Safety Assessment of *Zanthoxylum piperitum*-Derived Ingredients as Used in Cosmetics

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The Expert Panel for Cosmetic Ingredient Safety members are Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Wilbur Johnson, Jr., M.S., former Senior Scientific Analyst/Writer, and Regina Tucker, M.S., Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ◊ Washington, DC 20036-4702 ◊ PH 202.331.0651
cirinfo@cir-safety.org

ABBREVIATIONS

AICIS	Australian Industrial Chemicals Introduction Scheme
BoNT/A	botulinum toxin type A
CFR	Code of Federal Regulations
cGMP	current good manufacturing practices
CIR	Cosmetic Ingredient Review
CPSC	Consumer Product Safety Commission
Council	Personal Care Products Council
Da	Daltons
DMSO	dimethyl sulfoxide
dw	dry weight
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
GC-MS	gas chromatography-mass spectrometry
GRAS	generally recognized as safe
HRIPT	human repeated insult patch test
MS/MS	tandem mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
MW	molecular weight
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OECD	Organization for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
RC	relative content
RIFM	Research Institute for Fragrance Materials
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 4 *Zanthoxylum piperitum*-derived ingredients as used in cosmetic formulations. Collectively, these ingredients are reported to function as skin conditioning agents, skin protectants, biocides, astringents, and fragrance ingredients in cosmetic products. *Zanthoxylum piperitum*-derived ingredients comprise constituents that may cause adverse effects. Because final product formulations may contain multiple botanicals, each possibly containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. With *Zanthoxylum piperitum*-derived ingredients, the Panel was concerned about the presence of potential sensitizers (e.g., citronellol and geranyl acetate) in cosmetics. Additionally, industry should minimize impurities, such as heavy metals and pesticide residues, according to limits set by the US Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). The Panel considered the available data and concluded that the 4 *Zanthoxylum piperitum*-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing.

INTRODUCTION

The safety of the following 4 *Zanthoxylum piperitum*-derived ingredients as used in cosmetics is reviewed in this safety assessment.

Zanthoxylum Piperitum Fruit Extract
Zanthoxylum Piperitum Oil
Zanthoxylum Piperitum Peel Extract
Zanthoxylum Piperitum Peel Water

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), collectively, the *Zanthoxylum piperitum*-derived ingredients are reported to function as skin conditioning agents, skin protectants, cosmetic biocides, cosmetic astringents, and fragrance ingredients in cosmetic products (See Table 1).[1] The Panel routinely does not review ingredients that function only as fragrance ingredients, because, as fragrances, the evaluation of the safety of these ingredients is the purview of the Research Institute for Fragrance Materials (RIFM). However, although Zanthoxylum Piperitum Oil is only reported to function as a fragrance ingredient in cosmetics, the safety of this ingredient was neither previously nor currently the subject of review by RIFM; thus, it is included in this review.

These *Zanthoxylum piperitum*-derived ingredients may contain hundreds of constituents, some of which may have the potential to cause toxic effects. For example, terpenes (e.g., citronellol and geranyl acetate) are potential sensitizers. In this assessment, the Panel will review the potential toxicity of each of the *Zanthoxylum piperitum*-derived ingredients as a whole, complex mixture; toxicity from single components may not predict the potential toxicity of botanical ingredients.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. The published data in this document were identified by conducting an exhaustive search of the world's literature; this search was last conducted July 2023. A list of the search engines and websites that are used, and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is available on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data may be provided by the cosmetics industry, as well as by other interested parties and is included and summarized, where appropriate.

An assessment report on *Zanthoxylum piperitum* extract has been published by the National Industrial Chemicals Notification and Assessment Scheme (NICNAS; now known as the Australian Industrial Chemicals Introduction Scheme (AICIS)).[2] Because the ingredient in that assessment is identified as *Zanthoxylum piperitum* extract, it is possible that the data could pertain to either Zanthoxylum Piperitum Fruit Extract or Zanthoxylum Piperitum Peel Extract; although, it is not clear which ingredient is being reviewed specifically, these data are included in this review and may inform safety. Please note that this source provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when this source is cited.

The names of the ingredients in this report are written in accordance with the INCI naming conventions, i.e., capitalized without italics or abbreviations. When referring to the genus and species from which the ingredients are derived, the standard taxonomic practice of using italics is followed (e.g., *Zanthoxylum piperitum*). It is often not known how the substance being tested in a study compares to the cosmetic ingredient. In the report text, if it is known that the material being tested is a cosmetic ingredient, the INCI naming convention will be used (e.g., Zanthoxylum Piperitum Fruit Extract). However, if it is not known that the test substance is the same as the cosmetic ingredient, the taxonomic naming conventions (e.g., a *Zanthoxylum piperitum* fruit extract) will be used.

CHEMISTRY

Definition and Plant Identification

All of the *Zanthoxylum piperitum*-derived ingredients named in this assessment have the generic CAS No. 97404-53-0.[1] The definitions for the *Zanthoxylum piperitum*-derived ingredients are presented in Table 1. It should be noted that the chemical class for Zanthoxylum Piperitum Oil is essential oils and water.

Zanthoxylum piperitum (common names, Japanese pepper and Sichuan pepper)[1] is native to East Asia and prevalent in Japan.[3] It bears a tiny red fruit between August and September. The fruit includes the pericarp, which is a portion of the fruit that surrounds the seeds.

Chemical Properties

According to a submission to NICNAS, a *Zanthoxylum piperitum* extract (plant part not specified) has an average molecular weight (MW) of constituents equivalent to < 500 Daltons (Da) and a water solubility value of 5.69 mg/l - 1.56 g/l.[2] These and other properties are presented in Table 2.

Method of Manufacture

Zanthoxylum Piperitum Fruit Extract

A powdered extract of *Zanthoxylum piperitum* was prepared from 100 g of *Zanthoxylum* fruit.[4] It was extracted by reflux with 1 l of 50% ethanol for 4 h. The extract was concentrated under decompression and powdered by freeze drying at -80°C for 72 h. Eleven grams of *Zanthoxylum piperitum* fruit extract were obtained, resulting in 11% final yield of *Zanthoxylum piperitum*.

Two additional studies describe the manufacturing methods of *Zanthoxylum piperitum* fruit extract from dried fruit. In one instance, 100 g of *Zanthoxylum piperitum* fruit was soaked in 50% ethanol (1 l) at room temperature for 24 h.[5] The ethanol extract was filtered through filter paper and concentrated in a rotary vacuum evaporator for 30 min to remove the ethanolic base. The concentrated extracts were then freeze-dried. In the second study the dried fruits (1 kg) were soaked in 70% ethanol (10 l) at room temperature for 12 h.[6] The ethanol extract was filtered through filter paper, and concentrated under a vacuum at 40°C. The concentrated extracts were then freeze-dried and stored at -20°C until use.

