
Safety Assessment of Radish Root – Derived Ingredients as Used in Cosmetics

Status: Tentative Report for Public Comment
Release Date: January 13, 2022
Panel Meeting Date: March 7-8, 2022

All interested persons are provided 60 days from the above release date (i.e., March 14, 2022) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available for review by any interested party, and may be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 Executive Director, Dr. Bart Heldreth.

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.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Preethi Raj, Senior Scientific Analyst/Writer, CIR.

ABBREVIATIONS

ACP	acid phosphatase
AD	atopic dermatitis
ALT	alanine transaminase
ARE	antioxidant response element
ARE-Nrf2	antioxidants response elements – transcription factor Nrf2
AST	aspartate aminotransferase
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
<i>Dictionary</i>	<i>International Cosmetic Ingredient Dictionary and Handbook</i>
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPRA	direct reactivity peptide assay
EC	European Commission
ECHA	European Chemicals Agency
FDA	Food and Drug Administration
GRAS	generally recognized as safe
HaCaT	human keratinocyte cell line
HPLC	high performance liquid chromatography
HRIPT	human repeated insult patch test
IC ₅₀	half-maximal inhibitory concentration
IgE	immunoglobulin E
LD	lethal dose
LDH	lactate dehydrogenase
MIC	minimum inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide
N/A	not applicable
NR	not reported/none reported
OD	odds ratio
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate buffer solution
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 7 radish root-derived ingredients, most of which are reported to function as hair and skin conditioning agents in cosmetic products. Industry should use current good manufacturing practices to minimize impurities that could be present in botanical ingredients. The Panel reviewed the available data and concluded that these 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

This assessment reviews the safety of the following 7 radish root-derived ingredients as used in cosmetic formulations:

Lactobacillus/Radish Root Ferment Extract Filtrate
Lactobacillus/Radish Root Ferment Filtrate
Leuconostoc/Radish Root Ferment Filtrate
Leuconostoc/Radish Root Ferment Lysate Filtrate

Raphanus Sativus (Radish) Root Extract
Raphanus Sativus (Radish) Root Juice
Raphanus Sativus (Radish) Root Powder

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (WINCI; *Dictionary*), these ingredients are mostly reported to function in cosmetics as hair and skin conditioning agents (Table 1).¹ Some ingredients are also reported to function as antimicrobial agents, an anti-dandruff agent, an antifungal agent, and an antioxidant; Lactobacillus/Radish Root Ferment Filtrate is exclusively reported to function as a preservative. It should be noted that most of these other functions are not considered cosmetic functions in the United States (US), and therefore, use as such does not fall under the purview of the Expert Panel for Cosmetic Ingredient Safety (Panel).

Botanicals, such as radish root-derived ingredients, may contain hundreds of constituents. However, in this assessment, the Panel will assess the safety of each of the *Raphanus sativus*-derived ingredients as a whole, complex mixture; toxicity from single components may not predict the potential toxicity of botanical ingredients. Some of the ingredients reviewed in this safety assessment may be consumed as food, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Therefore, the primary focus in this assessment of these ingredients is to evaluate the potential for effects from topical exposures.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing 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 provided 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 are provided by the cosmetics industry, as well as by other interested parties.

The cosmetic ingredient names, according to the *Dictionary*, are written as listed above, without italics. In many of the published studies, it is not known how the substance being tested compares to the ingredient as used in cosmetics. Therefore, if it is not known whether the ingredients being discussed are cosmetic ingredients, the test substances will be identified by the standard taxonomic practice of using italics to identify genus and species (i.e., “*Lactobacillus*/radish root...”, “*Leuconostoc*/radish root...”, or “*Raphanus sativus* (radish)....” However, if it is known that the substance is a cosmetic ingredient, the International Nomenclature Committee (INC) terminology will be used (e.g., Raphanus Sativus (Radish) Root Extract).

CHEMISTRY

Definition and Plant Identification

The ingredients in this report are related as derivatives from the same species, *Raphanus sativus*. Additionally, only ingredients made from the root portion of the *Raphanus sativus* plant are being reviewed. The definitions of these radish root-derived ingredients are presented in Table 1.¹ Leuconostoc/Radish Root Ferment Lysate Filtrate and Raphanus Sativus (Radish) Root Extract have the CAS Nos. 1686112-10-6 and 84775-94-0, respectively. The other ingredients do not have CAS numbers assigned.

