Safety Assessment of Chamomile Ingredients as Used in Cosmetics

Status:	Scientific Literature Review for Public Comment
Release Date:	February 11, 2013
Panel Date:	June 10-11, 2013

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date to comment on this Tentative Report and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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

The 2012 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Manager/Lead Specialist and Bart Heldreth, Ph.D., Chemist.

1101 17TH STREET, NW, SUITE 412 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

Table of Contents

INTRODUCTION

This is a review of the available scientific literature relevant to evaluating the safety of chamomile (German chamomile [*Chamomilla recutita* (*matricaria*) and Roman chamomile [*Anthemis nobilis*]) ingredients as used in cosmetics. These function mostly as fragrance ingredients and skin conditioning agents in cosmetic products. In addition to being a skin conditioning agent, chamomilla recutita (matricaria) flower/leaf/stem extract, also functions as a flavoring agent and an oral care agent. It should be noted that chamomilla recutita (matricaria) flower oil and anthemis nobilis flower oil are also known as German chamomile oil and Roman chamomile oil, respectively.[1] These names are used frequently in the published literature.

CHEMISTRY

The definitions of the chamomile ingredients presented in this scientific literature review are included in Table 1.

Physical and Chemical Properties

Chemical and physical properties of chamomilla recutita (matricaria) flower oil and anthemis nobilis flower oil are included in Table 2.

Method of Manufacture

Chamomilla Recutita (Matricaria) Flower Oil

Chamomilla recutita (matricaria) flower oil resulted from the steam distillation of chamomile (*Chamomilla recutita*) flowers.[2,3] According to another publication, chamomilla recutita (matricaria) flower oil is prepared by steam distillation of the flowers and stalks of *Chamomilla recutita* (*Matricaria*).[4]

Anthemis Nobilis Flower Oil

The preparation of anthemis nobilis flower oil involves the steam distillation of the dried flowers of *Anthemis nobilis.*[5]

Composition/Impurities

Data on the composition of chamomilla recuita (matricaria) flower extract, chamomilla recuita (matricaria) flower oil, and anthemis nobilis flower oil are included in Table 3. Additional information relating to composition is included below.

Chamomilla Recutita (Matricaria)

The chamomile species *Chamomilla recutita* may be classified into 4 different chemotyopes, depending on the main constituent of the essential oil:[6] bisabolol, bisabolol oxide A, bisabolol oxide B, and bisabolone oxide A. A characteristic constituent of chamomile flowers is the essential oil with the bisabolol derivatives matricine, or its artifact (chamazulene), trans beta-farnesene, and cis- as well as trans-en-in-dicycloether as typical components. Other than the coumarins herniarin and umbelliferone, flavonoids are the main hydrophilic constituents. Pectin-like polysaccharides with a main chain of alpha-1→4-linked polygalacturonic acid and a highly branched polysaccharide with beta-1→4-linked xylose are also present.

A study was performed to qualify the individual variability of components in 10 selected lines that originate from the chamomile (*Chamomilla recutita*) population.[7] Seedlings were planted in Poland in October of 2000 and flower heads were harvested during the following year. For the 10 chamomile lines investigated, the essential oil content ranged from 0.25 to 0.55%. Of the 60 components of essential oil detected using gas chromatography, 19 were identified. The major components were identified as follows: bisabolol oxide B (24.08% to 33.75%), bisabolol oxide A (5.75% to 10.92%), chamazulene (30.42%), farnesene (3.89% to 5.90%), and spathulenol (3% to 4.90%), and spiroether (12.63% to 19.95%). Polyacetylene – spiroether is the component of chamomile essential oil that has anti-inflammatory activity. Concentration ranges for 2 other sesquiterpenes (minor components) were α-bisabolone oxide (2.53% to 7.52%) and α-bisabolol (0.12% to

0.73%). The monoterpenes, sabinene, limonene, and cineol were present in small amounts, and only traces of α-pinene, p-cymene, and γ-terpinene were detected.

A study relating to the following impurities in dry chamomile (*Chamomilla recutita*) from Croatia was identified: lead and cadmium heavy metals, and the herbicides linuron, fluazifop-p-butyl, and cycloxydim.[8] Cadmium and all 3 pesticide residues in dried samples of industrially grown dry chamomile were found to be above the suggested and accepted tolerance values.

In the post-harvest processing of *Chamomilla recutita*, drying is an important process for preserving plant material, in that it inhibits enzymatic degradation and limits microbial growth.[9] The phenolic content of Chamomilla recutita consists of the flavonoids, flavone glycosides (e.g., apigenin 7-glucoside) and flavonols (e.g., quercetin glycosides and luteolin glucosides). The effect of drying on the total phenol content of aqueous chamomile extracts has been reported. Freshly extracted chamomile flowers had a higher content of phenols (19.7 ± 0.5 mg/g dry weight (dw)) when compared to any of the dried samples, except for those freeze-dried ($p \leq 0.05$). There was no significant difference between the total phenol content in samples that were freeze-dried, air-dried, or oven-dried at 40ºC. However, a major decrease in the phenol concentration of chamomile flowers oven-dried at 80ºC (13 ± 1 mg/g dw; $p \leq 0.05$) was noted. Data showing the effect of drying on content of the flavonoid apigenin 7-glucoside were also presented. Extracts produced from fresh chamomile had an apigenin 7-glucoside content of 3.0 ± 0.4 mg/g dw, which was significantly higher than any of the dried samples ($p \leq 0.05$). There was no significant difference in the apigenin 7-glucoside content between the chamomile flowers that were freeze-dried, air-dried, or oven-dried at 40ºC (2.0 ± 0.4 mg/g dw). The greatest decrease in apigenin7-glucoside content (1.0 ± 0.3 mg/g dsw) was observed in samples oven-dried at 80ºC.[9]

Benzodiazepine-like compounds and gamma-amino butyric acid (GABA) have been found in the flower heads of Chamomilla recutita (Matricaria), used in folk medicine to prepare a spasmolytic and sedative tea.[10] The flower heads were extracted and purified by high performance liquid chromatography (HPLC). Several fractions were then tested for their ability, *in vitro*, to displace [^{3}H]flunitrazepam bound to its receptors in rat (strain not stated) cerebellar membranes, [^{3}H]muscimol linked to GABA receptors in rat (strain not stated) cortical membrane preparations, and [^{3}H]RO 5-4864, bound to the peripheral benzodiazepine binding sites in membrane preparations from rat (strain not stated) adrenal glands. It was noted that few of these fractions displaced both central and peripheral benzodiazepine binding sites and GABA receptors. After further HPLC analysis, GABA was identified as the main agent that was responsible for the displacing effect. Fractions containing GABA and both central and peripheral receptors displacing ligands or fractions containing only central and peripheral receptors displacing ligands were injected intracerebroventricularly (i.c.v., 5 µl) into rats (strain not stated) and locomotor activity was evaluated. Some of the fractions without GABA induced a statistically significant reduction in locomotor activity. The results of this study indicate that the flower heads of Chamomilla recutita (Matricaria) contain endogenous ligands that are able to displace real benzodiazepines from both central and peripheral benzodiazepine receptors.

The occurrence of formaldehyde in intact *Chamomilla recutita* (*Matricaria*) plants was evaluated. Wild *Chamomilla recutita* (*Matricaria*) and 2 varieties of this plant, BK-2 and *Degumil*, grown in Hungary were studied.[11] The BK-2 and Degumil varieties were grown in central Hungary, whereas, the wild type was grown in southern Hungary. Formaldehyde (HCHO) in dimedone adduct form (formaldemethone) was identified and quantified using automatic overpressured layer chromatography (OPLC). Plant samples were frozen, powdered, and treated with a 0.2% solution of dimedone in methanol. Each plant part (root, shoot, or inflorescence) suspension was then centrifuged and the supernatant was used for OPLC. The inflorescence (≈ 6.5 µg HCHO/g) and root (≈ 7 µg HCHO/g) samples of the intact, soil-grown *Degumil* variety contained the greatest quantity of HCHO, followed by the shoots and inflorescence of the cultivated BK-2 and *Degumil* varieties. The wild type contained similar amounts of HCHO in its inflorescence (≈ 5µg HCHO/g) and shoots (5µg HCHO/g). The amount of HCHO bound by the dimedone reagent increased as the concentration of dimedone increased, until a maximum was reached.

Chamomilla Recutita (Matricaria) Flower

Chamomilla recutita (matricaria) flowers contain a volatile oil (0.24 to 2.0%) that is blue in color.[12] The two key components (-)-alpha-bisabolol and chamazulene account for 50 to 65% of the total volatile oil content. Other components of the oil are as follows: (-)-alpha-bisabolol oxide A and B, (-)-alpha bisabolone oxide A, spiroethers (cis- and trans- en-yn-dicycloether, sesquiterpenes (anthecotulid), cadinene, farnesene, furfural, spathulenol, and proazulene (matricarin and matricin). Chamazulene is formed from natricin during steam distillation of the oil.

Chamomilla Recutita (Matricaria) Flower Oil

Chamomilla recutita (matricaria) flower oil contains anti-inflammatory and spasmolytic sesquiterpene lactones such as α-bisabolol, blue chamazulene (weaker anti-inflammatory effect), farnesene, polyenes, and several flavonoids.[13] *Chamomilla recutita* imported from Argentina may contain larger amounts of the strongly allergenic sesquiterpene lactone anthecotulide, and, additionally, may be contaminated with the morphologically similar dog fennel (*Anthemis cotula*), which contains up to 7.3% anthecotulide. However, *Chamomilla recutita* of European origin contains only traces of anthecotuliode.

The following are known essential components of chamomilla recutita (matricaria) flower oil:[14] azulene, sesquiterpenes, sesquiterpene alcohols, paraffin hydrocarbons, umbelliferone methyl ether, furfurol, and a fatty acid.

The essential oil production of cultivated (BK-2, Degumil) and wild chamomile populations of 4 typical chamomile-rich regions of Hungary.[15] The Hungarian BK-2 contained more chamazulene in its essential oil than the German Degumil type, which is cultivated mainly for α-bisabolol content. Both components have important anti-inflammatory activities. Wild populations can be easily distinguished from cultivated ones, based on their high content of bisaboloides. This is true particularly for the flower of Szabadkigyós wild type, for which the average content of biologically active (-)-α-bisabolol was 48%.

Chamomilla Recutita (Matricaria) Flower Oil
and Chamomilla Recutita (Matricaria) Flower Extract

Kamillosan® (an alcoholic extract of chamomile flowers that contains 150 mg of chamomilla recutita (matricaria) flower oil), the hydroalcoholic extract (42% ethanol) of chamomilla recutita (matricaria) flowers, and pure chamomilla recutita (matricaria) oil (plant part source not stated) were analyzed (using HPLC) to identify the coumarin derivatives umbelliferone and herniarin. Kamillosan® contained 41.8 µg umbelliferone/ml and 93.1 µg herniarin/ml, and the hydroalcoholic extract of chamomilla recutita (matricaria) flowers contained 36.0 µg umbelliferone/ml and 114.0 µg herniarin/ml. Pure chamomilla recutita (matricaria) oil contained 540 µg herniarin/ml. Information on Kamillosan® content is presented because it is tested in some of the studies included in this safety assessment.

USE

Cosmetic

The chamomile ingredients (chamomilla recutita (matricaria) and anthemis nobilis) function mostly as fragrance ingredients and skin conditioning agents in cosmetic products.[1] The only ingredient with different functions, in addition to being a skin conditioning agent, is chamomilla recutita (matricaria) flower/leaf/stem extract, which also functions as a flavoring agent and oral care agent. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2012, the following 10 chamomile ingredients have been used in cosmetic products:[16] **chamomilla recutita (matricaria) flower oil**, chamomilla recutita (matricaria) oil, chamomilla recutita (matricaria) extract, **chamomilla recutita (matricaria) flower**, **chamomilla recutita (matricaria) flower extract**, **chamomilla recutita (matricaria) flower/leaf extract**, chamomilla recutita (matricaria) flower water, **anthemis nobilis flower extract**, **anthemis nobilis flower oil**, and **anthemis nobilis flower water**.

Results from surveys of ingredient use concentrations provided by the Personal Care Products Council in 2009 and 2010 indicate that, collectively, the following 10 ingredients have been used at concentrations up to13%:[17,18] **chamomilla recutita (matricaria) flower oil**, chamomilla recutita (matricaria) flower powder, **chamomilla recutita (matricaria) flower**, **chamomilla recutita (matricaria) flower extract**, **chamomilla recutita (matricaria) flower/leaf extract**, chamomilla recutita (matricaria) flower/leaf/stem extract, chamomilla recutita (matricaria) leaf extract, **anthemis nobilis flower extract**, **anthemis nobilis flower oil**, and **anthemis nobilis flower water**. [Overlap between the FDA and industry survey data sets is represented in bold print.] The VCRP and Council survey data combined indicate that 13 chamomile ingredients have been used in cosmetic products, and these data are summarized in Table 2. Because current data from FDA and the Council are anticipated, the breakdown of use frequency and use concentration data into various categories based on exposure type (e.g., dermal contact, hair-coloring, baby products, etc.) does not appear in Table 2. However, information will be presented in this manner after current data have been received.

Cosmetic products containing chamomile ingredients may be applied to the skin and hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Non-Cosmetic

Chamomilla Recutita

The chamomile species used in medicine is *Chamomilla recutita,* and hydroalcoholic extracts of chamomile flowers in ointments or creams (e.g., Kamilosan^{R}) are often used. Additionally, bath additives (e.g. Kamillobad^{R}) and mouth sprays (e.g., Kamillosan M Spray) containing chamomile extracts as the active ingredient are offered for topical and oral treatment in appropriate dosage forms.[6] The use of chamomile in aroma therapy for the treatment of patients with dementia has also been reported.[19]

Reportedly, chamomile (*Matricaria recutita*) is listed as an official drug in the pharmacopoeias of 26 countries, including Germany, Belgium, France, and the United Kingdom.[20] With this in mind, reportedly, chamomile (*Matricaria recutita*) is used extensively for the treatment of a wide range of conditions, namely, upset stomach, ulcers of inflamed skin and mucous membranes, and mild nervous conditions.

Chamomilla recutita (matricaria) (German chamomile) and *Anthemis nobilis* (Roman chamomile) are listed among the spices and other natural seasonings and flavorings that are generally recognized as safe (GRAS) for their intended use in food for human consumption.[21] They are also listed among the spices and other natural seasonings and flavorings that are GRAS for their intended use in animal drugs, feeds, and related products.[22]

Chamomilla recutita (matricaria) flowers and *Anthemis nobilis* flowers are listed among the essential oils, oleloresins (solvent-free), and natural extractives (including distillates) that are GRAS for their intended use in food for human consumption.[23] They are also listed among the essential oils, oleloresins (solvent-free), and natural extractives (including distillates) that are GRAS for their intended use in animal drugs, feeds, and related products.[24]

Chamomile flowers have also been used as active ingredients of digestive aid drug products (over-the-counter drug products). However, the U.S. Food and Drug Administration has determined that the available data are inadequate for establishing general recognition of safety and effectiveness of these ingredients as used in digestive aid drug products.[25]

TOXICOKINETICS

Data on the absorption, distribution, metabolism, and excretion of chamomile ingredients were not found in the published literature.

TOXICOLOGY

Acute Toxicity

Oral

Chamomilla Recutita (Matricaria) Flower

The acute oral toxicity of a lyophilized infusion of chamomilla recutita (maricaria) flowers was evaluated using 2 groups of 12 female mice of the Swiss-NOS strain.[26] Preparation of the lyophilized infusion was in accordance with the following procedure: Boiling water (1000 ml) was poured on 50 g of tubular flowers. [26]The preceding step was followed by vacuum filtration and the extract was lyophilized, forming a dried product. The 2 groups received a single oral dose of 720 and 1440 mg/kg, respectively, and were observed for 24 h post-dosing. None of the animals died, and there was no evidence of acute toxicity.

Chamomilla Recutita (Matricaria) Flower Oil

The acute oral toxicity of chamomilla recutita (matricaria) flower oil (dose = 5 g/kg) was evaluated using 10 rats (strain not stated).[27] Dosing was followed by a 14-day observation period. None of the animals died, and an LD50 of > 5 g/kg was reported.

Acute oral LD50 values of 8,560 mg/kg and 10,000 mg/kg in rats have also been reported for chamomilla recutita (matricaria) flower oil .[28] Details relating to the test protocol and study results were not included.

Chamomilla Recutita (Matricaria) Flower Oil Extract

In an acute toxicity study, doses of chamomilla recutita (matricaria) flower oil extract (10, 100, 1000, 1600, 2900, 4300, and 5600 mg/kg) were administered orally to groups of male NIH mice (number per group not stated).[29] None of the animals died. This study was performed prior to the antigenotoxicity study summarized in the Genotoxicity section of this report.