Zanthoxylum Piperitum Peel Extract

Zanthoxylum Piperitum Peel Extract can be manufactured by extracting dried raw material with an ethanol solution (70%/vol), and afterwards allowing it to settle as a sediment.[7] The sediment is then filtrated and adjusted before being packaged. Zanthoxylum Piperitum Peel Extract can also be prepared by extracting the dried raw material with 1,3-butylene glycolic solution (50%/vol) and allowing it to deposit as a sediment. This sediment is again then filtrated and adjusted before being packaged.

The peel of *Zanthoxylum piperitum* fruit was extracted with purified water at 100°C for 1 h.[8] The soluble extract was then separated from the insoluble waste and concentrated by removal of water under reduced pressure. Spray drying was used to generate dried extract powder.

Zanthoxylum Piperitum Peel Water

In some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture. Zanthoxylum Piperitum Peel Water is the aqueous solution of the steam distillate obtained from the peel of *Zanthoxylum piperitum*.[1]

Composition/Impurities

There are more than 50 sanshools present in the *Zanthoxylum* genus.[9] The main pungent components of *Zanthoxylum piperitum* fruit are sanshool and sanshoolamide.[10] Sanshoamide has also been reported as a component from the fresh unripe fruits of *Zanthoxylum piperitum*.[11]

Zanthoxylum piperitum extract

According to NICNAS, the degree of purity of a *Zanthoxylum piperitum* extract (supercritical carbon dioxide extract, plant part not specified) is 100%, and it does not contain any additives/adjuvants.[2] The three constituents present at the highest concentrations in the *Zanthoxylum piperitum* extract tested are: linalyl acetate at 30 - 50% %, linalool at 10 - 20% and limonene at 5 - 10% , accounting for 56.13% (ranging from 45 - 80%) of the composition of the *Zanthoxylum piperitum* extract. Composition data on *Zanthoxylum piperitum* extract from the NICNAS report is included in Table 3.[2]

Zanthoxylum Piperitum Fruit Extract

Zanthoxylum piperitum pericarp extract was obtained utilizing chloroform, n-hexane, diethyl ether, and ethyl acetate, and its amides were isolated independently and analyzed.[12,13] The highest amide constituent of the pericarp was noted to be hydroxy-α-sanshool (1.89% of dry weight (dw)) and (6*RS*,11*SR*)-6,11-dihydroxy-*N*-(2-hydroxy-2- methylpropyl)-2,7,9-dodecatrienamide (0.41% composition of the extract). Details from these studies are summarized in Table 4.

The amount of tocopherols in the extract from the dried pericarp and seed was determined.[14] The following were found: α-tocopherol (3.2 mg/100 g dw), β-tocopherol (0.12 mg/100 g dw), γ-tocopherol (0.35 mg/100 g dw), and δ-tocopherol (0.27 mg/100 g dw) in the dried pericarp and α-tocopherol (0.12 mg/100 g dw) and γ-tocopherol (2.8 mg/100 g dw) in the seed.

In another study, the total polyphenol and flavonoid content of *Zanthoxylum piperitum* fruit extract was 742.8 ± 3.97 mg gallic acid equivalents/g and 486.8 ± 7.08 mg quercetin equivalents/g, respectively.[6] Although the individual polyphenols

and flavonoids were not specified, flavone was the major component among this chemical grouping. The dried fruit extract has reported concentrations of 40.342 ± 0.13 and 23.209 ± 0.04 μg/ml for hyperoside and quercitrin, respectively. The relative standard deviations of both compounds was less than 8.07%.[5] Additional composition data on *Zanthoxylum piperitum* fruit extracts are found in Table 5 and Table 6.

Zanthoxylum Piperitum Oil

Composition data on *Zanthoxylum piperitum* fruit oil from ripe fruit and from dried pericarp are found in Table 7.[15,16] Volatile components of *Zanthoxylum piperitum* fruit oil from the ripe fruit include hydrocarbons, alcohols, and esters, primarily D-limonene (11.5%), geraniol (11.0%), citronellal (16.2%) and geranyl acetate, respectively.[15] (Percent composition was calculated by adding all the totals of the relative content (RC) and dividing individual RC). Another study identified similar constituents of *Zanthoxylum piperitum* fruit oil. However, the main component was limonene (37.99%), with minor amounts of sabinene (13.30%), and β-myrcene (7.17%); these 3 represented almost 59% of all the volatile constituents.[17] Data on the major components of *Zanthoxylum piperitum* oil (from whole plant) are found in Table 8.[18]

Another study examined the constituents of *Zanthoxylum piperitum* pericarp steam distillate.[19] The following were identified: carveol, β-caryophyllene oxide, 1,8-cineole, citronellal, citronellol, citronellyl acetate, α-copaene, cuminaldehyde, cuminyl alcohol, *p*-cymene, geranyl acetate, limonene, linalool, linalool oxide, β-myrcene, α-pinene, β-pinene, piperitone, and, α-terpineol.

Constituents of the essential oil obtained from leaves of *Zanthoxylum piperitum* were determined via GC-MS analysis.[20] The major components were identified as D-limonene (18%), geranyl acetate (15.3%), cryptone (8.5%), citronellal (7.1%), cuminal (6.2%), and phellandral (5.2%).

Zanthoxylum Piperitum Peel Extract

The composition of Zanthoxylum Piperitum Peel Extract from an ethanol solvent contains triterpene and tannin (% composition not specified).[7] Impurities of this extract include heavy metals, not more than 20 ppm, and arsenic, not more than 2 ppm. The composition of the extract from the 1,3-butylene glycolic solution yield triterpenoids (% composition not mentioned) along with heavy metals, no more than 10 ppm and arsenic, no more than 2 ppm.

The volatile compounds from the skin of the mature fruit of *Zanthoxylum piperitum* extracted with methyl *t*-butyl ether were analyzed with gas chromatography – mass spectrometry (GC-MS).[3] The composition can be found in Table 9.

The composition of a methanolic extract of a *Zanthoxylum piperitum* peel was determined.[21] The results, obtained using GC-MS, are also shown in Table 9.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP data, Zanthoxylum Piperitum Fruit Extract is reported to be used in 183 cosmetic products (Table 10).[22] Although this ingredient has the highest reported frequency of use for the ingredients in this group, and it is used in numerous product categories in the VCRP, the results of a concentration of use survey provided by the Council in 2021 only report concentration of use data for Zanthoxylum Piperitum Fruit Extract in one product category; according to the survey, it is used at a maximum concentration of up to 0.01% in spray body and hand products.[23] Zanthoxylum Piperitum Peel Extract is the only other ingredient in this report that is reported to be in use; it is reported to be used in 19 formulations at maximum use concentrations up to 0.0022% in make-up and skin care preparations. According to VCRP and Council survey data, 2 of the 4 ingredients, i.e., Zanthoxylum Piperitum Oil and Zanthoxylum Piperitum Peel Water, are not currently in use in cosmetic products (Table 11).

