (after the volatile oil [1.1%] was removed) + CPA	Absolute ethanol	1,500 mg/kg bw/d in olive oil	Micronucleus test; dosed by gavage with the extract for 7 days, then given a single ip dose of 100 mg/kg bw CPA; bone marrow cells collected 24 hours 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	41
Rosmarinus officinalis (rosemary) leaf oil (contained 20.86% bornyl acetate; 16.24% L-camphor, and 8.25% borneol) + CPA	Hydrodistillation	1,100 mg/kg bw/d	Micronucleus test; dosed by gavage with the oil for 7 days, then given a single ip dose of 100 mg/kg bw CPA; bone marrow cells collected 24 hours 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	41

Abbreviations: B(a)P, benzo(*a*)pyrene; conc, concentration; CPA, cyclophosphamide; ip, intraperitoneal; 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 and normochromatic erythrocytes; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; stat sig, statistically significant; tBOOH, tert-butyl hydroperoxide.

Table 12. Effects on Tumor Promotion.

Test article	Extraction solvent/ method	Dose/exposure route	Species no./group	Tumor type	Carcinogenicity model	Results	Reference
Rosmarinus officinalis (rosemary) leaf extract (RE; 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; 30 F/grp	Skin	- Initiation: topical treatment with 200 nmol DMBA in 200 μL acetone - Promotion: after 1 week, 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), 2×/wk, for 20 weeks	1.2 mg: decreased tumor/mouse by 48%, 27%, and 28% after 7, 11, and 15 weeks TPA promotion 3.6 mg: decreased tumor/mouse by 84%, 37%, and 48% after 7, 11, and 15 weeks TPA promotion	[44]
As above	Methanol	1.2 or 3.6 mg; 5 minutes prior to B(a)P; dermal	CD-1 mice; 30 F/grp	skin	- Initiation: topical treatment with 200 μL acetone (controls) or with extract in 200 μL acetone (RE grp) 5 minutes prior to each 20 nmol application of B(a)P or 2 nmol DMBA, 1×/wk, for 10 weeks - Promotion: after 1 week, promotion with 15 nmol TPA in 200 μL acetone, 2×/wk, for 20 weeks	1.2 mg: decreased tumor/mouse by 15, 42, and 54% after 9, 13, or 21 weeks TPA promotion 3.6 mg: decreased tumor/mouse by 62, 63, and 64% after 9, 13, or 21 weeks TPA promotion	[44]
As above	Methanol	3.6 mg; dermal	CD-1 mice; 30 F/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 minutes before topical application of 200 nmol B(a)P in 200 μL acetone - Promotion: after 1 week, 15 nmol in 200 μL acetone, 2×/wk, for 20 weeks	Decreased tumor/mouse by 83%, 81%, and 58% after 9, 13, or 21 weeks TPA promotion	[44]
RE	DDW	500 mg/kg bw; gavage	Swiss albino mice; 12 M/grp	Skin	DMBA-initiated and croton oil-promoted skin tumorigenesis Grp 1: controls—topical treatment with 100 μL acetone; DDW by gavage for 15 weeks Grp 2: 500 mg/kg bw/d RE in 100 μL DDW for 15 weeks Grp 3: single topical dose 100 μg DMBA in 100 μL acetone; 2 weeks later, 1% croton oil in acetone, 3×/wk; also, 100 μL by gavage for 15 weeks 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 weeks after DMBA, 1% croton oil in acetone, 3×/wk Grp 5: single topical dose 100 μg DMBA in 100 μL acetone; after 2 weeks, 500 mg/kg bw RE extract by gavage for 15 days and 1% croton oil in acetone 3×/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 weeks after DMBA, 1% croton oil in acetone, 3×/wk	- A stat sig decrease in tumor number, diameter, and weight and a stat sig increase in the average latency period were 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	[55]

(continued)

Table 12. (continued)