Anthemis Nobilis Flower Oil

The acute oral toxicity of anthemis nobilis flower oil (dose = 5 g/kg) was evaluated using 10 rats (strain not stated).[30] Dosing was followed by a 14-day observation period. None of the animals died, and an LD50 of > 5 g/kg was reported.

In a study involving rabbits (strain not stated), the acute oral LD_{50} exceeded 5 g/kg.[5]

Dermal

Chamomilla Recutita (Matricaria) Flower Oil

The acute dermal toxicity of chamomilla recutita (matricaria) flower oil (dose = 5 g/kg) was evaluated using 6 rabbits (strain not stated).[27] None of the animals died, and an LD50 of > 5 g/kg was reported. The skin reactions observed are reported in the section on Skin Irritation.

Anthemis Nobilis Flower Oil

The acute dermal toxicity of chamomilla recutita (matricaria) flower oil (dose = 5 g/kg) was evaluated using 4 rabbits (strain not stated). None of the animals died, and an LD50 of > 5 g/kg was reported.[30] The skin reactions observed are reported in the section on Skin Irritation.

Repeated Dose Toxicity

Animal

Chamomilla Recutita (Matricaria) Flower

Data on repeated dose toxicity were presented in a study on the the effect of the herbal tea, chamomile tea on the activity of hepatic phase I and phase II metabolizing enzymes from the rat.[31] Results relating to effects on enzyme activity are summarized in the section on Enzyme Inhibition later in the report text. Chamomile tea is made from the dried flower heads of *Chamomilla recutita* (matricaria). Five female Wistar rats (8 to 9 weeks old) had free access to Chamomile tea solution (2% w/v), whereas the control group had access to water. After 4 weeks of treatment, the animals were killed. Dosing had no significant influence on body weight, and there were no signs of gross pathology of internal organs. Liver weight /body weight ratios of treated rats were not significantly different from control values.

Human

Fourteen health volunteers (7 males, 7 females) were given 200 ml of chamomile tea daily for 2 weeks. None of the subjects reported adverse effects after ingestion of the tea.[32] An analysis of urine samples collected before dosing, during the

dosing period, and after dosing indicated that depletion of creatinine and the elevation of hippurate and glycine were strongly associated with chamomile tea intake.

Chamomilla Recutita (Matricaria) Flower Extract

Sprague-Dawley rats of either sex (number not stated) were fed increasing doses (1, 2, 4, and 8 g/kg body weight) of chamomilla recutita (matricaria) flower extract, dissolved in water, for 14 days.[33] Neither signs of toxicity nor mortalities were observed at doses up to 4 g/kg body weight. Information relating to effects of the 8 g/kg dose was not included. All of the animals remained physically active.

Antimicrobial Activity

Chamomilla Recutita (Matricaria) Flower Oil

A study was performed to present quantitative data on the antimicrobial activity of chamomilla recutita (matricaria) flower oil.[34] The fungus *Candida albicans* and the following bacterial strains were tested: *Staphylococcus aureus* and *Bacillus subtilis* (both gram positive), and *Escherichai coli* and *Pseudomonus aeroginosa* (both gram negative). Marked bacteriostatic and bactericidal activity was observed in the fungus and gram positive bacteria, only at test concentrations greater than 0.05% v/v. Antimicrobial activity was not observed at concentrations less than 0.025%. Gram negative bacteria were found to be less sensitive to antimicrobial effects of the oil. Complete (100%) bactericidal activity was not achieved at concentrations of the oil as great as 8% v/v.

The disc agar diffusion method was used to evaluate the antibacterial activity of chamomilla recutita (matricaria) flower oil, grown in Iran, against the following strains: *Bacillus cereus* (MTCC430), *Bacillus subtilis* (MTCC441), and *Staphylococcus aureus* subsp. *aureus* (MTTCC2940) – all gram-positive, and *Klebsiella pneumonia* (MTCC109), *Escherichia coli* (MTCC443), *Proteus vulgaris* (MTCC426), and *Salmonella typhi* (MTCC733) – all gram-negative.[35] Aliquots (10 µl) of the oil at 1:2 dilutions in DMSO were placed on filter paper discs that were applied to agar plates. The plates were incubated for 24 h (at 37˚C) and inhibition zones observed were measured. The DMSO solvent (10 µl) and streptomycin (10 µl/disc) were applied to control discs, and inhibitory activity (% inhibition) was expressed in relation to the streptomycin standard. Results for chamomilla recutita (matricaria) flower oil in DMSO (1:2 dilutions) against gram positive bacteria were as follows: *Bacillus cereus* (153% inhibition), *Bacillus subtilis* (73% inhibition), and *Staphylococcus aureus* subsp. *aureus* (108% inhibition). The following results for gram negative cultures were comparable to those reported for gram positive bacteria: *Klebsiella pneumonia* (95% inhibition), *Escherichia coli* (62% inhibition), *Proteus vulgaris* (115% inhibition), and *Salmonella typhi* (94% inhibition).

The antimicrobial activity of chamomilla recutita (matricaria) flower oil against the following fungal cultures was studied using the disc diffusion technique: *Aspergillus* (*A*) *niger, A. fumigatus, A. flavus, A. flavipes, A. ochraceus, A. nidulans, A. glaucus, A. terrus, Paecilomyces* spp., and *Penicillium spp*.[36] Filter paper discs completely moistened with the oil (undiluted or 10% and 2% dilutions in acetone) were placed on the surface of the inoculated plates, which were refrigerated for 1 h to prevent diffusion of the oil. The plates were then incubated (28ºC) for 3 days, and the zone of inhibiton was measured. Crude chamomilla recutita (matricaria) flower oil had a moderate inhibitory effect against all tested fungi. The results of testing dilutions of the oil indicated that inhibitory activity was reduced as dilution was increased, except for the following fungi: *A. flavus, A. flavipes, A. ochraceus*, and *A. glaucus*. For these 4 fungi, the activity of 10% chamomilla recutita (matricaria) flower oil was greater than that of the crude oil. At a concentration of 2%, the oil had either no effect or a slight effect against the fungi tested.

In another study, the activity of chamomilla recutita (matricaria) flower oil against different thymidine-kinase-positive (acyclovir-sensitive) and thymidine-kinase-negative (acyclovir-resistant) herpes simplex virus type 1 (HSV-1) strains was evaluated.[37] Clinical HSV-1 isolates containing frameshift mutations in the thymidine kinase (TK) gene, an insertion or deletion, yield a non-functional thymidine kinase enzyme resulting in phenotypical resisitance against acyclovir. The inhibition of virus replication was measured using a plaque reduction assay involving RC-37 cells (African green monkey kidney cells), Vero cells, and MCDK cells. Each experiment, performed with 6 replicates, was repeated 3 times. Using the number of plaques observed in virus-infected controls (treated with 1% ethanol but no addition of the oil) as a reference, the concentration of the oil that inhibited the plaque number by 50% (IC50) was determined from dose-response curves. In order to determine the mode of antiviral action, the cells were pretreated with the oil prior to viral infection. The viruses were incubated with the oil before infection, and the cells and viruses were incubated together during adsorption or penetration of the virus into the host cells.

Chamomilla recutita (matricaria) flower oil had a high level of antiviral activity against acyclovir-sensitive HSV strain KOS and acyclovir-resistant clinical HSV isolates, as well as acyclovir-resistant srain Angelotti. At a maximum noncytotoxic concentration of the oil (10 ± 1.2 µg/mL), plaque formation was significantly reduced by 99.9%, that is, when herpes viruses were preincubated with drugs before attachment to host cells. No significant effect on viral infectivity was achieved by adding the oil during the replication phase. These results indicate that chamomilla recutita (matricaria) flower oil affected the virus by interrupting adsorption of herpes viruses, and in a manner different from that of acyclovir, which is effective after attachment inside of the infected cells. Thus, the oil was capable of exerting a direct effect on HSV, and might be useful in the treatment of drug-resistant viruses.[37]

Chamomilla Recutita (Matricaria) Flower Oil
and Chamomilla Recutita (Matricaria) Flower Extract

The following substances were evaluated for antimicrobial activity against *Escherichia coli Bs-1* (*rec+ exr-, hcr-*) in the presence of UV light (300 to 400 nm, peak at 350 nm; 48240 J/m^2 for 2 h) or in the dark:[38] Kamillosan® (1 ml; also defined in Chemistry section) - contained 150 mg of chamomilla recutita (matricaria) flower oil and the coumarin derivatives umbelliferone (41.8 µg) and herniarin (93.1 µg), the hydroalcoholic extract (42% ethanol) of chamomilla recutita (matricaria) flowers (0.2 ml) - contained umbelliferone (7.2 µg) and herniarin (22.8 µg), and herniarin (100 µg; used as standard). For all substances evaluated, antimicrobial activity (inhibition zones visible) was observed in the presence of UV light, but not in the dark.

The antimicrobial activity of chamomilla recutita (matricaria) flower extract (plant grown in Jordan, ethanolic extract) against the following bacterial strains was evaluated *in vitro* using the agar dilution technique: *Staphylococcus aureus*, *Candida albicans*, *Escherichai coli*, *Betula pubescens*, and *Pinus sylvestris*.[39] The extract was dissolved in DMSO and diluted to yield concentrations ranging from 2000 to 31.3 µg/ml. The minimum inhibitory concentration (MIC) was determined after 24 h of incubation at 37˚C. Chamomilla recutita (matricaria) flower extract had antimicrobial activity against *Staphylococcus aureus* (MIC = 32.5 µg/ml) and *Candida albicans* (MIC = 65 µg/ml). The extract was also said to have had noticeable activity against *Escherichia coli* (MIC = 250 µg/ml), *Betula pubescens* (MIC = 125 µg/ml), and *Pinus sylvestris* (MIC = 500 µg/ml).

Effect on Nervous System

Chamomilla Recutita (Matricaria) Flower

A lyophilized infusion of chamomilla recutita (maricaria) flowers was evaluated for its effect on the central nervous system. Preparation of the lyophilized infusioin was in accordance with the following procedure: Boiling water (1000 ml) was poured on 50 g of tubular flowers.[26] The preceding step was followed by vacuum filtration and the extract was lyophilized, forming a dried product. The infusion (suspended in phosphate buffer solution [PBS], pH 7.4) was administered i.p. to groups of female mice of the Swiss-NOS strain. Control mice were injected with a volume of PBS that was equivalent to that of the test material. The following tests were performed: spontaneous motor activity tests (long-term motility test: groups of 3 mice, single 360 mg/kg injection; short-term motility test: groups of 3 mice, logarithmic scale of 8 doses from 11.5 to 520 mg/kg), motor coordination test (rota-rod apparatus; number of mice not stated; dose of 360 mg/kg), exploratory activity test (hole-board test; groups of 16 mice, doses of 90, 180, and 360 mg/kg) and barbiturate potentiation (groups of 8 mice; doses of 20, 40, 80, 160, and 320 mg/kg). Basal motility was decreased in a dose-dependent manner (92% reduction at a dose of 360 mg/kg), without involving motor coordination and muscle relaxation. Both exploratory and motor activities registered in the hole-board test were significantly decreased. A mild, hypogenic effect was observed at doses of 160 and 320 mg, with significant potentiation of hexobarbital-induced sleep. It was concluded that the lyophilized infusion of chamomilla recutita (maricaria) flowers had a depressive effect on the central nervous system.

Chamomilla Recutita (Matricaria) flower Oil

The effect of chamomilla recutita (matricaria) flower oil on the central nervous system (CNS) was studied using groups of 7 adult male Balb/c mice.[40] Changes in spontaneous locomotor activities and motor coordination induced by chamomilla recutita (matricaia) flower oil (25, 50, and 10 mg/kg doses) and caffeine (reference drug, 25 mg/kg dose) were evaluated using activity cage measurements (for locomotor activities) and Rota-Rod tests (for motor coordination assessment). The following tests were used to assess the emotional state of the mice: open field, social interaction, and elevated plus-maze tests. A tail suspension test was performed to evaluate the effect of the oil on depression levels in the mice. A sunflower oil solution served as the control. Chamomilla recutita (matricaria) flower oil, caffeine, and the control solution were administered orally at 60 minutes before the experimental sessions. At doses of 50 and 100 mg/kg, chamomilla recutita (matricaria) flower oil had the following effects: significantly increased spontaneous locomotor

activities, exhibited an anxiogenic effect in the open field, elevated plus-maze and social interaction tests, and decreased the immobility times of mice in tail suspension tests. Dosing did not result in a change in the failing latencies in Rota-Rod tests. The 25 mg/kg dose of chamomilla recutita (matricaria) flower oil was ineffective in each test. The activity profile for chamomilla recutita (matricaria) flower oil (50 and 100 mg/kg doses) was similar to that of the drug, caffeine (25 mg/kg dose).

Ocular Irritation

Chamomilla Recutita (Matricaria) Flower

A conjunctival provocation test was performed on 7 hay fever patients who had experienced conjunctivitis after ocular rinsing with chamomilla recutita (matricaria) tea (from flower heads).[41] Chamomilla recutita (matricaria) tea extract (tea extracted in phosphate-buffered saline) was evaluated in the provocation test. Initially, one drop of the tea extract (1:1,000,000 wt/vol) was instilled into the conjunctival sac. If a reaction was not observed within 20 minutes, the next concentrations (progressively increased by ten-fold) were instilled into the conjunctival sac of the other eye. The conjunctivitis initially experienced after ocular rinsing with the tea was reproduced via conjunctival provocation. Two of the patients had a positive conjunctival response to very dilute solutions of the extract (1:100,000 wt/vol and 1:1,000,000 wt/vol, respectively). Three and two patients had positive responses to 1:1000 w/v and 1:100 w/v, respectively. Additionally, all 7 patients had positive skin prick tests to the tea extract. Only 2 of the 100 control hay fever patients had a positive conjunctival reaction to the tea extract, ruling out that these reactions were due to an irritating effect. It was concluded that ocular rinsing with chamomilla recutita (matricaria) tea can induce allergic conjunctivitis.

Chamomilla Recutita (Matricaria) Flower Oil

The hen's egg test – chorioallantoic membrane (HET-CAM assay) was used to determine the irritation potential of chamomilla recutita (matricaria) flower oil.[37] HET-CAM assays were performed with 6 replicates and repeated 3 times. The oil was applied to the CAM of fresh, fertile eggs that had been incubated for 72 h. Undiluted chamomilla recutita (matricaria) flower oil was not irritating to the hen's egg chorioallantoic membrane.

Skin Irritation

Chamomilla Recutita (Matricaria) Flower Oil

In the acute dermal toxicity study on chamomilla recutita (matricaria) flower oil involving 6 rabbits (strain not stated), summarized earlier, the following skin reactions were observed after dosing with 5 g/kg: slight redness (2 rabbits), moderate redness (4 rabbits), slight edema (2 rabbits), and moderate edema (4 rabbits).

Anthemis Nobilis Flower Oil

In the acute dermal toxicity study on anthemis nobilis flower oil involving 4 rabbits (strain not stated), summarized earlier, the following skin reactions were observed after dosing with 5 g/kg: slight redness (1 rabbit), moderate redness (2 rabbits), and moderate edema (4 rabbits).[27]

Skin Irritation and Sensitization

Animal

Chamomilla Recutita (Matricaria) Extract

The cross reactivity of carabron (sesquiterpene lactone isolated from *Arnica longifolia*) with chamomilla recutita (matricaria) extract was evaluated using 5 female albino guinea pigs of the Pirbright white strain.[42] The chamomilla recutita (maricaria) extract tested was a *Chamomilla recutita* (*Matricaria*) whole plant ether extract. A 10% acetone solution of carabron (0.05 ml) was applied daily (weekends excluded) to a 2 cm^2 area of the clipped and shaved flanks; slight erythema developed on day 9. Applications were continued for up to a period of 4 weeks and were discontinued when a strong inflammatory reaction (+++) was observed. The challenge phase was initiated 2 weeks after the end of the induction phase. Four different concentrations of carabron (0.3%, 1%, 3%, and 10%) were applied to the opposite flank. Challenge reactions (slight spotty erythema, down to the 0.3% dilution) were observed in all animals. The animals were also challenged with 10% chamomilla recutita (matricaria) extract, and there were no sensitization reactions in any of the 5 guinea pigs previously sensitized to carabron.

Chamomilla Recutita (Matricaria) Flower Oil

In a skin irritation study, undiluted chamomilla recutita (matricaria) flower oil was applied to the backs of hairless mice (number and strain not stated). Details relating to the test procedure were not included. The oil was classified as non-irritating.[5]

In another study, chamomilla recutita (matricaria) flower oil was applied (under occlusion) to intact or abraded skin of rabbits (number and strain not stated) for 24 h. The oil was classified as moderately irritating.[5]

Anthemis Nobilis Flower Oil

In a skin irritation study, undiluted anthemis nobilis flower oil was applied to the backs of hairless mice (number and strain not stated). Details relating to the test procedure were not included. The oil was classified as non-irritating.[5]

In another study, undiluted anthemis nobilis flower oil was applied (under occlusion) to intact or abraded skin of rabbits (number and strain not stated) for 24 h. The oil was classified as moderately irritating.[5]

The skin sensitization potential of anthemis nobilis flower oil was evaluated in the open epicutaneous test using at least 6 guinea pigs (males and females).[43] Using a pipette or syringe, anthemis nobilis flower oil (4% solution, 0.1 ml) was applied epicutaneously to an 8 cm^2 area of the clipped flank daily, and the test site remained uncovered for 24 h. These induction applications were repeated daily for 3 weeks. Reactions were scored either at the end of the application period or at the end of each week. The guinea pigs were challenged with the oil (on contralateral flank) on days 21 and 25. Ten guinea pigs served as controls. Anthemis nobilis flower oil was not allergenic in this study.