Raphanus sativus is a tap root from the Brassicaceae family, which has been historically cultivated in Asia and Europe.² It grows in temperate climates at altitudes between 190 and 1240 m, is 30 - 90 cm high, and has thick edible roots which have a pungent taste and are of various sizes, forms, and colors.³ Generically, the root is defined as the organ of a plant that absorbs and transports water and nutrients, lacks leaves and nodes, and is usually underground.¹

Four of these ingredients are filtrates of *Raphanus sativus* fermented with either the *Lactobacillus* or *Leuconostoc* microorganism. Both strains are gram-positive and anaerobic, occurring as non-spore forming rods and cocci, and are considered lactic acid bacteria because they consume carbohydrates to produce lactic acid.⁴ A lysate is obtained by breaking down cell outer membranes via chemical or physical processes.⁵ The filtrate ingredients in this report are made by removing the bacterial cells (alive or dead), potentially along with other molecules (like peptides), from the fermented products.⁵

Chemical Properties

Leuconostoc/Radish Root Ferment Filtrate

A supplier has indicated that 1 g of Leuconostoc/Radish Root Ferment Filtrate is specified to contain 48 – 52% solids (when observed for 1 h at 105 °C).⁶ The log K_{ow} of Leuconostoc/Radish Root Ferment Filtrate is -1.92.⁷ Additional physical and chemical properties are presented in Table 2.

Method of Manufacture

In some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture, and these are captured below. Additionally, some of the methods described are general to the processing of the radish root-derived ingredients, and it is unknown if they apply to cosmetic ingredient manufacturing.

Lactobacillus/Radish Root Ferment Extract Filtrate

Lactobacillus/Radish Root Ferment Extract Filtrate is a filtrate of the extract of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Lactobacillus*.¹

Lactobacillus/Radish Root Ferment Filtrate

Lactobacillus/Radish Root Ferment Filtrate is a filtrate of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Lactobacillus*.¹

Leuconostoc/Radish Root Ferment Filtrate

Leuconostoc/Radish Root Ferment Filtrate is a filtrate of the product obtained by the fermentation of *Raphanus sativus* roots by the microorganism, *Leuconostoc*.¹

Leuconostoc/Radish Root Ferment Lysate Filtrate

Leuconostoc/Radish Root Ferment Lysate Filtrate is a filtrate of a lysate of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Leuconostoc*.¹

Raphanus Sativus (Radish) Root Extract

Radish roots, sized 30 cm each, were made into powder by washing, cutting into ~ 3 mm pieces, being dried at 60 °C for 21 h, and then being blended and sieved with a 60 mesh sifter.⁸ The resulting powder was macerated at a 1:10 ratio at ~24 °C using 3 different solvents (hexane, ethyl acetate, and ethanol) for 8, 16, and 24 h. The resulting suspensions were filtered and evaporated at 45 °C.

One gram of powdered black *Raphanus sativus* roots was used to make an ethanolic radish root extract.⁹ Aqueous ethanol, 50 ml, 50% (v/v) was used to extract the powder on a magnetic stirrer for 120 min at room temperature, and then centrifuged at 5000 rpm for 10 min at 4 °C.

Raphanus Sativus (Radish) Root Juice

Fresh *Raphanus sativus* roots were washed well and processed in an electric blender to obtain 2 l of fresh root juice.¹⁰ The *Raphanus sativus* root juice was then filtered and concentrated in a rotary evaporator at 35 ± 5 °C under reduced pressure. The resulting material was freeze dried to obtain a semisolid mass of 40 g, 11.3% w/w, which was then dissolved in distilled water.

Raphanus Sativus (Radish) Root Powder

White radish roots were washed with water, sliced, and dried at 50 °C.¹¹ The dried slices of white radish were ground to a powder and sieved through a 40 mesh sifter. The resulting product was stored in a sealed bag and frozen at -20 °C until further extraction. In another study, peeled and unpeeled black radish roots were sliced and freeze-dried before being ground to a fine powder and sifted through a 0.5 mm mesh sieve; the powdered samples were stored in air-tight containers at 4 °C.⁹

Composition and Impurities

Leuconostoc/Radish Root Ferment Filtrate

A supplier has reported that a sample of Leuconostoc/Radish Root Ferment Filtrate, with a pH 4.0 – 6.0, comprises 48.80% water, 30.60% protein, 20.10% phenolics (tested as salicylic acid), and 0.50% polysaccharide content.¹² Specifications for this ingredient provide the following parameters: < 20 ppm heavy metals, < 10 ppm lead, < 2 ppm arsenic, and < 1 ppm cadmium.⁶ Additionally, the ingredient was specified to be positive to ninhydrin, and potentially contain 18-22% phenolics (tested as salicylic acid), and 0.10 - 0.50% bacteriocins (quantified via high-performance liquid chromatography).