Test article	Extraction solvent/ method	Dose/exposure route	Species no./group	Tumor type	Carcinogenicity model	Results	Reference
RE	DDW	1,000 mg/kg bw in DDW; gavage	Swiss albino mice; 12 M/grp	Skin	DMBA-initiated and croton oil-promoted skin tumorigenesis - Same protocol as above (grps 1-6), except 1,000 mg/kg bw RE was used	- Stat sig decrease in tumor burden and tumor yield, and a stat sig increase in average 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	56
R officinalis (rosemary) extract	Not specified	1.0%, in diet	Sprague Dawley rats; 20 F/grp	Mammary	- Rats were fed untreated or RE-supplemented diet throughout the study (16 weeks 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 between the 2 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	57

Abbreviations: B(a)P, benzo[a]pyrene; DDW, double distilled water; bw, body weight; DMBA, 7,12-dimethylbenz[a]anthracene; grp, group; F, female; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione-S-transferase; M, male; stat sig, statistically significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wt, weight.

Table 13. Dermal Irritations and Sensitization

Test Article	Concentration/dose	Test population	Procedure	Results	Reference
Nonhuman					
4.4% *Rosmarinus officinalis* (rosemary) leaf oil (and other essential oils)	Tested at concentrations up to 40%	Lewis rats	Ointment was applied to the shaved skin of Lewis rats twice daily, for 14 days	Not irritating No gross or microscopic lesions were reported in the skin	58
R officinalis (rosemary) leaf oil	Undiluted	Rabbits	Applications were made to intact and abraded rabbit skin under occlusion; no other details were provided	Moderately irritating	42
Human					
R officinalis (rosemary) leaves	Undiluted in sufficient petrolatum for binding	234 patients with contact dermatitis or eczema	Patch test	21 had +/− reactions; 18 had a + reaction; 5 had a ++ reaction; no patients had a +++ reaction	59
R officinalis (rosemary) leaves extracted with supercritical CO_2	Undiluted in petrolatum	20 patients	Epicutaneous test using Finn chambers	Weak irritant 1 positive reaction	9
R officinalis (rosemary) leaves as an absolute (soluble in hexane)	Undiluted in petrolatum	25 patients	Epicutaneous test using Finn chambers	Weak irritant 2 positive reactions	9
	2% and 10%	23 patients previously sensitized to peru balsam and/or perfumes or fragrance materials	Epicutaneous test using Finn chambers	Weak effect	
R officinalis (rosemary) leaves as a concrete (insoluble waxes) extracted in hexane	Undiluted in petrolatum	20 patients	Epicutaneous test using Finn chambers	No reactions	9
	2% and 10%	23 patients previously sensitized to peru balsam and/or perfumes or fragrance materials	Epicutaneous test using Finn chambers	Weak effect	
Cream containing 0.2% *R officinalis* (rosemary) leaf extract	Undiluted	20 patients	A 24-hour single insult occlusive patch test	Not an irritant No reactions were observed, and the primary irritation index was 0.00	60
Hair spray containing 0.0013% *R officinalis* (rosemary) leaf extract	Neat	102 patients	Modified Draize HRIPT Induction: Occlusive patches were applied for 24 hours, and the sites were scored prior to the application of the next patch; patches were applied 3×/wk for 3 weeks; the material was allowed to volatilize for 30 minutes prior to application Challenge: After a 2-week nontreatment period, challenge patches were applied to a previously untreated site; the test sites were scored 24 and 72 hours after application	Not an irritant or sensitizer Transient, barely perceptible to mild responses were observed in some patients, but were not considered related to skin irritation or an allergic reaction	62

(continued)

Table 13. (continued)