Human

Predictive Testing

Chamomilla Recutita (Matricaria) Flower Oil

The skin irritation potential of chamomilla recutita (matricaria) flower oil (4% in petrolatum) was evaluated in a 48-h closed patch test involving human subjects (number not stated). Skin irritation was not observed.[4]

The skin sensitization potential of chamomilla recutita (matricaria) flower oil was evaluated in the maximization test using 25 healthy volunteers (21 to 42 years old).[44] The test material (4% in petrolatum) was applied, under occlusion, to the volar forearm of each subject for a total of 5 alternate-day 48-h periods. The test site was pre-treated with 5% sodium lauryl sulfate (24-h application, under occlusion) prior to application of the test material. A 10-day non-treatment period was observed after the induction phase. Challenge patches were then applied, under occlusion, to new test sites for 48 h. The application of challenge patches was preceded by a 1-h application of 10% aqueous sodium lauryl sulfate (under occlusion). Reactions were scored at the time of challenge patch removal and 24 h later. There was no evidence of contact sensitization in any of the subjects tested.

Anthemis Nobilis Flower Oil

The skin irritation potential of anthemis nobilis flower oil (4% in petrolatum) was evaluated in a 48-h closed patch test involving human subjects (number not stated). Skin irritation was not observed.[5]

The skin sensitization potential of anthemis nobilis flower oil (4% in petrolatum) was evaluated in the maximization test using 25 healthy volunteers (21 to 44 years old) using the protocol described in the maximization test on chamomilla recutita (matricaria) flower oil in the preceding section.[45] There was no evidence of contact sensitization in any of the subjects tested.

Chamomile Essential Oil

In a skin irritation and sensitization study, chamomile essential oil (genus and species not stated) was initially applied to 113 healthy subjects (13 men, 100 women;18 to 69 years old), 110 of whom completed the study.[46] Three subjects withdrew for reasons unrelated to conduct of the study. The oil was applied, under an occlusive patch (volume and area not stated), between the scapulae of the upper back. Patches were applied to the same site on Mondays, Wednesdays, and

Fridays for a total of nine 24-h induction applications. Removal of patches on Tuesdays and Thursdays was followed by a 24-h non-treatment period. Patch removal on Saturdays was followed by a 48-h non-treatment period. Reactions were scored during non-treatment periods. The challenge phase was initiated at the end of a 2-week non-treatment period. Challenge patches were applied to new test sites, and reactions were scored at 24 h, 48 h, 72 h, and 96 h post-application. At most, mild erythema was observed in 5 subjects during the induction phase. During the challenge phase, 1 subject had mild erythema and edema at the 48-h reading. This reaction had increased to well-defined erythema by the 72-h reading, but had diminished to mild erythema by the 96-h reading. During re-challenge of this subject (semi-occlusive, occlusive, and open patches used), barely perceptible erythema was observed at 24 h (occlusive patch test only). There were no visible skin reactions at 48 h or 72 h following application of any of the 3 types of patches. It was concluded that chamomile essential oil did not demonstrate a potential for eliciting dermal irritation or sensitization.

The skin irritation and sensitization potential of chamomile essential oil (genus and species not stated) was evaluated in an RIPT that initially involved 122 healthy subjects (60 men, 32 women; 18 to 68 years old), 104 of whom completed the study.[47] Eighteen subjects withdrew for reasons unrelated to conduct of the study, one of whom withdrew due to a generalized pethechial response on most of the back. The oil (0.2 ml) was applied to a 2 cm x 2 cm semi-occlusive patch that was placed on the back (between the scapulae and waist, adjacent to the spinal midline) of each subject. The patches remained in place for 24 h. Removal of patches on Tuesdays and Thursdays was followed by a 24-h non-treatment period. Patch removal on Saturdays was followed by a 48-h non-treatment period. Reactions were scored during non-treatment periods. The test procedure was repeated on Mondays, Wednesdays, and Fridays for a total of 9 induction applications. The challenge phase was initiated at the end of a 2-week non-treatment period. Challenge patches were applied to new test sites, and reactions were scored at 24 h and 72 h post-application. Transient, barely perceptible erythema was observed in 8 of the 104 subjects during induction and/or challenge phases. These reactions were not classified as irritant or allergic in nature. It was concluded that chamomile essential oil did not induce skin irritation or allergenicity.

Provocative Testing

Chamomilla Recutita (Matricaria)
Chamomilla Recutita (Matricaria) Extract

The skin sensitization potential of chamomilla recutita (matricaria) extract (ether extract) was studied using 24 patients (men and women; age range: 23 to 82 years) with Compositae allergy.[48] The plant extract (1%) was applied to the back of each patient using Finn chambers on Scanpor®. Patch test reactions were scored at 2, 3, or 4 days, and, frequently, on days 5 to 7, according to the International Contact Dermatitis Research Group (ICDRG) grading scale. An additional group of 5 patients was also patch tested with the plant extract (2.5% in petrolatum). Of the 24 patients, 18 (i.e. 75%) had positive reactions to 1% chamomilla recutita (matricaria) ether extract. Most of the reactions were ++ (9 patients) and 2 patients had a +++ reaction. Additionally, 7 patients had a + reaction and 3 patients had a doubtful (?+) reaction. Of the 5 patients, 4 had positive reactions (scores not stated) to 2.5% chamomilla recutita (matricaria) ether extract. The 5 patients were also involved in a standard photopatch test, and the results are included in the section on Phototoxicity.

The frequency of allergic reactions to a Compositae plant mixture containing chamomilla recutita (matricaria) extract (ether extract) was evaluated using 3,851 patients (ages not stated) patch tested between 1985 and 1990.[49] Other components of the plant mixture included: Ether extracts of arnica, feverfew, tansy, and yarrow. Eighty-four patients (ages not stated) were patch-tested with chamomilla recutita extract (ether extract; test concentration = 2.5%) during the same period. The ether extract was prepared by cutting the fresh plant material (all above-ground parts) into 20 cm long pieces and extracting them with diethyl ether. Patches (Finn chambers on Scanpor) were secured to the back of each subject, using self-adhesive tape, for 24 h. Reactions were scored according to International Contact Dermatitis Research Group (ICDRG) recommendations. Positive reactions (at least ++) to the Compositae plant mixture were observed in 118 patients (3.1% of 3,851 patients tested). Of the 84 patients tested, there were 48 (0.56% of patients tested) positive reactions to chamomilla recutita extract.

Another study to investigate the frequency of *Compositae* (*Asteraceae*, daisy family) sensitivity was performed.[50] Thirty adult patients (24 females, 6 males; mean age = 34.7 years) with "extrinsic" atopic dermatitis were patch tested with chamomilla recutita (matricaria, 2.5% in petrolatum), sesquiterpene lactone mix (SL mix, 01% in petrolatum), and *Compositae* mix (C mix, 6% in petrolatum). The C mix contained the following ingredients: arnica (*Arnica Montana*) extract, chamomile (*Chamomilla recutita*) extract, tansy (*Tanacetum vulgare*) extract, feverfew (*Tanacetum parthenium*) extract, and yarrow (*Achillea millefolium*) extract. Patch testing was performed using Finn chambers on Scanpor® and Curatest®. Reactions were scored on days 2 and 3, and, where possible, on days 5 through 8 according to a grading scale (- to +++) recommended by the International Contact Dermatitis Research Group (ICDRG). A total of 9 patients reacted to SL

mix and/or C mix. Of these 9, 5 had positive reactions to chamomilla recutita (matricaria). All of the patients sensitive to chamomilla recutita (matricaria) were C mix positive.

Danish gardeners and greenhouse workers (19, ages not stated) with Compositae-related symptoms were patch tested with 2.5% Chamomilla recutita (matricaria) in petrolatum.[51] The test protocol was not included in this study. Positive reactions were observed in 11 of the 19 patients tested (58% sensitization rate).

Of the 36 patients (ages not stated) patch tested with ether extracts of chamomilla recutita (matricaria), 30 (or 94%) had positive patch test reactions.[52] The majority of these reactions (90%) were strongly positive (++ or +++ reactions); the relevance was most frequently recorded as unknown.

Chamomilla Recutita (Matricaria) Extract and Tea

The allergenicity of chamomilla recutita (matricaria) extract was evaluated using 9 patients (7 women, 2 men; mean age = 36 years).[53] These patients had a history of systemic allergic reactions after ingestion of honey and/or after drinking chamomilla recutita (matricaria) tea (from flower heads). To produce the plant extract, *Matricaria chamomilla* was defatted with acetone and macerated in phosphate buffered saline. The mixture was then stirred, centrifuged, and filtered. The extract (3.5 mg/ml) was applied to the volar surface of the forearm and a prick test was performed. Skin sites were examined after 15 minutes, and a positive reaction was defined as a wheal with a diameter > 3 mm. Twenty subjects (10 atopic, 10 nonatopic) served as controls. A positive reaction to chamomilla recutita (matricaria) extract was observed in all 9 patients. Results were negative in the 20 control subjects. A CAP inhibition assay (i.e., inhibition of binding of specific IgE to Andujar honey) was also performed. The Pharmacia CAP system (fluorometric assay) used is a system for titration of total and specific IgE. Pooled serum was obtained by mixing equal parts of serum from 5 of the 9 patients with the soluble extract of chamomilla recutita (matricaria) pollen (358 µg protein/ml). Duplicate 100-µl aliquots of serial two-fold dilutions (in phosphate buffered saline [PBS]) of the competing fluid-phase antigen were incubated (2-h incubation period) with an equal volume of serum from the serum pool. Fluorometric assay was performed at the end of the incubation period. Percent inhibition for each dilution was calculated, and the concentration of the extract that caused 50% inhibition of IgE binding to Andujar honey (C_{50}) was determined. A C_{50} of 45.72 µg/ml was reported for chamomilla recutita (matricaria) extract.

In the same study, the 9 patients were subjected to a conjunctival challenge with chamomilla recutita (matricaria) tea (from flower heads). One drop of phosphate buffered saline (PBS, negative control) was placed in the conjunctival sac. If a reaction was not observed, the tea (1 drop per dilution, every 15 minutes) was instilled as a series of 10-fold dilutions in PBS. The initial dilution instilled was $1:10^5$ (w/v). A positive reaction was defined by congestion of the conjunctival mucosa and itching of the eye. The same 20 subjects (10 atopic, 10 nonatopic) served as controls. A positive reaction to chamomilla recutita (matricaria) tea was observed in all 9 patients, only at low-level dilutions (1/10 or 1/100). Results were negative in the 20 control subjects.[53]

Chamomilla Recutita (Matricaria) Extract
Anthemis Nobilis Extract

The skin sensitization potential of chamomilla recutita (matricaria) extract was evaluated using 76 patients, all sensitive to 6% *Compositae* mix (contains chamomilla recutita (matricaria) extract) in petrolatum.[54] Chamomilla recutita (matricaria) extract (2.5% in petrolatum) was applied to the back for 2 days using Finn chambers on Scanpor® tape. Reactions were scored on days 3 to 5, and possibly, on day 7 according to ICDRG criteria. Of the 76 patients, 49 had positive reactions to the extract. In a subsequent test (same procedure), 52 of the 76 patients had positive reactions to the extract. The sensitization potential of anthemis nobilis extract in patients sensitive to 5% Compositae mix (also contains anthemis nobilis extract) in petrolatum was also evaluated in this study according to the same procedure. Anthemis nobilis extract (1% in petrolatum) was applied to the back of each of 29 patients (24 women [mean age = 56], 5 men [mean age = 55] for 2 days. There were no positive reactions to anthemis nobilis extract.

Chamomilla Recutita (Matricaria)
Chamomilla Recutita (Matricaria) Flower Extract

A skin sensitization study was performed using 35 patients (26 women, 9 men; mean age = 59) sensitive to sesquiterpene lactones mix and 22 control patients (17 women, 5 men; mean age = 52) who were not sensitive to sesquiterpene lactones mix.[55] All patients were patch tested with the following: chamomilla recutita (matricaria) flower extract (1, 3, 10, 32, and 100% aqueous extract;) and chamomilla recutita (matricaria) (2.5% w/w in petrolatum). Chamomilla recutita (matricaria) flower extract was actually an aqueous extract of chamomilla recutita tea (from dried flower

heads). Each test substance concentration (15 µl) was applied to the back using a Finn chamber (8 mm diameter) on Scanpor® tape. Chambers were removed after 2 days. Reactions were scored according to ICDRG recommendations on days 3 and 7. The numbers of patients with positive reactions to chamomilla recutita (matricaria) flower extract were as follows: 100% aqueous (30 patients; + to +++ reactions), 32% aqueous (27 patients; + to +++ reactions), 10% aqueous (21 patients; + to +++ reactions), 3% aqueous (14 patients; + to +++ reactions), and 1% aqueous (9 patients; + to +++ reactions). The number of patients with +++ reactions decreased with decreasing aqueous flower extract concentration. Of the 35 patients patch tested with chamomilla recutita (matricaria) (2.5% w/w in petrolatum), 22 had positive reactions (+ to +++). The following 2 of 22 control patients (not sensitive to sesquiterpene lactones mix) had positive reactions to chamomilla recutita (matricaria) flower extract: subject 1 (++ reaction to 100% aqueous and subject 2 (++ [100% aqueous], + [32% aqueous], ++ [10% aqueous], + [3% aqueous], and + [1% aqueous].

Chamomilla Recutita (Matricaria) Flower Extract

The sensitization potential of wild chamomilla recutita (matricaria) flower extract in 129 patients sensitive to compositae mix was evaluated.[56] Patches (Finn chambers on Scanpor) containing 2.5% chamomilla recutita (matricaria) flower extract in petrolatum remained in place for 2 days. Reactions were scored on days 2 to 4, and, whenever possible, on days 5 to 8 according to ICDRG recommendations. Of the 129 patients, 83 (64%) had positive reactions to the test material. When 74 chrysanthemum-positive patients were patch-tested with wild chamomilla recutita (matricaria) flower extract (2.5% in petrolatum), 58 (78%) had positive reactions.

The skin sensitization potential of aqueous extracts of chamomilla recutita (matricaria) tea (from flower heads) was evaluated using 20 patients (13 women, 7 men; mean age = 56 years) with known contact allergy to sesquiterpene lactone mix (containing altolactone, costunolide, and dehydrocostuslactone).[57] Aqueous extracts (1%, 10%, and 100%) of 2 different kinds of chamomilla recutita (matricaria) tea (identified as I and II) were tested. Each solution (15 µl) was applied to the back, using a Finn chamber on Scanpor tape, for 48 h. Reactions were scored on days 3 and 7 according to ICDRG recommendations. For 9 of the 20 patients, reactions were also scored on day 10. The following positive reactions to chamomilla recutita (matricaria) tea I were reported: 1% aqueous (2 reactions, + and ++), 10% aqueous (4 reactions, + to ++), and100% aqueous (11 reactions, ++ predominated). The following positive reactions to chamomilla recutita (matricaria) tea II were reported: 1% aqueous (1 reaction, ++), 10% aqueous (10 reactions, + to +++; mostly ++), and 100% aqueous (11 reactions, + to +++; mostly ++).

Chamomilla Recutita (Matricaria) Extract
Chamomilla Recutita (Matricaria) Flower Oil
Anthemis Nobilis

Up to 14 adult patients who had previously tested positive (at least a 2+ reaction) to ether extracts of *Chamomilla recutita* (2.5% in petrolatum) and/or arnica (0.5% in petrolatum) were patch tested with the following: *Chamomilla recutita* (2.5% in petrolatum), *Anthemis nobilis* (1% in petrolatum), and chamomilla recutita flower oil (1% and 4% in petrolatum).[58] A patch (Finn chambers on Scanpor® tape) containing either of the test materials was applied to the back for 2 days. Reactions were scored on day 3, and, possibly, day 7 according to ICDRG recommendations. Of the 10 patients patch tested with *Chamomilla recutita* (2.5% in petrolatum), 9 had positive reactions (+ to +++) and 1 had a doubtful positive follicular reaction. Only 2 of 14 patients had reactions to chamomilla recutita flower oil (doubtful positive reaction to 4 % [1 patient]; ++ reaction to 4% and 1% [1 patient]). Of the 14 patients patch tested with *Anthemis nobilis* (1% in petrolatum), 6 had reactions that were described as follows: 2 with ++ reactions, 2 with doubtful positive follicular reactions, 1 with a + follicular reaction, and 1 with a doubtful positive reaction.