Raphanus Sativus (Radish) Root Extract

In one study, a 16-h, crude ethyl acetate *Raphanus sativus* root extract contained the highest total phenolic and flavonoid content at 37.37 mg gallic acid equivalents (GAE)/g, and 5.74 mg quercetin equivalents (QE)/g, respectively.⁸ A compositional analysis of fresh radish root extracts yielded a flavonoid content of 267.47 ± 6.38 mg quercetin/100 g, total

phenolic content of 371.59 mg/100 g, and 380 ± 0.87 g/100g potassium (highest mineral content).¹³ Silica gel chromatography of a dichloromethane extract of *Raphanus sativus* roots yielded the following constituents: 3-(E)-(methylthio)methylene-2-pyrrolidinethione, a mixture of 4-methylthio-3-butenyl isothiocyanate and 4-(methylthio)butyl isothiocyanate, β -sitosterol, β -sitosteryl-3 β -glucopyranoside-6'-O-palmitate, monoacylglycerols, and a mixture of α -linolenic acid and linoleic acid.¹⁴ A methanolic extract of *Daikon* (vegetable; a *Raphanus sativus* var.) was the most constituent-rich, compared to extracts made with water, petrolatum, ethanol, and chloroform; phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and glycosides.¹⁵

High performance liquid chromatography (HPLC) analyses were used to compare glucosinolate, anthocyanin, and total isothiocyanate concentrations in 8 varieties of radish sprouts and 8-wk old radish tap roots.¹⁶ No anthocyanins were found in the mature tap roots; glucosinolate and isothiocyanate concentrations were significantly greater in the sprouts than in the mature tap roots.

Varying amino acid compositions were observed in anionic and cationic isoperoxidases isolated from crude *Raphanus sativus* enzyme extracts.¹⁷ In another amino acid sequence analysis, 3 isoferredoxin isoproteins were purified from white radish roots, while 2 isoferredoxin isoproteins were obtained from the leaves.¹⁸ Although the amino acid sequence of the root and leaf-derived isoferredoxin isoproteins differed, no significant physiological differences in the coupling activities of these ferredoxin isoproteins were measured in the NADP⁺-photoreduction system of radish chloroplasts and glutamate synthase.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2021 VCRP survey data, *Leuconostoc*/Radish Root Ferment Filtrate is reported to be used in 255 formulations, 104 of which are leave-on moisturizing products; *Lactobacillus*/Radish Root Ferment Filtrate and *Raphanus Sativus* (Radish) Root Extract are reported to have 2 uses each, in moisturizing and face and neck products, respectively (Table 3).¹⁹ The results of the concentration of use survey conducted by the Council indicate that *Raphanus Sativus* (Radish) Root Extract has the highest reported maximum concentration of use in leave-on products, at up to 6% in lipstick; *Leuconostoc*/Radish Root Ferment Filtrate is used at up to 1.1% in skin cleansing products.²⁰ The highest concentration of use reported for products resulting in leave-on dermal exposure is 0.03% *Leuconostoc*/Radish Root Ferment Filtrate in face and neck spray formulations. Use concentration data were not reported for *Lactobacillus*/Radish Root Ferment Filtrate, but uses were reported in the VCRP. *Lactobacillus*/Radish Root Ferment Extract Filtrate, *Leuconostoc*/Radish Root Ferment Lysate Filtrate, *Raphanus Sativus* (Radish) Root Juice, and *Raphanus Sativus* (Radish) Root Powder are not reported to be in use in the VCRP or industry survey (Table 4).

Radish root- derived ingredients have been reported to be used in products that may lead to incidental ingestion and exposure to mucous membranes; for example, *Raphanus Sativus* (Radish) Root Extract is reported to be used in a lipstick at up to 6%,²⁰ and *Leuconostoc*/Radish Root Ferment Filtrate is reported to be used at up to 0.01% in other eye makeup preparations.²⁰ Additionally, *Leuconostoc*/Radish Root Ferment Filtrate is reported to be used in products that could be potentially inhaled, e.g., *Leuconostoc*/Radish Root Ferment Filtrate is used in spray face and neck products at up to 0.03%.²⁰ In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μ m, with propellant sprays yielding a greater fraction of droplets/particles < 10 μ m compared with pump sprays.^{21,22} Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.^{23,24}

All of the radish root-derived ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.²⁵

Non-Cosmetic

According to the US FDA, commercially-produced products of carbohydrates, such as glucose, sucrose, or lactose, which undergo lactic acid fermentation, are generally recognized as safe (GRAS) for their intended use in foods [21CFR § 184.1016]. *Leuconostoc* is an approved bacterial strain used to produce a butter starter distillate [21CFR § 184.1848].