Test Article	Concentration/dose	Test population	Procedure	Results	Reference
Sunscreen cream containing 0.2% *R officinalis* (rosemary) leaf extract	Neat	27 patients	Maximization test Induction: An occlusive patch containing 0.1 mL of 0.25% aq SLS was applied to the upper outer arm, volar forearm, or back of each patient for 24 hours; the SLS patch was removed and an occlusive patch with 0.1 mL test material then applied for 48 or 72 hours; the patch was then removed and the test site examined; a total of 5 SLS/test material patches were applied during induction Challenge: After a 10-day nontreatment period, an occlusive patch with 0.1 mL of a 5% aq SLS solution was applied to a previously untreated site for 1 hour; this patch was removed and an occlusive patch containing 0.1 mL undiluted test material was then applied for 48 hours; the challenge site was graded 1 and 24 hours after patch removal	Not a contact sensitizer No reactions were observed	[61]
R officinalis (rosemary) leaf oil	10% in petrolatum	Not specified	48-hour closed patch test; details not provided	Not an irritant	[42]
R officinalis (rosemary) leaf oil	10% in petrolatum	25 patients	Maximization test; details not provided	Not a sensitizer	[42]
Leave-on massage oil containing 1.5% *R officinalis* (rosemary) leaf oil	Neat	104 patients	HRIPT Induction: An occlusive patch containing 50 μL of undiluted test material was applied for 48 hours; the patches were then removed and a new patch applied; 9 induction patches were applied. Challenge: Performed 12 to 14 days after induction at the original test site and a previously untested site for 48 hours; sites were scored at 48 and 96 hours. Patches of 0.5% SLS were used as a positive control, and deionized water as a negative control	Did not induce allergic contact dermatitis No reactions were observed at induction or challenge	[63]

Abbreviations: aq, aqueous; bw, body weight; HRIPT, human repeated insult patch test; SLS, sodium lauryl sulfate.

Table 14. 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 *R officinalis* (rosemary) leaf extract on D3	[64]
Occupational exposure to a *R 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 D3 and D7, + reaction to 1% on D3 and D7; controls were negative to 0.1% (n = 110) and 1% (n = 116) carnosol	[65]
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 patients, + in 1, among other positive reactions	[66]
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)	[67]
Picked rosemary leaves	Developed hand, forearm, and face dermatitis within hours	Prick-by-prick testing was negative at 15 minutes 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	[68]
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 tests with sage and oregano were negative - 5 controls were negative with all	[69]
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	[70]
Applied a poultice containing rosemary and thyme	After 24 hours, 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	[71]

(continued)

Table 14. (continued)

Mode of contact	Indication	Patch testing	Reference
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 *R 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	[72]

Abbreviation: aq, aqueous.

Rosmarinus officinalis (rosemary) extract is prepared by extraction from the leaves of *R officinalis* with acetone, ethanol, hexane, a combination of hexane and ethanol (in a 2-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 *R 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 *R 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 *R 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 some studies were performed for up to 3 months (dietary) with doses of up to 400 mg/kg bw/d. 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 with carbon tetrachloride, but not without, resulted in an increase in 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/d) of rosmarinus officinalis (rosemary) leave extract was a reproductive toxicant in a dietary study in male rats. In a study in gravid female Wistar rats, no statistically significant changes were observed after oral dosing with 26 mg/d 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 *R 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 *R officinalis* (rosemary) leaf 5:1 extract (and other botanical ingredients) in premenopausal women, a few changes were found. Overall, the changes were not remarkable.

In vitro, rosemary extract (solvent not specified) and rosmarinus officinalis (rosemary) leaf oil were not mutagenic in an Ames test, and rosmarinus officinalis (rosemary) leaf extract was not genotoxic in an Ames test, a chromosomal aberration assay in human lymphocytes, or a gene-locus mutation assay in human lymphocytes. In in vivo studies in mice and rats, oils that were extracted by hydrodistillation induced statistically significant increases in chromosomal aberrations without gaps in a chromosomal aberration assay at 2,000 mg/kg bw, increases in MNPCEs in several micronucleus tests at 1,000 and 2,000 mg/kg bw, and increases in DNA damage in a comet assay at ≥300 mg/kg bw; however, no genotoxic effects were seen in mice in a micronucleus test at 1,500 mg/kg bw/d with leaves extracted with absolute ethanol. A hydroalcoholic extract of rosemary was not genotoxic in a chromosomal aberration assay or a micronucleus test in rats. A mixture containing 19% *R officinalis* (rosemary) leaves, 71.5% St John's wort, and 9.5% spirulina induced in mice statistically significant increases in MNPCEs at 760 and 1,520 mg/kg bw/d in a micronucleus test; in frequency of aneuploidy, percent polyploidy, and total percent aberrations with 760 and 1,520 mg/kg bw/d in a chromosomal aberration assay; and in frequency of banana-shaped, swollen acrosome, and triangular head sperm

abnormalities and percent total spermatozoa abnormalities at 1,520 mg/kg bw/d in a spermatozoa abnormality assay.