Chamomilla Recutita (Matricaria) Flower Oil

The skin sensitization potential of chamomilla recutita (matricaria) flower oil (2% in yellow, soft paraffin) was evaluated using 74 patients (ages not stated), all negative to balsam of Peru.[59] Of the 74 patients, 3 were positive to chamomilla recutita (matricaria) flower oil. Though negative to balsam of Peru, these 3 patients were also positive to 1 or more of the 3 other balsams (colophony, turpentine, and wood tars: ol. Betule and ol. Fagi). Details relating to the test procedure were not stated.

Of 200 patients patch tested with chamomilla recutita (matricaria) flower oil in Poland, 2 positive reactions were reported.[60] Details relating to the patch test procedure were not included.

Eighty-six patients with positive reactions to a perfume mixture containing the following ingredients were tested with chamomilla recutita (matricaria) flower oil:[61] eugenol, isoeugenol, cinnamic aldehyde, geraniol, cinamic alcohol,

oakmoss absolute, hydroxycitronellal, and amyl cinnamic alcohol. Neither the test concentration of chamomilla recutita (matricaria) flower oil nor details relating to the test protocol were included. Two (or 3.4%) of the 86 patients were sensitive to the oil.

Case Reports

Chamomile/Chamomile Extract

Rapid onset of a transient rash, burning, stinging, and itching at the application sites were reported for a 24-year-old woman who had applied a cosmetic skin mask formulation to her face.[63] Components of the skin mask were as follows: whole egg, lecithin, allantoin, aloe gel, melissa extract, and chamomile extract. The genus and species of the chamomile extract were not stated. Open testing (i.e., without prick, scratch, or chamber) with 1% chamomile extract (in physiologic saline) produced an extensive wheal and flare reaction on intact forearm skin. Neither the passive transfer (PK Test) nor radioallergosorbent test (RAST) was performed. Open test results were negative for the saline control and 1% chamomile extract in 10 control subjects. The authors concluded that the patient appeared to have developed immunologic contact urticaria.

A 20-year-old woman complained of a short-lasting cough and rhinitis after inhaling fragrance from a chamomile-scented toilet paper.[64] The genus and species of the chamomile were not stated. Chamomile allergenicity was evaluated in a prick test and radioallergosorbent test (RAST). Results for the prick test (wheal mean diameter = 12 mm) and RAST (Pharmacia ImmunoCAP system (CAP system): 12.9 KU/l (v.n. < 0.35) were positive. Results were also positive when the chamomile-scented toilet paper was evaluated in a prick-by-prick test (mean diameter of wheal = 9 mm (toilet paper) and 5 mm (histamine). Two atopic subjects and 2 healthy subjects served as controls for the prick-by-prick test, and results were negative for the chamomile-scented tissue.

Chamomilla Recutita (Matricaria) Flower
Anthemis Nobilis Flower

Acute eczema on the forearms and hands was observed in a 50-year old metalworker after using a product for cleaning metallic items.[62] The patient had no personal or family history of atopy, but had psoriasis. Treatment of the eczema involved washing and applying compresses (over 2-month period) with chamomilla recutita (matricaria) tea (from flower heads) and, subsequently, with a tea made from chamomilla recutita (matricaria) (flower heads), anthemis nobilis (flower heads), and mallow herbs. Patch tests were performed using Finn chambers; neither the area of application nor test concentration was stated. The following positive reactions were reported: chamomilla recutita (matricaria) tea (+ on day 2; ++ on day 4) and anthemis nobilis tea (++ on days 2 and 4). Negative results were reported for 5 control subjects tested with each tea.

Chamomilla Recutita (Matricaria) Flower Extract
Chamomila Recutita (Matricaria) Flower Oil

An 8-year old boy with hay fever and bronchial asthma had a severe anaphylactic reaction after ingestion of a *Matricaria chamomilla*-tea (from flower heads) infusion for the first time.[65] At 2 weeks after the reaction occurred, the patient was subjected to a skin prick test, beginning with a 1:100,000 wt/vol concentration of *Matricaria chamomilla* tea extract (tea extracted in phosphate-buffered saline). Skin test sites were read after 15 minutes, and a wheal of at least 3 mm x 3 mm was considered a positive reaction. Ten patients with hay fever and 10 normal subjects served as controls. Testing at a concentration of 1:100 wt/vol elicited a 4 mm by 6 mm wheal. None of the control subjects reacted to the tea extract. The enzyme-linked immunosorbent assay (ELISA) was used to test the 8-year-old patient's serum for specific IgE antibodies to antigens contained in the tea extract. IgE activity toward the tea extract was noted; however, this was not true for serum samples from 22 healthy subjects or from 5 patients with hay fever.

A healthy, 35-year-old pregnant woman was given an enema containing glycerol and Kamillosan (oily extract of chamomilla recutita (matricaria) flowers).[66] Urticaria, larynx edema, tachycardia, and hypotension followed, indicative of an anaphylactic reaction. In the skin prick test, Kamillosan induced a 5 x 5 mm wheal reaction.

Eyelid angioedema was observed in a 23-year-old female after applying compresses of chamomile tea (obtained from the dried flower heads of *Chamomilla recutita.*[67] She had a history of seasonal rhinitis, conjunctivitis, and exercise-induced asthma. Prick test results were positive (++) for chamomilla recutita (matricaria) flower extract, and the level of IgE antibody was expressed as 3.37 kUA/l. In a subsequent oral challenge test performed with diluted chamomile tea,

generalized pruritus of the face was the only symptom observed. The patient was diagnosed as having immune-mediated contact urticaria.

Anthemis Nobilis Flower Oil

Severe exudative eczema of both nipples and areolae was observed in a 32-year-old woman who had been applying Kamillosan® ointment (containing extracts and oil of *Anthemis nobilis* 10.5%) to treat cracked nipples.[68] It should be noted that, reportedly, 2 ointments marketed under the name Kamillosan® are available in Europe, one containing anthemis nobilis and, the other, containing chamomilla recutita (matricaria). Patch testing of the ointment (Finn chambers on Scanpor® tape) identified a 3+ reaction to the ointment at 2 days. A 3+ reaction was also observed after patch testing with 0.1% anthemis nobilis flower oil in petrolatum; results were negative in 10 control subjects. Bilateral eczema of the nipples and areolae was also observed in a 38-year-old woman who had used the same ointment. Patch testing also revealed a 3+ reaction to 0.1% anthemis nobilis flower oil in petrolatum at 2 days.

A 34-year-old woman with a history of atopic dermatitis was hospitalized with acute generalized eczema, accentuated on the face.[69] Prior to the onset of symptoms, the patient had applied compresses of chamomile tea to her face and neck. Additionally, she drank chamomile tea regularly. Patch test results were as follows: 25% anthemis nobilis flower oil in olive oil (++ on day 2; +++ on day 3) and 4% anthemis nobilis flower oil in petrolatum (++ on day 2; +++ on day 3).

Anthemis Nobilis and Anthemis Nobilis Extract

A 55-year-old male employee of a magnet factory presented with crops of disseminated confluent erythroderma, initially on sun-exposed areas (face, neck, V of neck and acral) and then spreading to the remainder of the skin.[75] The lesions were described as itchy and scaly. The patient experienced exacerbation of these reactions after visiting an area where there were many and varied plants, even though there was no direct contact with the plants. Patch testing with *the Anthemis nobilis* plant as is yielded a +++ reaction on days 2 and 4. The same reactions were reported after patch testing with *Anthemis nobilis* ethyl ether extracts (stem and leaves). Photopatch testing (Finn chambers, UVA exposure) also yielded a +++ reaction to the plant as is and its ethyl ether extracts.

Matricaria Recutita and Anthemis Nobilis

Occupational dermatitis of the hands was observed in a 27-year old florist, and patch test results revealed positive reactions to the petals and leaves of *Matricaria recutita* and weaker reactions to *Anthemis nobilis* (plant parts not specified).[70] Details relating to the patch test procedure were not included.

Delayed-type contact dermatitis of the face was observed in a 62-year-old female who worked in a flower stall 1 day per week.[71] The patient presented with a relapsing dermatitis of the face for 1 year. Relapase of dermatitis was observed within 24 h of working a single afternoon in the shop. Patch test results indicated positive reactions to the flowers, petals, and stems of *Matricaria recutita*. Details relating to the patch test procedure were not included.

A 54-year-old female cosmetician complained of sneezing, coughing (with occasional dyspnea), orbital pruritus, dacryorrhea, and rhinitis.[72] Her work involved the preparation and application of herbal beauty masks containing 24% chamomile flower. Dermatitis of both hands, with intermittent vesiculation, was observed. Open patch testing (immediate reactions read after 30 and 60 minutes) revealed a positive reaction to chamomile flower. The diameter of the wheal was ≈1 cm. A positive prick test reaction (++) to chamomile pollen was also reported. A provocation test was performed using acoustic rhinometry, and the duration of exposure to chamomile flower was 3 minutes. Sneezing, dyspnea, and nasal chonchae swelling and hyperemia were reported. The decrease in volume of the nasal cavities was 3x that of the normal volume. Results of the provocation test were classified as strongly positive.

A 22-year-old female with facial eczema had been a frequent drinker of steaming-hot chamomile tea over the past year, which had immediately preceded at least some of the relapses of her facial eczema.[73] At times, the facial eczema was accompanied by lip swelling. Patch testing revealed a + D2/++ D4 reaction to 2.5% Chamomilla recutita in petrolatum. During follow-up at 4 months, the patient reported that she no longer drank chamomile tea and that there had been no further relapses of the eczema.

Work-related rhinoconjunctivitis and asthma were diagnosed in a 43-year-old man 11 years after he began working at a tea-packing plant.[74] The plant processed black tea (*Camellia sinensis*) as well as various herbal teas, including tea from chamomile flowers (*Chamomilla recutita*), lime (*Tilia cordata*), and dog rose. His symptoms occurred electively when chamomile tea was packaged. Furthermore, he became symptom-free when the production of herbal teas was transferred to

another factory. A skin prick test with chamomile extract at a concentration of 10 mg/ml elicited a 6-mm wheal response. Prick test results were negative for black tea and lime tea extracts.

A 41-year-old atopic woman with hand eczema reported that she had not used chamomile tea externally, but had used the tea when treating her dog's inflamed eyes.[52] When patch tested, a +? follicular reaction to *Chamomilla recutita* (2.5% in petrolatum) was reported. In a subsequent identical patch test one month later, a ++ reaction was reported.

Phototoxicity

Chamomilla Recutita (Matricaria) Extract

Five patients were initially patch tested (Finn chambers on Scanpor® tape) with 2.5% chamomilla recutita (matricaria) ether extract, and the results were positive in 4 patients. The 5 patients were also evaluated in a standard photopatch test. The first reading (day 1) was followed by UV-irradiation and a second reading at day 3. Additional details regarding the test procedure were not included. Photoaggravation (score not provided) was observed in one of the 5 patients.[48]

Chamomilla Recutita (Matricaria) Flower Oil

The phototoxicity of chamomilla (matricaria) flower oil was evaluated using 12 Skh:hairless-1mice and 2 miniature swine.[76] The light source was a 6-kW long-arc xenon high pressure burner (UVA and UVB proportions approximated those found in mid-latitude summer sun spectrum) or a bank of 4 fluorescent F40BL black light lamps (UVA region, centered over 350 nm). The 12 mice and 2 swine were treated with the non-viscous oil, tested as received. A single application of the oil (20 µl) was made to an area of the back that was approximately 2 cm^2. Six mice and 1 swine were then exposed to one of the light sources, and, the remaining 6 mice and 1 swine, to the other light source at 30 minutes post-application of the oil. The duration of exposure to the fluorescent blacklight source was 1 h (integrated UVA intensity = 3 W/m^2), and, 40 minutes (intensity of weighted erythemal energy = 0.1667 W/m^2), to the xenon lamp. If application of the oil elicited a response from skin exposure to the blacklight lamp or elicited more than a barely perceptible response to the xenon lamp, the oil was considered phototoxic. The area of skin treated with the oil, but not irradiated, served as the control for primary irritant reactions. One group of control mice was treated with 8-methoxypsoralen (8-MOP, 0.01% in methanol), and another group, with appropriate vehicle only. Exposure to the xenon lamp caused barely perceptible erythema in animals pretreated with vehicle only or with chamomilla (matricaria) flower oil. Parallel results were obtained using the blacklight lamp. 8-MOP was phototoxic.

Anthemis Nobilis Flower Oil

The phototoxicity of anthemis nobilis flower oil was evaluated, but neither the species tested, test concentration, nor the test protocol was stated.[77] Study results were negative.

Sensory Irritation

Chamomilla Recutita (Matricaria) Flower Oil

Chamomilla recutita (matricaria) flower oil (German chamomile oil, bisabololoxide A type) was evaluated for its effect on capsaicin-induced sensory irritation in mice.[78] The intradermal injection of capsaicin into the mouse paw resulted in dose-dependent, paw-licking behavior due to sensory irritation. Co-administration of the oil suppressed this behavior in a dose dependent-manner over the 1% to 5% concentration range. This Japanese publication would need to be translated for additional study details.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Chamomile

A study was performed to examine the use of herbal products among a sample of pregnant women in Italy and the potential influence of herbal consumption on pregnancy outcome.[79] The number of subjects (mostly between 31 and 40 years old) interviewed was 392. Of the 392 subjects, 109 reported having taken one or more herbal products during pregnancy; the remaining 283 were classified as non-users. The most frequently used herb was chamomile (48; 44% of the 109

subjects), followed by licorice (15; 13.8% of the 109 subjects). For the 37 regular users of chamomile and 14 regular users of licorice, there was a higher frequency of threatening miscarriages (21.6% and 35.7%, respectively) and preterm labors (21.6% and 16.7%, respectively) when compared to non-users. An unspecified cardiac malformation (thought to have been related to Down's syndrome) and an enlarged kidney were diagnosed in 2 neonates, following regular maternal consumption of chamomile. Regarding pregnancy outcome in the study population, no statistically significant differences were evident between users and non-users, except for a higher incidence of newborns small for gestational age (11.9% vs. 5.3%; $p = 0.039$). However, after further analysis of the data, it was noted that a possible influence of regular intake of 2 herbs (chamomile and licorice, taken from the beginning of pregnancy) on threatening miscarriages and preterm labors of low birth weight infants could be hypothesized.

GENOTOXICITY

Chamomilla Recutita (Matricaria) Flower Oil

The potential for chamomile recutita (flower) oil-induced inhibition of genotoxicity produced by daunorubicin (DAU, mutagen) was evaluated using the following groups of 5 male NIH mice:[80] control group administered corn oil orally (0.1 ml), positive control group treated with corn oil (0.1 ml) and DAU administered by intramuscular injection (10 mg/kg), a group administered chamomile recutita (flower) oil (500 mg/kg), and 3 groups treated with DAU and chamomile recutita (flower) oil (5, 50, and 500 mg/kg), respectively. Specifically, the effect of the 3 doses of essential oil on the rate of sister chromatid exchange (SCE) induced by DAU in spermatogonia was studied. Chamomile recutita (flower) oil was not genotoxic. However, dosing with this essential oil resulted in inhibition of SCE induced by DAU, and % inhibition was as follows at administered doses of the oil: 5 mg/kg (47.5% inhibition), 50 mg/kg (61.9% inhibition), and 500 mg/kg (93.5% inhibition).

Chamomilla Recutita (Matriaria) Flower Oil Extract

The genotoxicity of crude chamomile recutita (matricaria) flower oil extract was evaluated using 5 groups of five male NIH mice. Three groups of mice received oral doses of 10, 100, and 1000 mg/kg, respectively.[29] The negative control group was dosed orally with corn oil and the positive control group was dosed intraperitoneally (i.p.) with an aqueous solution of methyl methanesulfonate (25 mg/kg). Following injection with an aqueous suspension of 5-bromodeoxyuridine (BrdU) and then colchicine, the mice were killed and bone marrow cell suspensions prepared for microscopic examination. At each dose, the incidence of sister chromatid exchanges was comparable to that noted in bone marrow cells from control animals (i.e., not more than 1.1). A high incidence of SCE's was observed after dosing with MMS, and the difference between this incidence and that for animals dosed with corn oil was statistically significant ($p < 0.05$). Additionally, when compared to control values, chamomile recutita (matricaria) flower oil extract produced a non-significant cytotoxic effect. The results for an acute oral toxicity preliminary test on the crude oil are included in that section of this report.