Cosmetic products containing *Zanthoxylum piperitum*-derived ingredients may incidentally come in contact with the eyes or mucous membranes (concentration data for these formulation-types not provided). It should be noted that Zanthoxylum Piperitum Fruit Extract is reported to be used in 5 baby products (use concentration not provided). Additionally, some of the ingredients are used in cosmetic sprays and powders, and could possibly be inhaled; for example, Zanthoxylum Piperitum Fruit Extract and Zanthoxylum Piperitum Peel Extract are reported to be used in products that are

known to be sprayed (up to 0.01% in body and hand products and up to 0.0000018% in night products, respectively), and Zanthoxylum Piperitum Peel Extract is reported to be used in face powders at a maximum use concentration of 0.0000022%. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets /particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The *Zanthoxylum piperitum*-derived ingredients are not restricted from use in any way under the rules governing cosmetic products in the European Union.[24]

Non-Cosmetic

Zanthoxylum piperitum extract appears on the Flavor and Extract Manufacturers Association's (FEMA) list of flavoring ingredients that are classified as generally recognized as safe (GRAS) under the 1958 food additives amendment to the US Federal Food, Drug, and Cosmetics Act.[25] As a result of its lemon-like aroma and pungent taste, Japanese pepper (Rutaceae, *Zanthoxylum piperitum*) is commonly used in Japanese dishes as a spice and for seasoning to mask unpleasant odors that arise from fish and meat ingredients.[3] Specifically, the fresh young leaves of the plant, as well as the fruit pericarp, are used as spices in Japanese cuisine.[10] According to another source, fruit peels and leaves of *Zanthoxylum piperitum* (Rutaceae) have been used in Japan for centuries as spices to preserve foods.[21]

Zanthoxylum piperitum is among the Korean medicinal plants (Korean salad plants), so named due to their content of purported bioactive compounds, mainly antioxidant phenolics.[26] *Zanthoxylum piperitum* fruit extract has been indicated to alleviate rheumatoid arthritis, inhibit alveolar bone loss, reduce weight gain, decrease adipocytes and adipose tissue mass, and act as a therapeutic agent for osteoporosis.[4-6,27]

TOXICOKINETIC STUDIES

Dermal Penetration

Zanthoxylum piperitum extract

NICNAS noted that given the low molecular weight of the components of *Zanthoxylum piperitum* extract (supercritical carbon dioxide extract, < 500 Da; plant part not specified), its water solubility (5.69 mg/l - 1.56 g/l), and a log P_{ow} of 2.9 - 4.4, there is potential for *Zanthoxylum piperitum* extract to cross biological membranes.[2]

Absorption, Distribution, Metabolism, and Excretion

Zanthoxylum Piperitum Fruit Extract

The pharmacokinetics of a mixture containing *Zanthoxylum piperitum* fruit was studied using 16 subjects (fasted).[28] The mixture had the following composition: *Zanthoxylum piperitum* fruit, ginger, ginseng, and maltose. A randomized, open-label, three-arm, three-period protocol was used. The mixture was administered orally to each subject in doses of 2.5, 5, and 10 g. Blood samples were collected just before and at the following intervals after administration: 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24, and 48 h. Plasma fractions were stored prior to analysis by high performance liquid chromatography. Of the 6 compounds measured, hydroxy-α-sanshool, a constituent of *Zanthoxylum piperitum* fruit, had the highest plasma concentration of the mixture. The plasma concentration of hydroxy-α-sanshool reached the maximum concentration within 30 min after administration. Its median half-life was 1.6 to 1.7 h, indicating rapid absorption and elimination. The maximum concentration of hydroxy-α-sanshool in the plasma was 209 – 664 ng/ml. However, it is not mentioned if the compounds are directly from *Zanthoxylum piperitum* or from other ingredients of the mixture.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Data on the acute toxicity of *Zanthoxylum piperitum*-derived ingredients reviewed in this safety assessment were not found in the published literature, nor were these data submitted.

Short-Term, Subchronic, and Chronic Toxicity Studies

Data on the short-term, subchronic, and chronic toxicity of *Zanthoxylum piperitum*-derived ingredients reviewed in this safety assessment were not found in the published literature, nor were these data submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Data on the developmental and reproductive toxicity of *Zanthoxylum piperitum*-derived ingredients reviewed in this safety assessment were not found in the published literature, nor were these data submitted.

GENOTOXICITY STUDIES

The in vitro and in vivo genotoxicity studies summarized below are presented in Table 12.

A *Zanthoxylum piperitum* extract (supercritical carbon dioxide extract, in acetone, plant part not specified) was not mutagenic in an Ames test when tested at concentrations of up to 5000 µg/plate, with or without metabolic activation.[2] Results were also negative in an in vitro micronucleus test, whereby human lymphocytes were incubated with *Zanthoxylum piperitum* extract (supercritical carbon dioxide extract, in DMSO, plant part not specified) at concentrations up to 640 µg/ml (without metabolic activation) and up to 320 µg/ml (with metabolic activation). Neither a statistically nor biologically significant increase in the number of micronucleated cells was observed, and the test substance was neither clastogenic nor aneugenic to human lymphocytes. In an Ames test, *Zanthoxylum piperitum* essential oil was not mutagenic when tested at concentrations up to 1000 µg/ml with and without metabolic activation.[20] A mammalian chromosome aberration test of *Zanthoxylum piperitum* essential oil at concentrations up to 300 µg/ml was also negative in Chinese hamster lung cells. In a bone marrow micronucleus test, groups of 6 male and 6 female ICR mice were orally given up to 1000 mg/kg bw/d *Zanthoxylum piperitum* essential oil for 2 d; no abnormalities were observed, and the test article was not clastogenic.

CARCINOGENICITY STUDIES

Data on the carcinogenicity of *Zanthoxylum piperitum*-derived ingredients reviewed in this safety assessment were not found in the published literature, nor were these data submitted.

ANTI-CARCINOGENICITY STUDIES

Zanthoxylum Piperitum Fruit Extract

A *Zanthoxylum piperitum* fruit extract and its ability to induce autophagic cell death was examined.[8] Using phase-contrast microscopy, cells were treated for 24 h with 200 µg/ml of a *Zanthoxylum piperitum* fruit extract, and vacuoles were observed in the cytoplasm. Cell proliferation assays were performed after 48 h of treatment, and proliferation in at least 3 cell lines was inhibited. In a human colorectal cell line, after 72 h of treatment, the viability and number of cancer cells was reduced. To further confirm the induction of autophagy, Western blot analysis was performed to analyze the conversion of cytosolic LC3-1 into LC3 II in DLD-1 cells. This demonstrated autophagic activity. Quantitative RT-qPCR and Western blot were also utilized to examine the ability of an essential protein to prevent a *Zanthoxylum piperitum* fruit extract from inducing autophagic cell death. The phosphorylation of c-Jun-N-terminal kinase in 6 different types of cancer cells was also measured via Western blot analysis. To explore the effect of a *Zanthoxylum piperitum* fruit extract on normal cells, a rat intestinal cell line was treated with *Zanthoxylum piperitum* fruit extract and examined. *Zanthoxylum piperitum* fruit extract appears to induce JNK-dependent autophagic cell death.