Rosmarinus officinalis (rosemary) leaf extract was shown to have antimutagenic potential in vitro. 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 1.0% 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, *R officinalis* (rosemary) leaves produced irritation (scores of +/−, +, or ++) in 44 of 234 patients with contact dermatitis or eczema. A supercritical extract and the absolute of *R officinalis* (rosemary) leaves were considered weak irritants in a small study with test populations of 20 to 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-hour closed patch test or a sensitizer in a maximization study in 25 subjects; a formulation containing 1.5% rosmarinus officinalis (rosemary) leaf oil was not an irritant or a sensitizer in an HRIPT in 104 patients.

Several cases of allergic reactions to *R 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 0.1% and 1.0% carnosol resulted in positive reactions.

Discussion

Rosmarinus officinalis is GRAS as a spice, and although that mitigates much of the concern of oral exposure with cosmetic use, data on local effects, such as dermal irritation and sensitization data, are necessary to determine safety.

Additional information on the deodorizing process that is part of the preparation of some of the ingredients was not received. After further discussion, however, the Panel stated that because the deodorizing process is part of the preparation of food-grade rosmarinus officinalis (rosemary) extract, and because data are included in this safety assessment on some ingredients that were deodorized and no adverse effects were found, 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 in final formulation of plant constituents that may cause sensitization or other adverse effects. Specific examples of constituents that could possibly induce sensitization are linalool or monoterpenes, and those that could possibly cause adverse effects are caffeic acid and terpenes, such as thujone, limonene, and methyleugenol.

The 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 to limit impurities.

One study evaluated the irritation potential of *R officinalis* (rosemary) leaves in patients with contact dermatitis or eczema. The Panel stated that because the test participants were patients with eczematous skin, the report of irritation could not be interpreted for relevance to cosmetic use.

The Panel discussed the positive results observed in a reproductive toxicity study in male rats fed 500 mg/kg/d rosmarinus officinalis (rosemary) leaf extract, 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 at very high concentrations. Because these effects were observed only at very high concentrations, and because no statistically significant effects were reported in a study in rats dosed orally with 26 mg/d of a 30% aq extract of rosmarinus officinalis (rosemary) flower/leaf/stem extract, reproductive and developmental toxicity is not a concern with cosmetic use of *R officinalis* (rosemary)-derived ingredients, which are mostly used at very low concentrations.

Finally, the Panel discussed the issue of incidental inhalation exposure to *R officinalis* (rosemary)-derived ingredients. The Panel stated that although there were no inhalation data available, the *R officinalis* (rosemary)-derived ingredients are used at very low concentrations in products that could incidentally be inhaled; for example, rosmarinus officinalis (rosemary) leaf extract is used in other fragrance preparations and rosmarinus officinalis (rosemary) extract is used in face powders. The Panel noted that in aerosol products, 95% to 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 Cosmetic Ingredient Review Expert Panel concluded that the following 10 *R officinalis* (rosemary)-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be nonsensitizing:

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

*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.

Author's Note

Unpublished sources cited in this report are available from the Executive Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

Author contributions

M. Fiume and B. Heldreth contributed to conception and design, contributed to acquisition, analysis, and interpretation, drafted the manuscript, and critically revised the manuscript. L. Gill, W. Bergfeld, D. Belsito, R. Hill, C. Klaassen, D. Liebler, J. Marks, R. Shank, T. Slaga, and P. Snyder contributed to conception and design, contributed to analysis and interpretation, and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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