Antigenotoxicity studies were also performed using groups of 5 male NIH mice. When compared to mice dosed with corn oil**,** sister chromatid exchanges induced by daunorubicin were decreased in mice pre-treated with crude chamomile recutita (matricaria) flower oil extract at doses ranging from 5 to 500 mg/kg.[29] Administration of the crude oil to daunorubicin-treated mice caused a statistically significant, dose-dependent reduction in the genotoxic damage (SCE's). The antigenotoxic response corresponded to 25.7, 63.1, and 75.5% at doses of 5, 50, and 500 mg/kg, respectively. Similarly, a statistically significant, dose-dependent decrease in genotoxicity (SCE's) was observed in MMS-treated mice after dosing with the crude oil. The 3 doses of crude oil tested (250, 500, and 1000 mg/kg) induced 24.8, 45.8, and 60.6% inhibition of genotoxicity, respectively.

Chamomilla Recutita (Matricaria) Tea Extract

Modification of the *in vitro* activity of heterocyclic aromatic amines [HAA, in DMSO] with the hot water extract of chamomilla recutita (matricaria) tea was studied in the Ames plate incorporation test, with and without metabolic activation, using *Salmonella typhimurium* strain TA98.[81] Initially, measured volumes of the tea extract (usually 1, 5, 10, 50, and 100 µl) were plated in triplicate to establish a dose-response curve. DMSO served as the negative control, and there were 3 sets of positive controls, 2-amino-3- methylimidazo[4,5-*f*]quinolone (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinolone (MeIQ), and B[*a*]P. Test results were expressed as the induced number of revertants by subtracting the number of spontaneous revertants (20-38 revertants/plate) from the total number obtained on each plate. A sample was considered mutagenic if it produced a dose-related increase in the number of revertants, when compared to the control, and if the number of revertants

was at least 2.5 times greater than the spontaneous level. Chamomilla recutita (matricaria) tea (from flower heads) extract alone was not mutagenic.

HAAs were tested in combination with 2 doses of the tea extract, 10 and 50 mgEQ. All tests were performed in triplicate. At both doses, chamomilla recutita (matricaria) tea extract caused mild inhibition of the mutagenicity of IQ-type HAA (tested up to 0.5 ng/plate), but caused potentiation of the mutagenicity of 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx, tested at 5 ng/plate) and 4,7,8-TriMeIQx (tested at 10 ng/plate).[81]

Anthemis Nobilis Flower Oil

The genotoxicity of anthemis nobilis flower oil was evaluated in the rec-assay using *Bacillus subtilis* strains PB 1652 and PB 1791 and in the *Salmonella*/microsome reversion assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537.[82] In the rec-assay, 10-30 µl of the oil was applied to a sterile filter paper disk (9-mm diameter), placed on the surface of nutrient agar plates seeded with the tester strains. Following incubation, the diameter of the inhibition zones formed around the disk was measured. Methy, methanesulfonate (MMS), mitimycin C (MIT C), and adriamicin (ADR) served as positive controls. Ampicillin (AMP) and chloramphenicol (CAF) served as negative controls. Positive DNA-damaging activity was assumed if the ratio between the diameter of the inhibition zone of the rec^- mutant and that of the parental rec^+ strain exceeded a value of 1.2. Anthemis nobilis flower oil did not produce positive DNA-damaging activity in either *Bacillus subtilis* strain. All positive controls had positive DNA damaging activity, whereas, the 2 negative controls did not. In the *Salmonella*/microsome reversion assay (with and without metabolic activation), the oil (in DMSO) was evaluated at doses up to 1 µl/plate and was not found to be genotoxic.

CARCINOGENICITY

Carcinogenicity studies on the chamomile ingredients reviewed in this safety assessment were not found in the published literature.

Anticarcinogenicity

Chamomilla Recutita (Matriaria) Flower Extract

The anticancer properties of aqueous and methanolic extracts of chamomile recutita flowers against the following human cancer cells lines, human prostate cancer cells derived from different metastatic sites, were evaluated: LNCaP, PC-3, DU145, and PZ-HPV-7.[83] HPLC analysis of the extracts confirmed apigenin 7-O-glucoside as the major chamomile constituent. To prepare aqueous extracts, dry chamomile flowers were crushed to a powder and a 5% (w/v) suspension was prepared by adding boiling hot water. Filtered aqueous suspension was freeze-dried and stored before addition to the culture medium. To prepare methanol extracts, powdered chamomile was soaked in methanol (5% w/v); dried, filtered extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a 100 mg/mL stock solution that was later mixed with the culture medium.

The effect of chamomile extracts on cell viability was determined using an MTT assay, in which the freeze-dried aqueous extract was evaluated at concentrations of 1000, 2000, and 4000 µg/ml and the methanol extract was evaluated at concentrations of 100, 200, and 400 µg/ml. In the DNA fragmentation assay, cells were treated with the aqueous extract at a concentration of 2000 µg/ml or the methanol extract at a concentration of 200 µg/ml. In the cell death detection ELISA^{PLUS} assay, cells were harvested after treatment with the aqueous extract (2000 µg/ml) or the methanol extract (200 µg/ml) to determine the extent of apoptosis. Apoptosis was detected using fluorescence microscopy. Exposure to both extracts caused minimal growth inhibitory responses in normal cells, but a significant decrease in cell viability was observed in various human cancer cell lines. Differential apoptosis was observed in cancer cells, but not in normal cells at similar concentrations.[83]

The cytotoxic activity of the following chamomilla recutita (matricaria) flower extracts against Yoshida ascites sarcoma was evaluated using Wistar Glaxo albino rats: 4.27% chamomilla recutita (matricaria) flower (petroleum ether extract), 10.04% chamomilla recutita (matricaria) flower (ethanol extract), and 13.73% chamomilla recutita (matricaria) flower (distilled water extract).[84] The following procedure was followed prior to determining these 3 extract yields. Following filtration, the aqueous solutions were lyophilized or the organic solvents were removed in vacuo. The crude total extracts were then dissolved in phosphate buffer solution (pH 7.2) and sterilized by filtration. Ascites sarcoma cells were

transplanted by i.p. injection into the rats. At 7 to 8 days post-injection, ascitic fluid was drawn from each animal, centrifuged, and the sediment was resuspended in the original volume with phosphate buffer solution. The tumor cells were then washed and resuspended in the same buffer solution to obtain a final concentration of 15 x 10^5/ml. Cytotoxicity was evaluated using the dye test. Equal volumes (0.2 ml) of serially diluted extracts (50 to 6.25 mg/ml) and of cell suspensions were mixed and incubated for 60 minutes. Trypan blue solution was then added to the mixture, and the differential count of stained and unstained cells was performed. Cytotoxicity was expressed as the LD_{50}. All 3 extracts were classified as exhibiting a poor cytotoxic effect (LD_{50} > 10 mg/ml).

Chamomilla Recutita (Matriaria) Flower Oil

The anticancer activity of chamomilla recutita (matricaria) flower oil, from flowers cultivated in Egypt, against leukemia HL-60 and NB4 cells was evaluated *in vitro*.[85] The cells used were from human promyelocytic cell lines, and the oil was evaluated at concentrations of 25, 50, 75, 100, and 200 ppm in cells cultured for 24 h. At the highest test concentration, the percentage of dead cells at 24 h was 78.4% for HL-60 cells and 86.03% for NB4 cells. At the lowest test concentration, the percentage of dead cells at 24 h was 22.8% for HL-60 cells and 17.1% for NB4 cells. Data on the antioxidant activity of chamomilla recutita (matricaria) flower oil included in this study are summarized in that section earlier in the report text.

BIOLOGICAL ACTIVITY

Neutrophil Activation

Chamomilla Recutita (Matricaria) Flower Oil

The effect of chamomilla recutita (matricaria) flower oil (German Chamomile Oil, main constituent = camazulene) on neutrophil activation was evaluated to assess its anti-inflammatory activity.[86] The neutrophil adherence test was performed according to the method of Ohnishi et al.[87] The human recombinant tumor necrosis factor-alpha (TNF-α)-induced adherence reaction of human peripheral neutrophils was used to measure neutrophil activation. The test substance was diluted to a 50% solution in dimethyl sulfoxide (DMSO), and then to a concentration of 0.4% in RPMI 1640 medium containing 10% fetal calf serum (complete medium). The 0.4% concentration was further diluted, using complete medium containing 0.4% DMSO (D-medium), to test concentrations of 0.2%, 0.1%, 0.05%, and 0.025%. Following a 1 h incubation period, neutrophil adherence to the plates was evaluated by measuring the absorbance of triplicate samples at 620 nm (OD value). The mean concentration of chamomilla recutita (matricaria) flower oil that caused 50% inhibition of neutrophil adherence (IC50) was 0.020 ± 0.0042 %. Neutrophil adherence to a plastic plate is recognized as a parameter representing the priming stage of neutrophils in inflammatory processes. Suppressive activity for TNF-α-induced neutrophil adhesion is suggestive of the capacity to modulate, perhaps negatively, neutrophil function in inflammation.

Anti-inflammatory Activity

Anthemis Nobilis Flower Oil

The anti-inflammatory activity of athemis nobilis flower oil was evaluated using groups of 6 adult male Wistar rats.[88] The oil from 2 varieties of *Anthemis nobilis* that have been cultivated in Italy under the names "white-headed" (WH) or "double-flowered roman chamomile" and "yellow-headed roman chamomile" (YH) was tested. The oil from each flower type was administered i.p. at a dose of 350 mg/kg, and the animals were then dosed orally (gavage) with 5 ml water. Of the 2 control groups, 1 was injected i.p. with normal saline (dose not stated), and, the other, with indomethacin (14 µM/kg). The dosing of control animals i.p. was followed by oral dosing with water. At 30 minutes post-treatment, the right hind paw was injected with 0.1 ml of a 1% suspension of carragenin in normal saline to induce phlogosis. Each oil caused a considerable anti-inflammatory effect, particularly by 3 h post-injection.

Immunomodulatory Activity

Chamomilla Recutita (Matricaria) Extract

The immunomodulatory activity of chamomilla recutita (matricaria) extract (extracted with methanol/water 50%) was studied using groups of 6 Balb/c mice.[89] The plant was grown in Egypt. Each of 6 animals was dosed i.p. with the extract (20 mg/dose/animal) for 5 consecutive days. Six untreated mice served as controls, and received the solvent (not stated) used to dissolve the extract. Blood samples were collected from the retro-orbital plexus. Dosing with the extract

enhanced the total white blood cells count (up to 1.2 x 10^4 cells/mm^3). Bone marrow cellularity was also increased significantly ($P < 0.01$), and the same was true for spleen weight ($P < 0.01$). When 2 groups of mice were immunosuppressed with cyclophosphamide (200 mg/kg body weight), it was found that pretreatment of one of the groups with the extract restored the resistance of these mice against lethal fungal infection with the predominantly granulocyte-dependent *Candida albicans*. The results of this study confirmed the immunomodulatory activity of chamomilla recutita (matricaria) extract.

Chamomilla Recutita (Matricaria) Flower Oil

In another study, the efficacy of chamomilla recutita (matricaria) flower oil in alleviating atopic dermatitis-like immune alterations was evaluated using the following 4 groups of 10 BALB/c mice (7 weeks old):[90] normal group (saline applied throughout atopic dermatitis induction stage and oil treatment period), control group (saline applied following induction of atopic dermatitis), vehicle group (jojoba oil applied), and experimental group (3% chamomilla recutita (matricaria) flower oil applied after atopic dermatitis induction). Initially, the mice were sensitized twice per week with 1% 2,4-dinitrochlorobenzene (DNCB,100 µL), applied to dorsal skin (8 cm^2). During the following week, the animals were challenged twice with 0.2% DNCB (100 µL) for atopic dermatitis induction. Next, 3% chamomilla recutita (matricaria) flower oil (70 µL) was applied daily (6 times per week) for 4 weeks. Control mice were treated with saline or jojoba oil. Blood samples were collected following the second DNCB challenge and at 2 and 4 weeks after application of the oil.

When compared to the jojopba oil or saline control groups, the application of chamomilla recutita (matricaria) flower oil resulted in significant reduction ($p < 0.05$) of serum IgE levels at the end of the 4-week application period. When compared to 2 weeks of application (1.80 mg/mL reduction), 4 weeks of oil application caused a 31% (13.75 mg/mL) reduction in the serum IgG1 level. Additionally, when compared to the saline control group (37.43 ng/mLserum histamine level) or jojoba oil control group (30.60 ng/mL serum histamine level or 40% lower) at 2 weeks, application of the oil resulted in a significantly lower (18.45 ng/mL or 51% lower, $p < 0.05$) serum histamine level. The frequency of scratching following application of the oil was significantly lower when compared to either control group. The immunoregulatory potential of chamomilla recutita (matricaria) oil for alleviating atopic dermatitis through influencing of Th2 cell activation was demonstrated in this study.[90]

Antispasmodic Activity

Chamomilla Recutita (Matricaria) Flower Oil

The antispasmodic activity of chamomilla recutita (matricaria) flower oil was evaluated in the presence of the spasmogen, barium chloride (1 x 10^{-4} g/ml) using the isolated guinea pig ileum.[6] ED50 (effective dose for 50% suppression of twitch tension) values were determined. A mean ED50 value of 3.84 (range: 3.19 to 4.62) x 10^{-5} g/ml was reported for chamomilla recutita (matricaria) flower oil. The ED50 for papaverine, known antispasmodic agent, was 1.64 (range: 1.16 to 1.84) x 10^{-6} g/ml.

Effect on Smooth Muscle Contraction

Chamomilla Recutita (Matricaria) Flower Oil

In an experiment using the isolated guinea pig ileum, chamomilla recutita (matricaria) flower oil (German chamomile essential oil, bisabololoxide A type) induced a dose-dependent inhibition of capsaicin-induced smooth muscle contraction.[78] These results are from a summay in a Japanese publication.

The effect of chamomilla recutita (matricaria) flower oil on guinea pig tracheal muscle (from 6 guinea pigs) and longitudinal ileal smooth muscle (from 6 guinea pigs) *in vitro* was investigated.[91] EC_{50} values (EC_{50} = concentration eliciting 50% of maximal response [mg/l]) were determined. Chamomilla recutita (matricaria) flower oil had a relaxant effect (i.e., inhibition of muscle contraction) in the tracheal smooth muscle (EC_{50} = 55 mg/l) and ileal smooth muscle (EC_{50} = 10.5 mg/l) preparations. Other oils were also evaluated in this study. Angelica root oil was most potent in the tracheal smooth muscle preparation (EC_{50} = 2.5 mg/l) and elecampane root oil was most potent in the ileal smooth muscle preparation (EC_{50} = 4.5 mg/l).

Enzyme Inhibition

Chamomilla Recutita (Matricaria) Extract

Serial dilutions of camomilla recutita (matricaria) extract (ethanol extract; 100% to 1.56% dilutions) were analyzed for inhibitory activity against cytochrome P450 3A4 (CYP3A4) using a fluorometric microtitre plate assay.[92] The test protocol was a modification of the procedure by Crespi et al.[93] Inhibition of CYP3A4-mediated metabolism of the test substrate, 7-benzyloxyresorufin was analyzed. The test material had an IC_{50} (median inhibitory concentration) value of 1.48% (% full strength).

Chamomilla Recutita (Matricaria) Flower

The effect of the herbal tea, chamomile tea on the activity of hepatic phase I phase II metabolizing enzymes from rat microsomes was evaluated.[31] Chamomile tea is made from the dried flower heads of *Chamomilla recutita* (matricaria). Five female Wistar rats (8 to 9 weeks old) had free access to Chamomile tea solution (2% w/v), whereas the control group had access to water. After 4 weeks of treatment, the animals were killed; livers were removed and microsomes prepared. Dosing had no significant influence on body weight, and there were no signs of gross pathology of internal organs. Liver weight /body weight ratios of treated rats were not significantly different from control values. There also was no significant difference in hepatic P450 content between treated and control rats. The following enzyme activities were determined: CYP1A2 (high affinity component of phenacetin-O-deethylase), CYP3A, CYP2E, CYP2D (debrisoquinine 4-hydroxylase), glucuronosyl transferase, and glutathione-S-transferase. The activity of CP1A2 in the liver of rats that received chamomile tea was significantly decreased ($P < 0.05$) to 39% of the control value. No statistically significant alterations in the activities of CYP2D, CYP2E, CYP3A, glucuronosyl transferase, or glutathione-S-transferase were observed.

Chamomilla Recutita (Matricaria) Flower Extract

A study was performed to determine whether chamomilla recutita (matricaria) flower extract interferes with the cyclogenase COX-2 pathway.[94] To prepare aqueous extracts, dry chamomile flowers were crushed to a powder and a 5% (w/v) suspension was prepared by adding boiling hot water. Initially, the effect of aqueous chamomile extract on the inhibition of endogenous prostaglandin E_2 (PGE_2) levels in murine RAW 264.7 macrophages was determined. Cellular respiration, an indicator of cell viability, was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, the endpoint of which is the mitochondrial-dependent reduction of MTT to formazan. Treatment with the aqueous extract caused a decrease in endogenous PGE_2 levels, which was more pronounced at 20 and 40 µg/ml concentrations than at concentrations of 5 or 10 µg/ml. The release of lipopolysaccharide (LPS)-induced PGE_2 in RAW 264.7 macrophages was inhibited, and this effect was due to inhibition of of COX-2 enzyme activity by chamomilla recutita (matricaria) flower extract. Treatment did not affect cell viability at test concentrations up to 40 µg/ml.