Zanthoxylum piperitum extract

Zanthoxylum piperitum extract and its ability to display anti-cancer activity in human cell lines (Calu-6 for human pulmonary carcinoma and SMU-601 for human gastric carcinoma) was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.[26] Serial dilutions of *Zanthoxylum piperitum* extract (dried methanol extract) were prepared by dissolving the extract in DMSO, followed by dilution with medium to yield the following final concentrations: 25, 50, 100, 200, 400, and 800 µg/ml. Optical density was recorded using a micro plate reader at 540 nm. Distilled water served as the positive control, and DMSO served as the solvent control. Controls and samples were assayed in duplicate for each concentration and replicated three times for each cell line. Cytotoxicity was obtained by comparing absorbance between the samples and the control. The values obtained were then used to calculate the concentration of *Zanthoxylum piperitum* extract required to cause a 50% reduction (IC_{50}, in µg/ml) in growth (cell number) for each cell line. In the Calu-6 cell line, the IC_{50} value for *Zanthoxylum piperitum* extract was 470.4 ± 13.1 µg/ml. In the SMU-601 cell line, the IC_{50} value for *Zanthoxylum piperitum* extract was 349.0 ± 9.1 µg/ml. Additionally, a dose-dependent inhibition of cell proliferation was observed in this study.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Human

Zanthoxylum piperitum extract

The skin sensitization potential of 2% *Zanthoxylum piperitum* extract (super critical carbon dioxide extract; plant part not specified) in ethanol:diethyl phthalate (1:3 w/w) was evaluated in a human repeated-insult patch test (HRIPT) involving 110 subjects.[2] Two different samples of the test substance were tested on each subject. During induction, the test substance (on a 3.62 cm^2 occlusive patch) was applied to the same location on the back of each subject 3 times per week for a total of 9 applications. Test sites were examined for dermal irritation at each visit prior to re-application of the test substance.

Approximately 10 to 21 d after the final visit of the induction phase, the challenge phase was initiated. The test substance was applied for ~ 24 h to a new site on the back. Test sites were examined for signs of dermal irritation or sensitization. The test substance did not elicit skin irritation or sensitization during the challenge phase and was classified as a non-sensitizer.

OCULAR IRRITATION STUDIES

Data on the ocular irritation potential of the *Zanthoxylum piperitum*-derived ingredients reviewed in this safety assessment were not found in the published literature, nor were these data submitted.

CLINICAL STUDIES

Zanthoxylum Piperitum Fruit Extract

The mechanisms, components, synergistic effects, and topical effects of a *Zanthoxylum piperitum* fruit extract were all examined in one study to determine methods to reduce wrinkles non-invasively via mechanisms similar to botulinum toxin type A (BoNT/A) injection.[29] Two in vitro assays (a co-cultured cell-based muscle contraction assay and a muscle contraction assay in *Caenorhabditis elegans*) were performed and *Zanthoxylum piperitum* was identified as a BoNT/A-like reagent that induced a 27.7% decrease in muscle contraction rates at a concentration of 1000 ppm. A test performed with the Neurotransmitter Transporter Uptake Assay Kit with modifications to assess the ability of *Zanthoxylum piperitum* fruit extract to regulate signal transduction in neurons indicated that muscle contraction is inhibited by attenuating electric signal transduction in presynaptic neurons. Furthermore, two components of a *Zanthoxylum piperitum* fruit extract, quercitrin and hyperoside, were examined for their role in muscle contraction. Quercitrin was found to be responsible for muscle contraction inhibition.

Finally, the effect of topical treatment of a *Zanthoxylum piperitum* fruit extract on facial wrinkles was determined. Twenty-three women aged 38 and older completed the study. The study was randomized, double-blind, and placebo-controlled. The participants were divided into a placebo group (n = 8), a *Zanthoxylum piperitum* fruit extract-treated group (n = 7, 60 ppm), and a *Zanthoxylum piperitum* fruit extract (60 ppm) with acetyl hexapeptide-8 (50 ppm)-treated group (n = 8). Lateral canthal rhytides were evaluated after daily application for 0, 4, 8, and 12 wk using a 3D skin imaging system. Compared to placebo treatment, *Zanthoxylum piperitum* fruit extract treatment for 12 wk ameliorated lateral canthal rhytides. The topical treatment of *Zanthoxylum piperitum* fruit extract improved the appearance of lateral canthal rhytides by 11.4%. It was also determined that *Zanthoxylum piperitum* fruit extract and acetyl hexapeptide-8 have synergistic effects on wrinkle improvement.

SUMMARY

The safety of the following 4 *Zanthoxylum piperitum*-derived ingredients as used in cosmetics is reviewed in this safety assessment: Zanthoxylum Piperitum Fruit Extract, Zanthoxylum Piperitum Oil, Zanthoxylum Piperitum Peel Extract, and Zanthoxylum Piperitum Peel Water. According to the *Dictionary*, collectively, the *Zanthoxylum piperitum*-derived ingredients are reported to function as skin conditioning agents, skin protectants, biocides, astringents, and fragrance ingredients in cosmetic products.

Zanthoxylum piperitum (i.e., Japanese pepper; Rutaceae) is native to East Asia and prevalent in Japan. It bears a tiny red fruit between August and September. The available composition data indicate that *Zanthoxylum piperitum*-derived ingredients consist of numerous volatile aromatic and aliphatic hydrocarbons.

According to 2023 VCRP data, Zanthoxylum Piperitum Fruit Extract is reported to be used in 183 cosmetic products. The results of a concentration of use survey provided by the Council in 2021 only reported maximum use concentration data for Zanthoxylum Piperitum Fruit Extract in one product category (i.e., at up to 0.01% in body and hand spray products). Zanthoxylum Piperitum Peel Extract is the only other ingredient in this report for which use concentration data are being reported; this ingredient is being used at maximum use concentrations of up to 0.0022% in make-up and skin care preparations.

Zanthoxylum piperitum extract appears on the FEMA list of flavoring ingredients that are classified as GRAS under the 1958 food additives amendment to the US Federal Food, Drug, and Cosmetics Act.

NICNAS noted that given the low molecular weight of the components of *Zanthoxylum piperitum* extract (supercritical carbon dioxide extract, < 500 Da, plant part not specified), its water solubility (5.69 mg/l - 1.56 g/l), and a log P_{ow} of 2.9 - 4.4, there is potential for *Zanthoxylum piperitum* extract to cross biological membranes.

The pharmacokinetics of a mixture containing *Zanthoxylum piperitum* fruit was studied using 16 subjects (fasted). The mixture was administered orally to each subject in doses up to 10 g. Of the 6 compounds measured, hydroxy-α-sanshool, a constituent of *Zanthoxylum piperitum* fruit, had the highest plasma concentration (maximum concentration range: 209 to 664 ng/ml) of the mixture. Its median half-life was 1.6 to 1.7 h, indicating rapid absorption and elimination.