Chamomilla Recutita (Matricaria) Flower Oil

The inhibitory effect of crude chamomilla recutita (matricaria) flower oil on the following 4 human recombinant cytochrome P450 enzymes was investigated: CYP1A2, CYP2C9, CYP2D6, and CYP3A4.[3] The assay was conducted in 96-well microtiter plates, and was based on the formation of fluorescent metabolites from substrates in the presence of CYP enzymes. The substrates, with each positive control, were identified as: 5 µM CEC (for CYP1A2; positive control = 100 µM furafylline); 75 µM MFC (for CYP2C9; positive control = 10 µM sulfaphenazole); 1.5 µM AMMC (for CYP2D6; positive control = 0.5 µM quinidine); and 50 µM BFC (for CYP3A4; positive control = 5 µM ketoconazole). The oil (5 mg/ml) was dissolved in acetonitrile, and increasing test concentrations (between 0.045 and 100 µg/ml) were incubated with individual, recombinant CYP isoforms. Enzyme inhibition was expressed as the IC_{50} in relation to positive controls. Crude chamomilla recutita (matricaria) flower oil inhibited each of the 4 enzymes, with CYP1A2 being more sensitive (i.e., highest oil-induced inhibition) than the other isoforms. Mean IC_{50} values (n = 4) for the crude oil and positive control values were as follows: 1.59 ± 0.27 µg/ml (for CYP1A2; furafylline = 3.04 ± 0.32 µM); 14.29 ± 5.16 µg/ml (for CYP2C9; sulfaphenazole = 0.24 ± 0.04 µM); 8.49 ± 0.88 µg/ml (for CYP2D6); quinidine = 0.004 ± 0.001 µM); and 4.97 ± 0.13 µg/ml (for CYP3A4; ketoconazole = 0.018 ± 0.006 µM).

Antioxidant Activity

Chamomilla Recutita (Matricaria) Flower Oil

The antioxidant activity of chamomilla recutita (matricaria) flower oil against di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) radical ($DPPH^{\bullet}$) was evaluated *in vitro*.[85] Chamomilla recutita (matricaria) flower oil

was evaluated at concentrations of 25, 50, 75, 100, and 200 ppm, and radical scavenging activity of the oil against the stable $DPPH^{\bullet}$ was determined spectrophotometrically. The percentages of $DPPH^{\bullet}$ inhibition (radical scavenginig activity) were 19.57% at a concentration of 25 ppm and 94.03% at a concentration of 200 ppm. Chamomilla recutita (matricaria) flower oil was classified as having good antioxidant capacity. Data on the anticancer activity of chamomilla recutita (matricaria) flower oil from this study are included earlier in the report text.

Hypnotic/Sedative Activity

Animal

Chamomilla Recutita (Matricaria) Flower Extract

The hypnotic activity of chamomilla recutita (matricaria) flower extract in sleep-disturbed rats was studied using 32 male Wistar rats (4 groups of 8).[95] One of the 4 groups served as the control; however, whether or not this group was untreated or treated with vehicle was not stated. The extract (suspended in 0.5% carboxymethyl cellulose solution) was administered orally at a dose of 30, 100, or 300 mg/kg. Sleep latency was defined as the time from test substance administration up to the first 12 consecutive 10-s epochs of sleep. Compared to the control group, a statistically significant ($p < 0.05$) decrease in sleep latency was observed only at a dose of 300 mg/kg. No significant effects on total times of wakefulness, non-rapid eye movement (non-REM) sleep, and REM sleep were observed. It was concluded that chamomilla recutita (matricaria) flower extract had benzodiazepine-like hypnotic activity.

Anthemis Nobilis Flower Oil

The sedative effect of athemis nobilis flower oil was evaluated using groups of 6 adult male Wistar rats.[88] The oil from 2 varieties of *Anthemis nobilis* that have been cultivated in Italy under the names "white-headed" roman chamomile (WH) or "double-flowered roman chamomile" and "yellow-headed roman chamomile" (YH) was tested. The oil from each flower type was administered i.p. at a dose of 350 mg/kg, and sedation (reduction of motility) was evaluated using a motility gauge. The scoring of movements was initiated at 15 minutes post-dosing and was continued over a series of three 20-minute periods. Control groups were treated with saline. The WH oil significantly reduced the mobility of rats at 35 and 55 minutes post-dosing; however, this effect disappeared within 75 minutes. The YH oil induced sedation only during the first 35 minutes.

Human

Chamomilla Recutita (Matricaria) Flower

A group of 12 hospitalized patients with heart disease (5 men, 7 women) underwent right and left ventricular catheterizations, primarily as an aid to their clinical management.[14] The patients drank chamomile tea (from dried flower heads of *Chamomilla recutita* (matricaria)) from a 6-oz cup containing 2 bags of tea in hot water in less than 10 minutes. Cardiac and arterial pressures and cardiac output were measured 30 minutes later. The average cardiac index decreased minimally from 3.0 to 2.8 liters/min/m^2, and this change was not statistically significant. However, a significant increase in mean brachial artery pressure from 91 to 98 mm Hg ($P < 0.05$) was reported. Approximately 10 minutes after tea ingestion, 10 of the patients fell into a deep sleep. Once awakened, they immediately fell into a deep sleep again, which lasted until the end of cardiac catheterization (~ 90 min).

Antidiuretic Effect

Anthemis Nobilis Flower Oil

The effect of anthemis nobilis flower oil on diuresis was evaluated using groups of 12 adult male Wistar rats.[88] The oil from one variety of *Anthemis nobilis* that has been cultivated in Italy, "white-headed" (WH) or "double-flowered roman chamomile" was tested. After an 18-h fast (water *ad libitum* up to 1h before testing), each animal received water in 3 doses (5 ml/kg body weight per dose). Anthemis nobilis flower oil was then administered (subcutaneously [s.c., 350 mg/kg] or i.p. [350 mg/kg] dosing) to 2 groups, and the amount of water eliminated by the animals was measured. The control group received water only. Rats dosed s.c. eliminated 46.85% of the water administered, and rats dosed i.p. eliminated 28.10% of ther water administered. Control rats eliminated 93.04% of the water administered. It was concluded that anthemis nobilis flower oil had an antidiuretic effect in Wistar rats.

Wound Healing Activity

Chamomilla Recutita (Matricaria) Flower Extract

The wound healing activity of chamomilla recutita (matricaria) flower extract was evaluated using 2 groups of 6 Sprague-Dawley rats. [33] One group of rats received chamomilla recutita (matricaria) flower extract (aqueous extract) in drinking water at a dose of 120 mg/kg/day. The wound closure rate was assessed by tracing the wound on days 1, 5, 10, and 15 post-wounding. Epithelialization was said to have occurred when the eschar fell off without leaving a residual raw wound. Control rats were maintained on plain drinking water. Healing was assessed by the rate of wound contraction, period of epithelialization, wound-breaking strength, granulation tissue weight, and hydroxyproline content. When compared to controls on day 15, test animals had a greater reduction in wound area (61% - test; 48% - controls), faster epithelialization, and a statistically significantly higher wound-breaking strength ($p < 0.002$). Wet and dry granulation tissue weight and hydroxyproline content were also significantly higher in test animals. It was concluded that chamomilla recutita (matricaria) flower extract facilitated wound healing.

SUMMARY

The safety of chamomile (German chamomile [*Chamomilla recutita* (*matricaria*) and Roman chamomile [*Anthemis nobilis*]) ingredients is reviewed in this assessment. These ingredients function mostly as fragrance ingredients and skin conditioning agents in cosmetic products. The VCRP and Council survey data combined indicate that the following 13 chamomile ingredients have been used in cosmetic products: chamomilla recutita (matricaria) flower oil, chamomilla recutita (matricaria) flower powder, chamomilla recutita (matricaria) flower, chamomilla recutita (matricaria) flower water, chamomilla recutita (matricaria) flower extract, chamomilla recutita (matricaria) flower/leaf/stem extract, chamomilla recutita (matricaria) flower/leaf extract, chamomilla recutita (matricaria) leaf extract , chamomilla recutita (matricaria) extract, chamomilla recutita (matricaria) oil, anthemis nobilis flower extract, anthemis nobilis flower oil, and anthemis nobilis flower water. The highest ingredient use concentration in this group has been reported as 13% (for anthemis nobilis flower extract).

Sesquiterpenes, sesquiterpene alcohols, and paraffin hydrocarbons are among the essential components of chamomilla recutita (matricaria) flower oil. The following impurities have been identified in dry chamomile (*Chamomilla recutita*) from Croatia: lead and cadmium heavy metals, and the herbicides linuron, fluazifop-p-butyl, and cycloxydim. Formaldehyde has been detected in intact *Chamomilla recutita* (*Matricaria*) plants grown in Hungary.

UV spectral analyses have indicated absorption maxima of 285 nm and ~225 nm for chamomilla recutita (matricaria) flower oil and anthemis nobilis flower oil, respectively. Additionally a logP value of 5.29 has been reported for chamomilla recutita (matricaria) flower oil.

The following ingredients did not induce acute toxicity when administered orally to mice or rats: chamomilla recutita (matricaria) flower, chamomilla recutita (matricaria) flower oil, chamomilla recutita (matricaria) flower oil extract, and anthemis nobilis flower oil. The same was true for chamomilla recutita (matricaria) oil and anthemis nobilis flower oil when administered dermally to rabbits. Chamomile recutita (matricaria) flowers (in the form of herbal tea) did not induce oral toxicity when consumed repeatedly by rats or humans. Chamomilla recutita (matricaria) flower extract also did not induce oral toxicity in rats when administered repeatedly.

The antimicrobial activity of chamomilla recutita (matricaria) flower oil has been demonstrated using various bacterial and fungal strains.

Seven hay fever patients experienced conjunctivitis after ocular rinsing with chamomilla recutita (matricaria) tea (from flowers). The results of a provocation test involving the tea extract confirmed that the tea induced allergic conjunctivitis. Chamomilla recutita (matricaria) flower oil was not irritating to the hen's egg chorioallantoic membrane in the HET-CAM *in vitro* assay for assessing ocular irritation potential.

Skin irritation was observed in acute dermal toxicity studies on chamomilla recutita (matricaria) flower oil) and anthemis nobilis flower oil involving rabbits. Chamomilla recutita (matricaria) flower oil was classified as non-irritating to the skin of hairless mice and moderately irritating to the skin of rabbits. The same was true for anthemis nobilis flower oil in rabbits and hairless mice. Anthemis nobilis flower oil (4%) did not induce skin sensitization in guinea pigs. Cross-reactivity of chamomilla recutita (matricaria) extract with carabron (sesquiterpene lactone) was not demonstrated in a guinea pig skin sensitization study.

In human predictive patch tests, chamomilla recutita (matricaria) flower oil (4%) and anthemis nobilis flower oil (4%) were not skin irritants in subjects tested or skin sensitizers in maximization tests involving 25 subjects. Chamomile essential oil (genus and species not stated) did not have skin irritation or sensitization potential in the 2 human repeated insult patch tests involving 110 and 104 subjects.

In provocative tests, skin sensitization was observed in 18 of 24 patients patch tested with 1% chamomilla recutita (matricaria) ether extract and in 4 of 5 patients and 48 of 84 patients patch tested with 2.5% chamomilla recutita (matricaria) ether extract in petrolatum. Five of 9 patients with positive patch test reactions to sesquiterpene lactone mix also had an allergic reaction to 2.5% chamomilla recutita (matricaria) [plant part(s) not specified] in petrolatum. Skin sensitization was also observed in 19 gardeners and greenhouse workers with compositae-related symptoms who were patch tested with 2.5% chamomilla recutita (matricaria) in petrolatum. Of 36 patients patch tested with ether extracts of chamomilla recutita (matricaria), 30 had positive patch test reactions, most of which were ++ or +++. Similarly, of the 35 patients patch tested with 2.5% chamomilla recutita (matricaria) in petrolatum, 22 had sensitization reactions (+ to +++). The number of patients (group of 35, sesquiterpene lactones mix sensitive) with positive reactions to chamomilla recuita (matricaria) flower aqueous extract decreased with decreasing test concentrations (100% [30 patients] to 1% [9 patients]). Of 129 patients (sensitive to compositae mix) patch-tested with 2.5% chamomilla recutita (matricaria) flower extract, 83 had sensitization reactions. In the prick test, chamomilla recutita (matricaria) extract (applied to forearm, 3.5 mg/ml) induced wheal formation in all 9 patients.

Provocative testing also yielded patch test reactions to chamomilla recutita flower oil, a doubtful positive reaction in 1 of 14 patients (4% concentration) and a ++ reaction to 4% and 1% in a second patient. Patch test reactions to *Anthemis nobilis* (plant part(s) not specified; 1% in petrolatm) were described as ++ reactions (2 of 14 patients) and doubtful positive follicular reactions (2 patients). Patch testing also resulted in a low incidence of skin sensitization to chamomilla recutita (matricaria) flower oil in 3 of 74 patients (2% in yellow soft paraffin), 2 of 200 patients, and 2 of 86 patients. The 86 patients were also sensitive to a perfume mixture. Positive reactions to chamomile ingredients (*Chamomilla recutita (matricaria*) and *Anthemis nobilis*) were also observed in a number of case reports.

Barely perceptible erythema was observed in hairless mice and miniature swine treated with chamomilla recutita (matricaria) flower oil in a photoxicity study. Photoxicity test results were classified as negative in animals treated with anthemis nobilis flower oil. Photoaggravation was observed in 1 of 5 patients tested with 2.5% chamomilla recutita (matricaria) ether extract in a standard photopatch test.

For 37 regular users of chamomile (herbal product, genus and species not stated) there was a higher frequency of threatening miscarriages (21.6%) and preterm labors (21.6%) when compared to non-users (283 subjects). Chamomilla recutita (matricaria) flower extract induced an incidence of sister chromatid exchanges that was comparable to that induced in bone marrow cells from control mice. Anthemis nobilis flower oil was not genotoxic in the rec-assay (no positive DNA-damaging activity) or Ames test. The antigenotoxic activity of chamomilla recutita (matricaria) flower oil and chamomilla recutita (matricaria) flower oil extract was also demonstrated *in vitro*. Carcinogenicity data on chamomile ingredients were not found in the published literature. However, chamomilla recutita (matricaria) flower extract and chamomilla recutita flower oil caused a significant decrease in cell viability in human cancer cell lines.

Various biological effects of chamomile ingredients (*Chamomilla recutita (matricaria)* and *Anthemis nobilis*), such as, anti-inflammatory activity, hypnotic/sedative activity, and enzyme inhibition, have been identified in the published literature.