A *Zanthoxylum piperitum* extract (supercritical carbon dioxide extract, in acetone, plant part not specified) was not mutagenic in an Ames test when tested at concentrations of up to 5000 µg/plate, with or without metabolic activation. Results were also negative in an in vitro micronucleus test, whereby human lymphocytes were incubated with *Zanthoxylum*

piperitum extract (supercritical carbon dioxide extract, in DMSO, plant part not specified) at concentrations up to 640 µg/ml (without metabolic activation) and up to 320 µg/ml (with metabolic activation). Neither a statistically nor biologically significant increase in the number of micronucleated cells was observed, and the test substance was neither clastogenic nor aneugenic to human lymphocytes. In an Ames test, *Zanthoxylum piperitum* essential oil was not mutagenic when tested at concentrations up to 1000 µg/ml with and without metabolic activation. A mammalian chromosome aberration test of *Zanthoxylum piperitum* essential oil at concentrations up to 300 µg/ml was also negative in Chinese hamster lung cells. In a bone marrow micronucleus test, groups of 6 male and 6 female ICR mice were orally given up to 1000 mg/kg bw/d *Zanthoxylum piperitum* essential oil for 2 d; no abnormalities were observed, and the test article was not clastogenic.

The ability of a *Zanthoxylum piperitum* fruit extract to induce autophagic cell death was studied. Using phase-contrast microscopy, cells were treated for 24 h with 200 µg/ml of the *Zanthoxylum piperitum* fruit extract, and vacuoles were observed in the cytoplasm. Cell proliferation in at least 3 cell lines was inhibited. In a human colorectal cell line, the viability and number of cancer cells was reduced. The phosphorylation of c-Jun-N-terminal kinase in 6 different types of cancer cells was also measured. *Zanthoxylum piperitum* fruit extract appears to induce JNK-dependent autophagic cell death.

Apoptosis of *Zanthoxylum piperitum* extract in human cancer cell lines (Calu-6 for human pulmonary carcinoma and SMU-601 for human gastric carcinoma) was measured using the MTT assay to evaluate anti-cancer activity potential. The following concentrations (in DMSO) were tested: 25, 50, 100, 200, 400, and 800 µg/ml. In the Calu-6 cell line, the IC_{50} value for *Zanthoxylum piperitum* extract was 470.4 ± 13.1 µg/ml. In the SMU-601 cell line, the IC_{50} value for *Zanthoxylum piperitum extract* was 349.0 ± 9.1 µg/ml. Additionally, a dose-dependent inhibition of cell proliferation was observed.

The skin sensitization potential of 2% *Zanthoxylum piperitum* extract (supercritical carbon dioxide extract, plant part not specified) in ethanol:diethyl phthalate (1:3 w/w) was evaluated in an HRIPT involving 110 subjects. During induction, the test substance (on a 3.62 cm^2 occlusive patch) was applied repeatedly to the back. At challenge, the test substance was applied for ~ 24 h to a new site on the back. The test substance induced neither skin irritation nor sensitization.

The mechanisms, components, synergistic effects, and topical effects of a *Zanthoxylum piperitum* fruit extract were examined in one study to determine methods to reduce wrinkles non-invasively via mechanisms similar to BoNT/A injection. The *Zanthoxylum piperitum* fruit extract improved the appearance of lateral canthal rhytides in humans.

DISCUSSION

This assessment reviews the safety of 4 *Zanthoxylum piperitum*-derived ingredients as used in cosmetic formulations. The Panel concluded that the available data are sufficient for determining safety of all 4 ingredients for use in cosmetic products when the products are formulated to be non-sensitizing.

Because final product formulations may contain multiple botanicals, each possibly containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *Zanthoxylum piperitum*-derived ingredients, the Panel was concerned about the presence of multiple terpenes (e.g., citronellol and geranyl acetate) in cosmetics, which could result in sensitization reactions. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects. An HRIPT of *Zanthoxylum piperitum* extract (super critical carbon dioxide extract; plant part not specified) in ethanol at 2% showed no evidence of sensitization or irritation. The need for additional sensitization data was mitigated by the caveat in the conclusion that products containing *Zanthoxylum piperitum*-derived ingredients must be formulated to be non-sensitizing.

Additionally, the Panel noted that although there was a lack of general toxicity data, *Zanthoxylum piperitum* extract is classified as GRAS, and its GRAS status mitigated toxicity concerns. Its use in foods also alleviated concerns regarding composition and the presence of impurities. The Panel noted these ingredients have low reported maximum concentrations of use (i.e., 0.01% in skin preparations). Also, the Panel found that because the composition of a *Zanthoxylum piperitum* essential oil derived from the leaf is similar to the *Zanthoxylum piperitum*-derived ingredients reviewed in this report, genotoxicity studies on the oil and extract served to further inform safety.

The Panel also expressed concern about heavy metals, pesticide residues, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to minimize impurities in cosmetic formulations according to limits set by the US FDA and EPA.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (for example, Zanthoxylum Piperitum Fruit Extract is reported to be used at up to 0.01% in spray body and hand products). Inhalation toxicity data were not available. However, the Panel noted that the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and

summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

Finally, the Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 4 *Zanthoxylum piperitum*-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing:

Zanthoxylum Piperitum Fruit Extract
Zanthoxylum Piperitum Oil*
Zanthoxylum Piperitum Peel Extract
Zanthoxylum Piperitum Peel Water*

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in the product categories and at concentrations comparable to others in this group.*

TABLES

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

Ingredient/CAS No.	Definition & Structures	Function(s)
Zanthoxylum Piperitum Fruit Extract 97404-53-0 (generic)	Zanthoxylum Piperitum Fruit Extract is the extract of the fruit of *Zanthoxylum piperitum.*	Skin-Conditioning Agents - Miscellaneous
Zanthoxylum Piperitum Oil 97404-53-0 (generic)	Zanthoxylum Piperitum Oil is the oil obtained from the fruit and fruit pericarp of *Zanthoxylum piperitum*.	Fragrance Ingredients
Zanthoxylum Piperitum Peel Extract 97404-53-0 (generic)	Zanthoxylum Piperitum Peel Extract is the extract of the peels of *Zanthoxylum piperitum*.	Cosmetic Biocides
Zanthoxylum Piperitum Peel Water 97404-53-0 (generic)	Zanthoxylum Piperitum Peel Water is the aqueous solution of the steam distillate obtained from the peel of *Zanthoxylum piperitum*.	Cosmetic Astringents; Fragrance Ingredients; Skin Protectants; Skin-Conditioning Agents - Miscellaneous

Table 2. Chemical properties of a *Zanthoxylum piperitum* extract (supercritical carbon dioxide)[2]

Property	Value
Physical Form (@ 20ºC and 101.3 kPa)	liquid
Molecular weight (Da; average of constituents)	< 500
Density (g/ml)	0.8984 – 0.9284
Water solubility (g/l)	0.00569 – 1.56 (estimated)
Partition coefficient (log P_{ow})	2.9 – 3.9 (aliphatic terpene constituents) (estimated) 4.2 - 4.4 (aliphatic cyclic constituents) (estimated)
Vapor pressure (kPa, @ 24 °C)	0.0249
Melting point (°C)	< -20 – 156 (based on primary constituents)
Boiling point (°C)	176 – 421 (based on primary constituents)
Flash point (ºC, @ 101.3 kPa)	39

Table 3. Composition data on a *Zanthoxylum piperitum* extract[2]

***Zanthoxylum piperitum* extract (supercritical carbon dioxide extract)**	
Constituents	**Composition (%)**
linalyl acetate	30 - 50
linalool	10 - 20
limonene	5 - 10
3-cyclohexene-1-methanol, α, α,4-trimethyl-, 1-acetate	1 – 5
bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1- methylethyl)-, 2- acetate; bicyclo [3.1.0]hexan-2-ol, 2-methyl-5-(1- methylethyl)-, 2- acetate, (1*R*,2*S*,5*S*)- *rel*-	5 - 15
2,6,8,10-dodecatetraenamide, *N*-(2-hydroxy-2- methyl propyl)-, (2*E*,6*E*,8*E*,10*E*)-	1 - 10

Table 4. Amide composition of *Zanthoxylum piperitum* fruit pericarp extract[12,13]

***Zanthoxylum piperitum* fruit (fruit pericarp ethyl acetate extract)**			
Constituents	**Quantity (mg)**	**% composition***	**% of dry weight****
(6RS) -(2E,7E,9E)-6-hydroxy-*N*-(2-hydroxy-2-methylpropyl)-11-oxo-2,7,9-dodecatrienamide	5.4	0.13	
(11RS)- (2E,7E,9E)-11-hydroxy-*N*-(2-hydroxy-2-methyl-propyl)-6-oxo-2,7,9-dodecatrienamide	4.8	0.11	
(10RS,11SR)-dihydroxy-*N*-(2-hydroxy-2-methylpropyl)-2,6,8-dodecatrienamide	10.1	0.24	
(10RS,11RS) -(2E,6Z,8E)- dihydroxy-*N*-(2-hydroxy-2-methylpropyl)-2,6,8-dodecatrienamide	4	0.10	
(6RS,11SR)-6,11-dihydroxy-*N*-(2-hydroxy-2- methylpropyl)-2,7,9-dodecatrienamide)	17.2	0.41	
(6RS,11RS) -(2E,7E,9E)-6,11-dihydroxy-*N*-(2-hydroxy-2- methylpropyl)-2,7,9-dodecatrienamide)	9.5	0.23	
α-sanshool			0.32
γ-sanshool			0.21
hydroxy-γ-sanshool			0.08
hydroxy-α-sanshool			1.89

* percent composition calculated from 4.2 grams of extract

** percent of dry weight pericarp (37 – 150 g pericarp used in this experiment)

Table 5. Composition data on *Zanthoxylum piperitum* fruit extract (methanol extract).[27]

Constituents	Area (%)*
myrcene	1.49
3(5)-[[1,2-dihydroxy-3-propoxy]methyl]-4hydroxy-1h-pyrazole-5(3)-carboxamide	0.09
β-phellandrene	4.28
hex-3-yne	0.10
3-hydroxycyclohexanone	0.06
isopropyl hexanoate	0.12
terpinolene	0.59
vinylcyclooctane	0.07
2-tetradecynoic acid	0.10
citronellal	2.75
3-hydroxy-2,3-dihydromaltol	0.67
pulegol	0.29
octanoic acid	0.24
(e)-4-undecenal	0.10
4-isopropyl-2-cyclohexenone	1.04
citronellol	1.20
(e)-beta-ocimene	0.39
3,7-dimethylocta-2,6-dien-1-ol	0.65
spiro[4.4]nona-1,3-diene, 1,2-dimethyl-	0.21
piperitone	0.41
nonanoic acid	0.42
8,8-dimethoxy-2,6-dimethyloct-2-ene	0.98
p-isopropylbenzyl formate	0.40
citronellic acid	0.55
α-terpinene	0.24
2,6-octadiene, 2,6-dimethyl-	1.60
terpinyl propionate	0.43
geranyl acetate	4.60
3-methylcyclohexene	0.21
1,4-dimethyl-4beta-methoxy-2,5cyclohexadien-1α-ol	0.33
2-propenoic acid, 3-phenyl-, methyl ester	0.49
6-methylenespiro[4.5]decane	0.07
β-caryophyllene	0.67
bergamotane	0.09
3-methyl-4,7-dioxo-oct-2-enal	0.30
2,6-dimethyl-3,5,7-octatriene-2-ol, z,z-	0.24
2-dodecenoic acid	0.12
1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-	0.35
1-methyldecahydronaphthalene	0.46
cadina-1(10),4-diene	0.34
2-(4-methylcyclohexyl)prop-2-en-1-ol	0.42
tetradec-13-enal	0.24
9-octadecenoic acid	0.15
1,2-di-but-2-enyl-cyclohexane	0.10
4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.04,6]dodecane	0.11
3,4-*o*-isopropylidene-d-galactose	0.08
2-hexenoic acid, 6-cyclohexyl-	0.22
heptadec-8-ene	0.35
octane	0.35
myristic acid	0.44
D-(-)-kinic acid	1.35
nonadecanoic acid	0.35
10-bromoundecanoic acid	0.77
stearic acid	0.23
cysteamine s-sulfate	1.27
limonene dioxide	0.23
2,6-dimethyl-4-nitro-3-phenyl-cyclohexanone	0.26
methyl palmitate	0.46
2,6-dimethyl-1,3,6-heptatriene	0.68
palmitic acid	2.65
neral	1.58
2-methyl-6-methylene-1,7-octadien-3-one	0.75
bis(3-benzyl-2,4pentanedionato)palladium(ii)	1.03
pentamethylbenzenesulfonyl chloride	6.52
Myrtenal	3.77

Table 5. Composition data on *Zanthoxylum piperitum* fruit extract (methanol extract).[27]

Constituents	Area (%)*
n,n-dimethyl-2-phenylethen-1-amine	20.61
allyl(chloromethyl)dimethylsilane	7.64
cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	1.64
3-epicycloeucalenol	1.09
2,5-furandione, 3-dodecenyl-	0.61
1-cinnamyl-3-methylindole-2-carbaldehyde	1.35
glyceryl palmitate	4.82
2-methyl-z,z-3,13-octadecadienol	0.37
pentadeca-2,3,6,9,12,13-hexaen-8-one, 2,5,5,11,11,14-hexamethyl-	0.51
6-(3,4-dimethoxy-phenyl)-8-ethoxy-1,3-dimethyl-cyclohepta[c]furan-4-one	1.04
monoolein	2.02
cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	1.56
cedrane-8,13-diol	0.12
26,27-dinorergosta-5,23-dien-3β-ol	0.18
cholest-4-en-3-one, 14-methyl-	0.07
(+)-sesamolin	0.08
5,5'-[tetrahydro-1h,3h-furo[3,4-c]furan-1,4-diylbis(oxy)]bis(2h-1,3-benzodioxole)	0.32
campesterol	0.12
stigmasta-5,22-dien-3-ol	0.06
clionasterol	0.15

* Peak area in GC-MS

Table 6. Composition data on *Zanthoxylum piperitum* fruit extract in 50% methanol [4]