Table 1. Definitions and functions of the ingredients in this safety assessment[1]

Ingredient, CAS No.	Definition	Function
Chamomilla Recutita		
Chamomilla Recutita (Matricaria) Extract	Chamomilla Recutita (Matricaria) Extract is the extract of the whole plant, *Chamomilla recutita*.	Skin-conditioning agents-miscellaneous
Chamomilla Recutita (Matricaria) Flower	Chamomilla Recutita (Matricaria) Flower is the flower of *Chamomilla recutita*.	Skin-conditioning agents-miscellaneous
Chamomilla Recutita (Matricaria) Flower Extract [84082-60-0]	Chamomilla Recutita (Matricaria) Flower Extract is the extract of the flowerheads of the matricaria, *Chamomilla recutita*.	Fragrance ingredients; skin-conditioning agents-miscellaneous; skin conditioning agents-occlusive
Chamomilla Recutita (Matricaria) Flower/Leaf/Stem Extract	Chamomilla Recutita (Matricaria) Flower/Leaf/Stem Extract is the extract of the leaves, flowers and stems of *Chamomilla recutita.*	Flavoring agents; oral care agents; skin conditioning agents-miscellaneous
Chamomilla Recutita (Matricaria) Flower/Leaf/Stem Water	Chamomilla Recutita (Matricaria) Flower/Leaf/Stem Water is an aqueous solution of the steam distillate obtained from the flowers, leaves and stems of *Chamomilla recutita*.	Fragrance ingredients
Chamomilla Recutita (Matricaria) Flower Oil [8002-66-2]	Chamomilla Recutita (Matricaria) Flower Oil is the volatile oil obtained from the flowers of *Matricaria recutita*.	Fragrance ingredients; skin-conditioning agents-miscellaneous
Chamomilla Recutita (Matricaria) Flower Powder	Chamomilla Recutita (Matricaria) Flower Powder is the powder obtained from the dried, ground flowers of *Chamomilla recutita.*	Skin-conditioning agents-miscellaneous
Chamomilla Recutita (Matricaria) Flower Water	Chamomilla Recutita (Matricaria) Flower Water is an aqueous solution of the steam distillate obtained from the flowers of *Chamomilla recutita*.	Fragrance ingredients
Chamomilla Recutita (Matricaria) Leaf Extract [84082-60-0]	Chamomilla Recutita (Matricaria) Leaf Extract is the extract of the leaves of *Chamomilla recutita.*	Fragrance ingredients; skin-conditioning agents-miscellaneous
Chamomilla Recutita (Matricaria) Oil	Chamomilla Recutita (Matricaria) Oil is the volatile oil obtained from the whole plant, *Chamomilla recutita.*	Fragrance ingredients
Anthemis Nobilis		
Anthemis Nobilis Flower Oil [8015-92-7]	Anthemis Nobilis Flower Oil is the volatile oil distilled from the dried flower heads of *Anthemis nobilis.*	Fragrance ingredients; skin-conditioning agents-miscellaneous
Anthemis Nobilis Flower Powder	Anthemis Nobilis Flower Powder is the powder obtained from the dried, ground flowers of *Anthemis nobilis.*	Skin-conditioning agents-miscellaneous
Anthemis Nobilis Flower Water	Anthemis Nobilis Flower Water is an aqueous solution of the steam distillates obtained from the flowers of *Anthemis nobilis.*	Fragrance ingredients; skin-conditioning agents-miscellaneous

Table 2. Chemical and Physical Properties[28,96,77,97]

Properties	Chamomilla Recutita (Matricaria) Flower Oil	Anthemis Nobilis Flower Oil
Form	Deep blue or blue-green liquid with strong, characteristic odor	Light blue or light green-blue liquid with strong, aromatic odor
logP	5.29	
Specific gravity	Between 0.910 and 0.950	Between 0.892 and 0.910
Refractive Index		Between 1.440 and 1.450 at 20°
Solubility	Soluble in most fixed oils and in propylene glycol. Insoluble in glycerin and in mineral oil	Soluble in most fixed oils and almost completely soluble in mineral oil. Soluble in propylene glycol, but insoluble in glycerin
Acid value	Between 5 and 50	Not more than 15.0
Ester value	Between 65 and 155	Between 250 and 310
Saponification number	≈ 43	
UV absorption maximum	285 nm	~ 225 nm

Table 3. Composition of Chamomile Ingredients[28,2,98,99,15,100,35,101,40,88]

Composition Data	Ingredients		
Components	**Chamomilla Recutita (Matricaria) Flower Extract**	**Chamomilla Recutita (Matricaria) Flower Oil**	**Anthemis Nobilis Flower Oil**
Apigenin	3.0 to 95.1 µmol/l		
Apigenin-7-glucoside	94.1 to 216.2 µmol/l		
Artemesia alcohol		< 0.1% to 0.2%	
Artemisia ketone		< 0.1 to 7.8%	
Azulene		0.40%	
Benzaldehyde		< 0.1%	
Benzyl alcohol		< 0.1%	
cis-En-yn-bicycloether		3.6 to 17.7%	
Bicyclogermacrene		0.10%	
β-Bisabolenal		0.80%	
cis-α-Bisabolene		0.30%	
cis-α-Bisabolene epoxide		< 0.05% to 3.8%	
α-Bisabolene oxide A		1.31 to 10%	
β-Bisabolene		0.2 to 19.6%	
(Z)-γ-Bisabolene		0.50%	
trans-γ-Bisabolene		0.10%	
α-Bisabolol		0.7 to 13.15%	
(-)-α-Bisabolol		1.59 to 41.45%	
α-Bisabolol acetate		1.80%	
α-Bisabolol oxide A		< 0.05% to 55.9%	
Bisabolol-oxide A		0.42 to 36.27%	
Bisabolol oxide B		4.64% to 11.17%	
α-Bisabolol oxide B		1.2 to 25.1%	
β-Bisabolol		0.1 to 2.5%	
Bisabolon-oxide		0.55 to 4.13%	
α-Bisabolone oxide A		< 0.05% to 13.6%	
Borneol		0.80%	
n-Butylangelate + hexyl acetate			14.5 to 34.2%
Butyl phthalate		15.10%	
Cadina-1,4-diene		< 0.1%	
α-Cadinene		0.2 to 3.75%	
δ-Cadinene		0.1 to 5.20%	
γ-Cadinene		0.1 to 2.25%	
Caffeic acid	1.2 to 5.1 µmol/l		
α-Calacorene		< 0.1%	
trans-Calamenene		< 0.1%	
Camphor		≤ 0.1%	
trans-Carveol		0.10%	
β-Caryophyllene		< 0.1 to 0.9%	
Caryophyllene oxide		0.70%	

Table 3. Composition of Chamomile Ingredients[28,2,98,99,15,100,35,101,40,88]

Composition Data	Ingredients		
Components	**Chamomilla Recutita (Matricaria) Flower Extract**	**Chamomilla Recutita (Matricaria) Flower Oil**	**Anthemis Nobilis Flower Oil**
Chamazulene		0.2 to 24.50%	
Chamo-spiroether		4.71%	
Chlorogenic acid	7.3 to 310.3 µmol/l		
cis-Chrysanthenol		0.10%	
1,8-Cineole		< 0.1% to 2.1%	
α-Copaene		0.2% to 0.24%	
ar-Curcumene		< 0.1%	
p-Cymene		0.05% to 1.1%	
para-Cymene-8-ol		0.70%	
Daucene		0.50%	
Decanoic acid		0.3 to 3.7%	
Dendrolasin		0.50%	
trans-Dicycle-ether		3.20%	
2,5-Dihydro-2,5-dimethylfuran		< 0.1%	
2,6-Dimethyl-5-heptenal		< 0.1%	
β-Elemene		< 0.1% to 0.9%	
δ-Elemene		0.10%	
γ-Elemene		0.70%	
Ethyl decanoate		< 0.1%	
Ethyl hexanoate		< 0.1%	
Ethyl 2-methybutyrate		< 0.1%	
ethyl isovalerate		< 0.1%	
γ-Eudesmol		1.50%	
α-Farnesene		0.15 to 27.72%	
(E,E)-α-Farnesene		3.10%	
β-Farnesene		52.30%	
(E)-β-Farnesene		0.9 to 10.9%	
cis-β-farnesene		0.90%	
tr-β-Farnesene		7.2 to 12.8%	
trans-β-Farnesene		5.20%	
(Z)-β-Farnesene		< 0.1% to 15.97%	
Furfural		< 0.1%	
Geraniol		< 0.1%	
Germacrene-D		0.16 to 5.78%	
2-Heptanone		< 0.1%	
Hexadecanoic acid		0.3 to 23%	
Hexanal		< 0.1%	
(Z)-3-Hexanol		0.10%	
(E)-2-Hexenal		< 0.1%	
(E)-β-Ionone		0.10%	

Table 3. Composition of Chamomile Ingredients[28,2,98,99,15,100,35,101,40,88]

Composition Data	Ingredients		
Components	**Chamomilla Recutita (Matricaria) Flower Extract**	**Chamomilla Recutita (Matricaria) Flower Oil**	**Anthemis Nobilis Flower Oil**
Isoamyl angelate			19.4 to 22.8%
Isoamyl tigliate			0.6 to 0.8%
Isbutyl butyrate			0.6 to 1.5%
Isobutyl isovalerate			3.5 to 3.8%
Isorhamnetin	0.1 to 3.6 µmol/l		
Juniperol		0.90%	
Kaempferol	0.2 to 0.9		
Ledol		< 0.1%	
Limonene		0.1% to 0.2%	
Linalool		0.10%	
Linaool acetate (dihydro)		3.39%	
Cis-Linalool oxide (furanoid)		< 0.1%	
trans-Linalool oxide (furanoid)		< 0.1%	
cis-Linoleic acid		< 0.05% to 11.9%	
Luteolin	0.6 to 9.2 µmol/l		
2-Methylbutyl 2-methylbutyrate			7.3% to 9.2%
Methyl decanoate		< 0.1%	
Methyl guaiacol		< 0.1%	
6-Methyl-5-hepten-2-ol		< 0.1%	
6-Methyl-5-hepten-2-one		0.10%	
Methyl hexadecanoate		2.60%	
5-Methyl-2-hexanal		< 0.1%	
Methyl linoleate		1.00%	
Methyl linolenate		1.10%	
α-Muurolene		0.8 to 3.41%	
γ-Muurolene		1.31%	
α-Muurolol		0.30%	
Myrcene		< 0.1%	
(E)-Nerolidol		0.20%	
Nonanal		< 0.1%	
n-Nonanal		0.10%	
Nonanoic acid		0.30%	
3-Nonen-2-one		< 0.1%	
(E)-β-Ocimene		0.10%	
(Z)-β-Ocimene		0.20%	
trans-β-Ocimene		1.73%	
(E,E)-3,5-Octadien-2-one		< 0.1%	
Octanal		< 0.1%	
2-Octanol		< 0.1%	
3-Octanol		< 0.1%	

Table 3. Composition of Chamomile Ingredients[28,2,98,99,15,100,35,101,40,88]

Composition Data	Ingredients		
Components	**Chamomilla Recutita (Matricaria) Flower Extract**	**Chamomilla Recutita (Matricaria) Flower Oil**	**Anthemis Nobilis Flower Oil**
1-Octen-3-ol		< 0.1%	
3-Octen-2-one		< 0.1%	
2-Phenylethanol		0.20%	
α-Pinene		< 0.1% to 0.12%	
β-Pinene		< 0.1%	
Pinocarvone		< 0.1%	
Quercetin	0.5 to 6.5 µmol/l		
Quercetin-3-glucoside	1.7 to 10.6 µmol/l		
Quercitrin	limit of detection		
Rutin	0.7 to 2.9 µmol/l		
cis-Sabinene hydrate		0.20%	
Sabinene		< 0.1%	
Safrole		< 0.1%	
Salvial-4(14)-en-1-one		0.1 to 4.1%	
(Z)-β-Santalol		1%	
β-Selinene		1%	
Spathulenol		0.46 to 9.4%	
Spiroether		1.10%	
cis-Spiroether		3.43 to 7.48%	
cis-en-in-Spiroether		0.73%	
trans-Spiroether		0.9 to 6.01%	
Terpinen-1-ol		< 0.1%	
Terpinen-4-ol		< 0.1%	
γ-Terpinene		< 0.1% to 0.3%	
α-Terpineol		0.10%	
4-Terpineol		0.10%	
α-Thujone		< 0.1%	
2,2,6-Trimethylhexanone		< 0.1%	
Umbelliferone	1.0 to 53.1 µmol/l		
α-Ylangene		< 0.1%	
Yomogi alcohol		< 0.1%	

Table 4. Frequency and Concentration of Use Data[16,17,18]

Ingredients	Use Frequency (Totals)	Maximum Use Concentrations (%)
Chamomilla Recutita (Matricaria) Flower Oil	**121**	**0.0004 to 3**
Chamomilla Recutita (Matricaria) Flower Powder	**NR**	**0.001 to 1**
Chamomilla Recutita (Matricaria) Oil	**10**	**NR**
Chamomilla Recutita (Matricaria) Extract	**79**	**NR**
Chamomilla Recutita (Matricaria) Flower	**26**	**3**
Chamomilla Recutita (Matricaria) Flower Extract	**883**	**0.00001 to 7**
Chamomilla Recutita (Matricaria) Flower/Leaf Extract	**380**	**0.001 to 0.1**
Chamomilla Recutita (Matricaria) Flower/Leaf/Stem Extract	**NR**	**0.0005**
Chamomilla Recutita (Matricaria) Leaf Extract	**NR**	**0.0006 to 0.6**
Chamomilla Recutita (Matricaria) Flower Water	**7**	**NR**
Anthemis Nobilis Flower Extract	**464**	**0.00003 to 13**
Anthemis Nobilis Flower Oil	**216**	**0.00002 to 6**
Anthemis Nobilis Flower Water	**2**	**5**

NR = Not Reported

References

1. Gottschalck, T. E. and Breslawec, H. P. International Cosmetic Ingredient Dictionary and Handbook. 14 *ed*. Washington, DC: Personal Care Products Council, 2012.

2. Povh, N. P. Garcia C. A. Marques M. O. M. and Meireles M. A. A. Extraction of essential oil and oleoresin from chamomile (Chamomila recutita [L.] Rauschert) by steam distillation and extraction with organic solvents: a process design approach. *Plantas Medicinais*. 2001;4(1):1-8.

3. Ganzera, M., Schneider, P., and Stuppner, H. Inhibitory effects of the essential oil of chamomile (Matricaria recutita L.) and its major constituents on human cytochrome P450 enzymes. *Life Sci.* 2006;78(8):856-861.

4. Opdyke, D. L. J. Monographs on fragrance raw materials. Chamomile oil German. *Food and Cosmetics Toxicology*. 1974;12:851-852.

5. Opdyke, D. L. J. Monographs on fragrance raw materials. Chamomile oil Roman. *Food and Cosmetics Toxicology*. 1974;12:853.

6. Carle, R. and Gomaa K. Chamomile: A pharmacological and clinical profile. *Drugs of Today*. 1992;28(8):559-565.

7. Weglarz, Z. and Roslon W. Individual variability of chamomile (chamomilla recutita (L.) Rausch.) in respect of the content and chemical composition of essential oil. *Herba Polonica*. 2002;48(4):169-173.

8. Momcilovic, B., Ivicic, N., Bosnjak, I., Stanic, G., Ostojic, Z., and Hrlec, I. G. "More does not mean better"risk assessment of heavy metals lead and cadmium and herbicides linuron, fluazifop-P-butyl, and cycloxydim in dry true chamomile (Chamomilla recutita L. Rauschert). *Arhiv.Za Higijenu.Rada I Toksikologiju.* 1999;50(2):201-210.

9. Harbourne, N. Jacquier J. c. and O'Riordan D. Optimisation of the extraction and processing conditions of chamomile (*Matricaria chamomilla L.*) for incoroporation into a beverage. *Food Chemistry*. 2009;115(1):15-19.

10. Avallone, R. Zanoli P. Corsi G. Cannazza G. and Baraldi M. Benzodiazepine-like compounds and GABA in flower heads of *Matricaria chamomilla*. *Phytotherapy Research*. 1996;10:S177-S179.

11. Máday, E. Tyihák E. and Szöke É. Occurrence of formaldehyde in intact plants, micropropagated plants and hairy root cultures of chamomile (*Matricaria recutita* L.). *Plant Growth Regulation*. 2000;30(2):105-110.

12. Matricaria chamomilla (German chamomile). Monograph. *Altern Med Rev.* 2008;13(1):58-62.

13. Paulsen, E. Contact sensitization from Compositae-containing herbal remedies and cosmetics. *Contact Dermatitis*. 2002;47(4):189-198.

14. Gould, L., Reddy, C. V. R., and Gomprecht, R. F. Cardiac effects of chamomile tea. *J.Clin.Pharmacol.New Drugs*. 1973;13(11):475-479.

15. Szõke, É Máday E. Marczal G. and Lemberkovics É. Proceedings of the international Conference on Medicinal and Aromatic Plants. Part II. Analysis of biologically active essential oil components of chamomiles in Hungary (In vivo - In Vitro). *Acta Horticulturae*. 2003;597:275-284.

16. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2012. Washington, D.C.: FDA.

17. Personal Care Products Council. Concentration of use by FDA product category. Chamomilla Recutita (Matricaria) ingredients. Unpublished data submitted by the Personal Care Products Council on 09-17-2009. 2009.

18. Personal Care Products Council. Concentration of use by FDA product category. Anthemis nobilis-derived ingredients. Unpublished data submitted by the Personal Care Products Council on 2-25-2010. 2010.

19. Shirakawa, S. Mizuno K. Mizuno K. Komada Y. Takahara M. and Hirose K. Dimentia and fragrance. *Aroma Research*. 2008;9(1):73-77.

20. Ross, S. M. Chamomile: A spoonful of medicine. *Holistic Nursing Practice*. 2008;22(1):56-57.

21. Food and Drug Administration (FDA). Substances generally recognized as safe. *Matricaria chamomilla* and *Anthemis nobilis*. 21 CFR 182.10. 2012.

22. Food and Drug Administration (FDA). Substances generally recognized as safe. *Matricaria chamomilla* and *Anthemis nobilis*. 21CFR 582.10. 2012.

23. Food and Drug Administration (FDA). Substances generally recognized as safe. *Matricaria chamomilla* flowers and *Anthemis nobilis* flowers. 21CFR 182.20. 2012.

24. Food and Drug Administration (FDA). Substances generally recognized as safe. *Matricaria chamomilla* flowers and *Anthemis nobilis* flowers. 21CFR 582.20. 2012.

25. Food and Drug Administration (FDA). New drugs. Chamomile flowers. 21CFR 310.545. 2012.

26. Della Loggia, R. Traversa U. Scarcia V. and Tubaro A. Depressive effects of *Chamomila recutita* (L.) Rausch, tubular flowers, on central nervous system in mice. *Pharmacological Research Communications*. 1982;14(2):153-162.