Constituents	MS/MS product ions*
adenosine	136.0625, 119.0357
quinic acid	111.0454, 83.0519, 69.0382, 95.0510
phenylalanine	120.0818, 103.0562, 149.0598, 131.0486
neochlorogenic acid	163.0399, 145.0292, 135.0451, 117.0349
procyanidin b1	127.0393, 409.0886, 287.0541
chlorogenic acid	163.0398, 145.0294, 135.0449, 117.0344
procyanidin b2	127.0404, 139.0407, 409.0897, 427.0983
magnoflorine	297.1112, 265.0851, 282.0878, 58.0690
epicatechine	139.0398, 123.0450, 147.0445, 161.0603, 207.0652
3-o-feruloylquinic acid	177.0548, 145.0288, 117.0343
rutin	303.0505, 465.1005, 129.0556, 85.0310
procyanidin b4	127.0403, 409.0878, 287.0529
hyperoside	303.0494, 229.0497, 257.0430, 91.0413
isoquercetin	303.0494, 229.0484, 145.0498, 85.0310
quercetin	229.0500, 257.0422, 201.0625, 285.0370
kaempferol	287.0551, 153.0185
astragalin	287.0546, 153.0209, 85.0299, 97.0323
isorhamnetin-3-o-galactorhamnoside	317.0648, 479.1149, 129.0554
quercitrin	303.0494, 85.0316, 71.0522, 129.0552
hesperidin	303.0855, 153.0184, 465.1347, 413.1184, 195.0285
kaempferol-3-o-α-l-rhamnoside	287.0550, 129.0554, 85.0308, 71.0519
hydroxyl-sanshool	105.0705, 91.0562,139.1002
unknown1	234.1490, 182.1179, 121.0662,278.1748
unknown2	284.2216, 302.2326, 266.2114, 248.2008
unknown3	105.0713, 117.0706, 145.1012
unknown4	128.0629, 143.0863, 121.0661, 119.0867
unknown5	262.1794, 105.0710, 149.0961, 95.0508
unknown6	117.0708, 145.0654, 115.0550, 159.0803
unknown7	347.0750, 332.0515
unknown8	369.0563, 329.0842, 613.1490

* Product ions via tandem mass spectrometry

Table 7. Composition data on *Zanthoxylum piperitum* fruit oil[15,16]

	Ripe Fruit	Dried Pericarp
Constituents	**Relative Content****	**Relative Content****
Hydrocarbons		
aromadendrene	0.01	-
2-carene	0.01	trace
β-caryophyllene	0.23	0.08
α-copaene	-	trace
β-cubebene	0.02	0.01
p-cymene	trace	trace
decane	0.01	trace
β-elemene	0.02	0.01
p-ethyltoluene	0.01	trace
(*E, E*)-α-farnesene	-	0.03
germacrene D	0.23	0.12
α-humulene	0.06	0.01
isomer of farnesene	-	0.01
D-limonene	6.04	5.55
(E)-β-ocimene	0.01	0.01
p-mentha-1,4,8-triene	0.02	0.01
4-methyldecane	*-	*-
myrcene	0.92	0.83
(Z)-β-ocimene	0.02	trace
(*E*)-β-ocimene	0.01	0.01
β-phellandrene	3.64	3.35
α-pinene	0.02	0.01
β-pinene	0.01	0.01
sabinene	0.03	0.03
α-selinene	0.02	-
β-selinene	0.01	-
α-terpinene	trace	trace
γ-terpinene	-	trace
terpinolene	-	trace
toluene	0.01	-
undecane	0.08	0.01
camphene	* -	* -
α-phellandrene	* -	* -
α-calacorene	* -	* -
calamenene	* -	* -
γ-cadinene	* -	* -
δ-cadinene	* -	* -
α-muurolene	* -	* -
γ_2-cadinene	* -	* -
Alcohols		
8-acetoxylinalool	0.06	0.06
benzyl alcohol	-	trace
bisabolol	0.10	0.12
δ-cadinol	trace	0.11
(*E*)-carveol	0.01	0.01
(*Z*)-carveol	0.01	0.01
citronellol	0.28	0.05
3,7-dimethyl-1,5-octadiene-3,7-diol	0.01	0.01
elemol	0.11	0.03
endo-1-bourbonanol	0.05	0.03
β-eudesmol	0.02	0.01
geraniol	5.81	1.67
(Z)-3-hexenol	-	trace
1-hydroxylinallol	0.06	0.06
isopulegol	0.05	0.05
ledol	0.01	trace
linalool	0.44	0.15
p-mentha-(E)-2,8(9)-dienol	-	0.01
4-(1-methylethyl) benzenemethanol	0.04	0.01
1-methyl-4-(1-methylethyl) 2-cyclohexen-1-ol	0.12	0.05
2-methylpropanol	-	trace
myrtenol	0.02	0.01
piperitol	0.04	0.01
1,2-propanediol	-	0.34
spathulenol	0.03	0.01
terpinen-4-ol	0.03	0.01
1-terpineol	0.06	0.03
α-terpineol	0.01	0.05

Table 7. Composition data on *Zanthoxylum piperitum* fruit oil[15,16]

	Ripe Fruit	Dried Pericarp
Constituents	**Relative Content****	**Relative Content****
δ-terpineol	0.03	0.01
trans-2,8-p-menthadien-1-ol	* -	* -
cis-2,8-*p*-menthadien-1-ol	* -	* -
methyl chavicol	* -	* -
limonen-4-ol	* -	* -
β-caryophylenne alcohol	* -	* -
cuminalcohol	* -	* -
1(7),8-*p*-menthadien-trans-2-ol	* -	* -
nerolidol	* -	* -
Aldehydes		
citronellal	8.55	1.36
4-ethylbenzaldehyde	-	-
geranial	1.79	0.06
(*E,E*)-2,4-hexadienal	0.01	trace
neral	0.31	0.04
perillaldehyde	* -	* -
cuminaldehyde	* -	* -
phellandral	* -	* -
Esters		
cinnamyl acetate	0.02	0.01
citronellyl acetate	0.11	0.07
ethyl hexanoate	0.01	-
geranyl acetate	21.10	3.33
geranyl butyrate	0.03	0.02
isobutyl hexanoate	0.09	0.02
linalyl acetate	0.33	0.33
methyl benzoate	0.01	-
methyl cinnamate	0.56	0.16
methyl hexanoate	0.01	-
neryl acetate	0.02	-
α-terpinenyl acetate	0.12	0.12
isobutyl isovarelate	* -	* -
isobutyl caproate	* -	* -
Ketones		
cryptone		0.06
1-(3,4-dimethylphenyl) ethanone	0.03	Trace
isomer of ethylacetophenone	0.03	0.01
piperitone	0.31	0.08
valeranone	0.04	0.03
dihydrocarvone	* -	* -
carvone	* -	* -
Acids		
acetic acid	0.02	Trace
heptanoic acid	0.01	0.01
hexanoic acid		0.02
3-hexenoic acid	0.04	0.01
octanoic acid	0.01	0.01
Others		
caryophyllene oxide	0.02	0.01
1,8-cineole	0.15	-
2,5-dihydro-3-methyl furan	0.02	Trace
β-terpinen-3,4-oxide	*-	* -
trans and cis Limonen-1,2-oxide	* -	* -
terpinolene-4,8-oxide	* -	* -

* constituent identified via gas liquid chromatography, infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectroscopy.
** = Relative content; average values calculated by comparing the peak are area of each compound with that of the internal standard, which is assigned the numerical value of 1, $n = 3$
- Quantity unlisted

Table 8. Composition data on a *Zanthoxylum piperitum* whole plant oil [18]

Constituents	Composition (%)
citronellal	7.1
citronellyl acetate	-
cryptone	8.5
cuminal	6.2
geranyl acetate	15.3
limonene	18.0
linalool	-
β-myrcene	-
phellandral	5.2
β-phellandrene	-

Table 9. Composition data on *Zanthoxylum piperitum* peel extract [3,21]

***Zanthoxylum piperitum* fruit peel extract (methyl *t*-butyl ether extract) [3]**	
Constituents	**Composition (%)**
β-caryophyllene	1.1%
citronellal	1.9%
D-limonene	44.3%
β-phellandrene	24.8%
volatile terpenes	0.012 (fresh weight)
***Zanthoxylum piperitum* fruit peel extract (methanol extract) [21]**	
Constituents	**Quantity (mg)**
3-*O*-caffeoylquinic acid	24.6
4-*O*-caffeoylquinic acid	8.3
(+)-catechin	10.1
(-)-epicatechin	27.8
procyanidin B1	14.2
procyanidin B2	24.7
procyanidin B4	17.6
hyperin	27.2
quercitrin	3.7
proanthocyanidin	2.10

Table 10. Frequency (2023) and concentrations of use (2021) according to likely duration and exposure and by product category[22,23]

	Zanthoxylum Piperitum Fruit Extract		Zanthoxylum Piperitum Peel Extract	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Totals*	**183**	**0.01**	**19**	**0.0000018-0.0022**
summarized by likely duration and exposure**				
Duration of Use				
Leave-On	*159*	*0.01*	*11*	*0.0000018-0.0022*
Rinse-Off	*24*	*NR*	*8*	*0.0022*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	4	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	81[a];51[b]	0.01	4 [a]	0.0000018
Incidental Inhalation-Powder	51[b]	NR	NR	0.0000022; 0.0022[c]
Dermal Contact	174	0.01	10	0.0000018-0.0022
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	7	NR	9	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	6	NR	1	NR
Baby Products	5	NR	NR	NR
as reported by product category				
Baby Products				
Baby Lotions/Oils/Powders/Creams	2	NR		
Other Baby Products	3	NR		
Eye Makeup Preparations				
Eye Lotion	3	NR		
Other Eye Makeup Preparations	1	NR		
Hair Preparations (non-coloring)				
Hair Conditioner			2	NR
Shampoos (non-coloring)	4	NR	3	NR
Tonics, Dressings, and Other Hair Grooming Aids	3	NR		
Other Hair Preparations			4	NR
Makeup Preparations				
Face Powders			NR	0.0000022
Foundations			3	0.0022
Makeup Bases	2	NR		
Personal Cleanliness Products				
Bath Soaps and Detergents	1	NR	1	NR
Douches	2	NR		
Other Personal Cleanliness Products	3	NR		
Skin Care Preparations				
Cleansing	13	NR	1	0.0022
Face and Neck (exc shave)	45	NR	NR	0.0022 (not spray)
Body and Hand (exc shave)	6	0.01 (spray)	NR	0.0022 (not spray)
Moisturizing	72	NR	3	0.0022 (not spray)
Night	3	NR	NR	0.0000018 (spray)
Paste Masks (mud packs)	1	NR	1	NR
Skin Fresheners	3	NR	1	NR
Other Skin Care Preparations	16	NR		
Suntan Preparations				
Suntan Gels, Creams, and Liquids			NR	0.00022 (not spray)

NR – not reported
* because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses
**likely duration and exposure are derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 11. *Zanthoxylum piperitum*-derived ingredients with no reported uses[22,23]

Zanthoxylum Piperitum Oil
Zanthoxylum Piperitum Peel Water

Table 12. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Ref.
			IN VITRO			
Zanthoxylum piperitum extract (carbon dioxide extract)	0, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate	acetone	*S. typhimurium* strains TA1535, TA1537, TA98, and TA100, and *E. coli* strain WP2uvrA (tests 1 and 2). *S. typhimurium* strains TA100 and TA1537 (test 3)	Doses with and without metabolic activation (tests 1 and 2). Doses without metabolic activation (test 3). Positive controls with metabolic activation: 2-aminoanthracene and benzo[a]pyrene. Positive controls without metabolic activation: 9-aminoacridine and 4-nitroquinoline-1-oxide	No biologically relevant increases in frequency of revertant colonies for any bacterial strain, either with or without metabolic activation. Two instances of slight increase in revertants (in different tests). These findings not dose-related and were not considered biologically relevant because they were within the range of historical negative controls. Test substance classified as non-genotoxic	[2]
Zanthoxylum piperitum extract (carbon dioxide extract)	Concentrations up to 260 µg/ml and up to 640 µg/ml (without metabolic activation). Concentrations up to 320 µg/ml (with metabolic activation)	DMSO	Human lymphocytes	Mammalian cell micronucleus test (OECD TG 487).	Inhibition of the cytokinesis block proliferation index at all test conditions. No statistically- or biologically significant increase in number of micronucleated cells with or without metabolic activation. Negative and positive controls performed as expected. Test substance not clastogenic or aneugenic to human lymphocytes	[2]
Zanthoxylum piperitum essential oil	0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/ml		*S. typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2uvrA	Bacterial reverse mutation assay Doses with and without metabolic activation. Positive controls with sodium azide (100.1%), 2-nutrofluorene (98.1%), 9-aminoacridine (97.7%), 4-nitro-quinoline 1 oxide (99%), 2-amino-anthracene (99.8%), and benzo[a]pyrene (99.8%).	No mutagenic activity observed for any concentration.	[20]
Zanthoxylum piperitum essential oil	100, 200, 300 µg/ml		Chinese hamster lung cells	Mammalian chromosome aberration test (OECD TG 473) with and without metabolic activation. Positive control with metabolic activation was cyclophosphamide monohydrate (99.7%) and positive control without metabolic activation with ethyl methanesulfonate.	No chromosomal aberrations noted.	[20]
			IN VIVO			
Zanthoxylum piperitum essential oil	250, 500, 1000 mg/kg bw/d		6 male and female ICR mice/group	Bone marrow micronucleus test. Mice were administered *Zanthoxylum. piperitum* essential oil orally for 2 d. Negative controls were given corn oil and positive controls were given cyclophosphamide monohydrate. Polychromatic, normochromatic, and micronucleated polychromatic erythrocytes were counted.	No significant differences between test groups; no cytotoxic effects noted.	[20]

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