27. MB Research laboratories, Inc. Chamomile oil, German. Acute oral toxicity test in rats and acute dermal toxicity test in rabbits. Report submitted to the Research Institute for Fragrance Materials (RIFM), Inc. Unpublished data submitted by RIFM on 10-11-2012. 1973. pp.1

28. Pauli, A. Relationship between lipophilicity and toxicity of essential oils. *International Journal of Essential Oil*. 2008;2(2):60-68.

29. Hernandez-Ceruelos, A. Madrigal-Bujaidar E. and de la Cruz C. Inhibitory effect of chamomile essential oil on the sister chromtid exchanges induced by daunorubicin and methyl methanesulfonate in mouse bone marrow. *Toxicol.Lett.* 2002;135(1-2):103-110.

30. MB Research Laboratories, Inc. Chamomile oil, Roman. Acute oral toxicity test in rats and acute dermal toxicity test in rabbits. Report to the Research Institute for Fragrance Materials (RIFM), Inc. Unpublished data submitted by RIFM on 10-11-2012. 1973. pp.1

31. Maliakal, P. P. and Wanwimolruk S. Effect of herbal teas on hepatic drug metabolizing enzmes in rats. *Pharmacy and Pharmacology*. 2001;53(10):1323-1329.

32. Wang, Y. Tang H. Nicholson J. K. Hylands P. J. Sampson J. and Holmes E. A metabonomic strategy for the detection of the metabolic effects of chamomile (*Matricaria recutita L.*) ingestion. *J.Agric.Food Chem.* 2005;53(2):191-196.

33. Shivananda, N. B. Sivachandra R. S. and Chalapathi R. A. V. Wound healing activity of *Matricaria recutita L*. extract. *Journal of Wound Care*. 2007;16(7):298-302.

34. Aggag, M. E. and Yousef R. T. Study of antimicrobial activity of chamomile oil. *Planta Medica*. 1972;22(2):140-144.

35. Alireza, M. Antimicrobial activity and chemical composition of essential oils of chamomile from Neyshabur, Iran. *Journal of Medicinal Plants Research*. 2012;6(5):820-824.

36. Ahmed, F. H., El-Badri, A. A., Ibrahim, M. Mk, El-Shahed, A. S., and El-Khalafawy, H. Mm. Comparative studies of antifungal potentialities for some natural plant oils against different fungi isolated from poultry. *Grasas Y.Aceites*. 1994;45(4):260-264.

37. Koch, C., Reichling, J., Kehm, R., Sharaf, M. M., Zentgraf, H., Schneele, J., and Schnitzler, P. Efficacy of anise oil, dwarf-pine oil and chamomile oil against thymidine-kinase-positive and thymidine-kinase-negative herpesviruses. *J Pharm Pharmacol*. 2008;60(11):1545-1550.

38. Ceska, O Chaudhary S. K. Warrington P. J. and Ashwood-Smith M. J. Coumarins of chamomile, *Chamomilla recutita*. *Fitoterapia*. 1992;63(5):387-394.

39. Bahtiti, A. and Hassan N. Chemical analysis and biological activity of Jordanian chamomile extracts. *Advance Journal of Food Science and Technology*. 2012;4(1):22-25.

40. Can, O. D. Õzkay U. D. Kiyan H. T. and Demirci B. Psychopharmacological profile of *Chamomile* (*Matricaria recutita L.*) essential oil in mice. *Phytomedicine*. 2012;19(3-4):306-310.

41. Subiza, J., Subiza, J. L., Alonso, M., Hinojosa, M., Garcia, R., Jerez, M., and Subiza, E. Allergic conjunctivitis to chamomile tea. *Ann Allergy*. 1990;65(2):127-132.

42. Hausen, B. M., Herrmann, H. D., and Willuhn, G. The senstitizing capacity of Compositae plants. I. Occupational contact dermatitis from Arnica longifolia Eaton. *Contact Dermatitis*. 1978;4(1):3-10.

43. Klecak, G. The Freund's complete adjuvant test and the open epicutaneous test. *Curr.Probl.Derm.* 1985;14:152-171.

44. Kligman, A. M. Maximization test on chamomile oil, German. Report to the Research Institute for Fragrance Materials (RIFM), Inc. Unpublished data submitted by RIFM on 10-12-2012. 1973. pp.1-2.

45. Kligman, A. M. Maximization test on chamomile oil, Roman. Report to the Research institute for Fragrance materials (RIFM). Unpublished data submitted by RIFM on 10-11-2012. 1973. pp.1-2.

46. Clinical Research laboratories, Inc. Final report. Repeated insult patch test with re-challenge. 2001.59212.001 essential oil. Unpublished data submitted by the Research Institute for Fragrance Materials (RIFM) on 10-12-2012. 2001. pp.1-15.

47. Reliance Clinical Testing Services, Inc. Final Report. Clinical safety evaluation. Repeated insult patch test. Test article: 2001.592I.004 (chamomile essential oil). Unpublished data submitted by the Research Institute for Fragrance Materials (RIFM) on 10-12-2012. 2002. pp.1-7.

48. Paulsen, E., Andersen, K. E., and Hausen, B. M. Compositae Dermatitis in a Danish Dermatology Department in One Year. (I). Results of Routine Patch Testing with the Sesquiterpene Lactone Mix Supplemented with Aimed Patch Testing with Extracts and Sesquiterpene Lactones of Compositae Plants. *Contact Dermatitis*. 1993;29(1):6-10.

49. Hausen, B. M. A 6-year experience with compositae mix. *Am J Contact Dermat.* 1996;7(2):94-99.

50. Jovanovic, M. Poljacki M. Duran V. Vujanovic L. Sente R. and Stojanovic S. Contact allergy to compositae plants in patients with atopic dermatitis. *Medicinski Pregled*. 2004;57(5-6):209-218.

51. Paulsen, E. Søgaard J. and Andersen K. E. Occupational dermatitis in Danish gardeners and greenhouse workers (III). Compositae-related symptoms. *Contact Dermatitis*. 1998;38(3):140-146.

52. Paulsen, E., Otkjaer, A., and Andersen, K. E. The coumarin herniarin as a sensitizer in German chamomile [Chamomilla recutita (L.) Rauschert, Compositae]. *Contact Dermatitis.* 2010;62(6):338-342.

53. Florido-Lopez, J. F. Gonzalez-Delgado P. Saenz de San Pedro B. Perez-Miranda C. Arias de Saavedra J. M. and Marin-Pozo J. F. Allergy to natural honeys and camomile tea. *International Archives of Allergy and Immunology*. 1995;108(2):170-174.

54. Paulsen, E. and Andersen K. E. Patch testing with constituents of Compositae mixes. *Contact Dermatitis*. 2012;66(5):241-246.

55. Lundh, K., Gruvberger, B., M"ller, H., Persson, L., Hinds,n, M., Zimerson, E., Svensson, A., and Bruze, M. Patch testing with thin-layer chromatograms of chamomile tea in patients allergic to sesquiterpene lactones. *Contact Dermatitis.* 2007;57(4):218-223.

56. Paulsen, E. Andersen K. E. and Hausen B. M. Sensitization and cross-reaction patterns in Danish compositae-allergic patients. *Contact Dermatitis*. 2001;45(4):197-204.

57. Lundh, K., Hinds,n, M., Gruvberger, B., M''ller, H., Svensson, A., and Bruze, M. Contact allergy to herbal teas derived from Asteraceae plants. *Contact Dermatitis.* 2006;54(4):196-201.

58. Paulsen, E., Chistensen, L. P., and Andersen, K. E. Cosmetics and herbal remedies with Compositae plant extracts - are they tolerated by Compositae-allergic patients? *Contact Dermatitis.%2008., Jan.* 58(1):15-23.(1:15-23):Contact.

59. Rudzki, E. and Grzywa Z. Balsam of Peru as screening agent for essential oils sensitivity. *Dermatologica*. 1977;155:115-121.

60. Rudzki, E. Grzywa Z. and Bruo W. S. Sensitivity to 35 essential oils. *Contact Dermatitis.* 1976;2:196-200.

61. Rudzki, E. and Gryzwa, Z. Allergy to perfume mixture. *Contact Dermatitis*. 1986;15(2):115-116.

62. Pereira, F., Santos, R., and Pereira, A. Contact dermatitis from chamomile tea. *Contact Dermatitis.* 1997;36(6):307.

63. West, I. and Maibach, H. I. Contact urticaria syndrome from multiple cosmetic components. *Contact Dermatitis*. 1995;32(2):121.

64. Scala, G. Acute, short-lasting rhinitis due to camomile-scented toilet paper in patients allergic to compositae. *Int Arch Allergy Immunol.* 2006;139(4):330-331.

65. Subiza, J., Subiza, J. L., Hinojosa, M., Garcia, R., Jerez, M., Valdivieso, R., and Subiza, E. Anaphylactic reaction after the ingestion of chamomile tea: a study of cross-reactivity with other composite pollens. *J Allergy Clin Immunol.* 1989;84(3):353-358.

66. Jensen-Jarolim, E., Reider, N., Fritsch, R., and Breiteneder, H. Fatal outcome of anaphylaxis to camomile-containing enema during labor: a case study. *J Allergy Clin Immunol.* 1998;102(6):1041-1042.

67. Foti, C., Nettis, E., Panebianco, R., Cassano, N., Diaferio, A., and Pia, D. P. Contact urticaria from Matricaria chamomilla. *Contact Dermatitis.* 2000;42(6):360-361.

68. McGeorge, B. C. and Steele, M. C. Allergic contact dermatitis of the nipple from Roman chamomile ointment. *Contact Dermatitis.* 1991;24(2):139-140.

69. Giordano-Labadie, F. Schwarze P. and Bazex J. Allergic contact dermatitis from camomile used in phytotherapy. *Contact Dermatitis*. 2000;42(4):247.

70. van Ketel, W. G. Allergy to matricaria-chamomilla. *Contact Dermatitis*. 1982;8(2):143.

71. van Ketel, W. G. Allergy to matricaria-chamomilla. *Contact Dermatitis*. 1987;16(1):50-51.

72. Rudzki, E., Rapiejko, P., and Rebandel, P. Occupational contact dermatitis, with asthma and rhinitis, from camomile in a cosmetician also with contact urticaria from both camomile and lime flowers. *Contact Dermatitis.* 2003;49(3):162.

73. Rycroft, R. J. Recurrent facial dermatitis from chamomile tea. *Contact Dermatitis.%2003, Apr*. 48(4):229.(4:229):Contact.

74. Vandenplas, O., Pirson, F., D'Alpaos, V., Vander, Borght T., Thimpont, J., and Pilette, C. Occupational asthma caused by chamomile. *Allergy*. 2008;63(8):1090-1092.

75. Manzano, D. Aguirre A. Gardeazabal J. Oleaga J. M. Izyu R. Zabala R. and Pérez J. L. D. Airborne allergic contact dermatitis due to wild plants. *Contact Dermatitis*. 1994;31:188-189.

76. Forbes, P. D. Urbach F. and Davies R. E. Phototoxicity testing of fragrance raw materials. *Fd.Cosmet.Toxicol.* 1977;15(1):55-60.

77. Urbach, F. and Forbes P. D. Phototoxicity study on Roman chamomile oil. Report to the Research Institute for Fragrance Materials (RIFM) on July 18, 1973. Unpublished data submitted by RIFM on 10-12-2012. 1973. pp.1-3.

78. Kobayashi, Y. Suppression of sensory irritation by bisabololoxide A, a major component of German chamomile essential oil, and its comparison with (-)-α-bisabolol. *Aroma Research*. 2008;9(2):107-112.

79. Cuzzolin, L., Francini-Pesenti, F., Verlato, G., Joppi, M., Baldelli, P., and Benoni, G. Use of herbal products among 392 Italian pregnant women: focus on pregnancy outcome. *Pharmacoepidemiol.Drug Saf.* 2010;19(11):1151-1158.

80. Hernandez-Ceruelos, A., Madrigal, Santill, Morales, Gonz, Chamorro-Cevallos, G., Cassani-Galindo, M., and Madrigal-Bujaidar, E. Antigenotoxic Effect of Chamomilla recutita (L.) Rauschert Essential Oil in Mouse Spermatogonial Cells, and Determination of Its Antioxidant Capacity in Vitro. *Int J Mol Sci*. 2010;11(10):3793-3802.

81. Stavric, B. Matula T. I. Klassen R. and Downie R. H. The effect of teas on the *in vitro* mutagernic potential of heterocyclic aromatic amines. *Food and chemical Toxicology*. 1996;34(6):515-523.

82. Zani, F. Massimo G. Benvenuti S. Bianchi A. Albasini A. Melegari M. vampa G. Bellotti A. and Mazza P. Studies on the genotoxic properties of essential oils with *Bacillus subtilis* rec-assay and *Salmonella*/microsome reversion assay. *J.Planta Medica*. 1991;57(3):237-241.

83. Srivastava, J. K. and Gupta, S. Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. *J Agric Food Chem.* 2007;55(23):9470-9478.

84. Trovato, A., Monforte, M. T., Rossitto, A., and Forestieri, A. M. In vitro cytotoxic effect of some medicinal plants containing flavonoids. *Boll.Chim.Farm.* 1996;135:263-266.

85. Romeilah, R. M. Anticancer and antioxidant activities of *Matricaria chamomilla L*. and *Marjorana hortensis* essential oils. *Research Journal of Medicine and Medical Sciences*. 2009;4(2):332-339.

86. Shigeru, A. Naho M. Kazumi H. Hiroko I. Shigeharu I. Haruyuki O. and Hideyo Y. Suppression of tumor necrosis factor-alpha-induced neutrophil adherence responses by essential oils. *Mediators of Inflammation*. 2003;12(6):323-328.

87. Ohnishi, M. Kimura S. Yamazaki M. Abe S. and Yamaguchi H. Characterization of immunological activity of a low toxicity antitumor lipopolysaccharide from Bordetella pertussis. *Microbiol.Immunol.* 1994;38:733-739.

88. Rossi, T. Melegari M. Bianchi A. albasini A. and Vampa G. Sedative, anti-inflammatory and anti-diuretic effects induced in rats by essential oils of varieties of Anthemis nobilis: A comparative study. *Pharmacological Reearch Communications*. 1988;20(5):71-74.

89. Ghonime, M., Eldomany, R., Abdelaziz, A., and Soliman, H. Evaluation of immunomodulatory effect of three herbal plants growing in Egypt. *Immunopharmacol Immunotoxicol.* 2011;33(1):141-145.

90. Lee, S.-H. Heo Y. and Kim Y. H. Effect of German chamomile oil application on alleviating atopic dermatitis-like immune alterations in mice. *J.Vet Sci.* 2010;11(1):35-41.

91. Reiter, M. and Brandt W. Relaxant effects on tracheal and ileal smooth muscles of the guinea pig. *J.Drug.Res.* 1985;35(1):408-414.

92. Budzinski, J. W., Foster, B. C., Vandenhoek, S., and Arnason, J. T. An in vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. *Phytomedicine.* 2000;7(4):273-282.

93. Crespi, C. L. Miller V. P. and Penman B. W. Microtitre plate assays for inhibition of human, drug-metabolizing cytochromes P450. *Anal.Biochem.* 1997;248:188-190.

94. Srivastava, J. K., Pandey, M., and Gupta, S. Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity. *Life Sci.* 2009;85(19):663-669.

95. Shinomiya, K. Inoue T. Utsu Y. Tokunaga S. Masuoka T. Ohmori A. and Kamei C. Hypnotic activities of chamomile and passiflora extracts in sleep-disturbed rats. *Biol.Pharm.Bull.* 2005;28(5):808-810.

96. Urbach, F. and Forbes P. D. Phototoxicity study on German chamomile oil. Report to the Research Institute for Fragrance Materials (RIFM) on July 18, 1973. Unpublished data submitted by RIFM on 10-12-2012. 1973. pp.1-3.

97. The United States Pharmacopeial Convention. Food Chemicals Codex. 6th *ed*. Rockville: The United States Pharmacopeial Convention, 2009.

98. Nováková, L. Vildová A. Mateus J. P. Gonçalves T. and Solich P. Development and application of UHPLC-MS/MS method for the determination of phenolic compounds in Chamomile flowers and Chamomile tea extracts. *Talanta*. 2010;82(4):1271-1280.

99. Raal, A. Orav A. Puessa T. Valner C. Malmiste B. and Arak E. Content of essential oil, terpenoids and polyphenols in commercial chamomile (Chamomilla recutita L. Rauschert) teas from different countries. *Food Chemistry*. 2012;131(2):632-638.

100. Pino, J. A. Bayat F. Marbot R. and Aguero J. Essential oil of Chamomile Chamomilla recutita (L.) Rausch. from Iran. *J.Essent.Oil Res.* 2002;14(6):407-408.

101. Matos, F. J. A. Machado M. I. L. Alencar J. W. and Craveiro A. A. Constituents of Brazilian chamomile oil. *J.Essent.Oil Res.* 1993;5(3):337-339.