Furthermore, *Raphanus sativus* roots are consumed as cruciferous vegetables worldwide, both raw and cooked, in pickles, salads, and curries.²⁶ Of note, *Raphanus sativus* fermented with *Lactobacillus* strains is consumed as a non-salted dish called Sinki in South Asia.²⁷ The Korean dish, kimchi, comprises variations of a mixed vegetable brine fermentation (achieved with lactic acid bacteria, such as *Lactobacillus* or *Leuconostoc*), and often includes radish roots.²⁸ Generally, *Lactobacillus* and *Leuconostoc* strains are used in the lactic acid fermentation of dairy, sauerkraut, and various food products.^{29,30}

TOXICOKINETIC STUDIES

No relevant toxicokinetics studies on radish root-derived ingredients were found in the published literature, and unpublished data were not submitted. In general, toxicokinetics data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Subchronic Toxicity Studies

Oral

Raphanus Sativus (Radish) Root Extract

Groups of albino rats were dosed with 0, 150, 250, 350, 450, or 550 mg/kg bw of methanolic *Daikon* (vegetable; a *Raphanus sativus* var.) extract, in the diet, for 90 d.¹⁵ Body weight, as well as various hematological parameters and enzymes, including red blood cell count, hemoglobin, white blood cell count, aspartate aminotransferase (AST), alanine transaminase (ALT), acid phosphatase (ACP), urea, uric acid, and protein were measured and compared at 30 and 90 d of treatment. Upon sacrifice, heart, kidney, liver, spleen, and brain weights were also measured, and those of treated animals were compared to controls. No statistically significant differences were observed between the mean body weights, organ weights, and measured hematological parameters in treated animals, compared to controls, throughout the experiment.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity studies were not found in the published literature, and unpublished data were not submitted.

GENOTOXICITY STUDIES

Leuconostoc/Radish Root Ferment Filtrate

The genotoxicity potential of Leuconostoc/Radish Root Ferment Filtrate was evaluated in a bacterial reverse mutation assay (Ames test) at concentrations of 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate, in distilled water, using the following strains: *Salmonella typhimurium* TA98, TA100, TA1535, TA 1537, and *Escherichia coli* WP2 *uvrA*.³¹ Distilled water served as the negative control and appropriate positive controls were used. The test substance did not induce a mutagenic effect in the presence or absence of metabolic activation.

Raphanus Sativus (Radish) Root Juice

In a Comet assay, the genotoxic potential of radish juice made from white, red, and large red *Raphanus sativus* tubers, as well as dichloromethane extracts of hydrolyzed *Raphanus sativus* white and cherry belle, red tubers, was tested in breast adenocarcinoma (MCF-7), chronic myelogenous leukemia (K562), and colorectal cancer (HT-29) cell lines.³² Each cell line was incubated with 500 µl of the root juice and 50 µg/ml of the dichloromethane juice extract; porcine aortic endothelial (PAE) cell lines were used as the negative control and immortalized cell lines exposed to 0.01% hydrogen peroxide for 20 min were used as positive controls. Tail length, percent deoxyribonucleic acid (DNA), and tail moment measurements were used to evaluate the extent of DNA damage. Juices from all 3 tubers exhibited significantly lower DNA damage in the porcine aortic endothelial cells, compared to positive controls. The breast adenocarcinoma cell line, MCF-7, showed the greatest amount of genetic fragmentation among all cancer cells, and the white tuber root juice was the most genotoxic towards aberrant cell lines.

CARCINOGENICITY STUDIES

Carcinogenicity studies were not found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Antioxidant and Radical Scavenging Potential

Raphanus Sativus (Radish) Root Extract and Raphanus Sativus (Radish) Root Juice

A freeze-dried juice and methanolic extract of white *Raphanus sativus* roots were evaluated for tyrosinase inhibition, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, cytotoxicity, and L-ascorbic acid content.³³ The ability of *Raphanus sativus* root extract and root juice to scavenge DPPH, superoxide anion, and singlet oxygen was measured in triplicate and used to calculate average half-maximal inhibitory concentration (IC₅₀) values, compared to L-ascorbic acid and Trolox®, a water soluble analog of Vitamin E. Various concentrations of the *Raphanus sativus* root extract and root juice in 20% v/v propylene glycol (in water) were tested, in tandem with L-ascorbic acid and licorice extract as reference tyrosinase inhibitors, using the DOPACHrome method. Lactate dehydrogenase (LDH) activity in fibroblasts treated with the root extract and root juice, compared to L-ascorbic acid and sinapic acid, was used to measure cytotoxic activity. Five replicates of the root extract and root juice were titrated with 0.1 N iodine to determine the L-ascorbic acid or vitamin C content. The freeze-dried juice showed higher potential for tyrosinase inhibition compared to the methanolic extract (IC₅₀ = 3.09 mg/ml vs. IC₅₀ = 9.62

mg/ml). The radical scavenging activity of the freeze-dried juice on DPPH radical, superoxide anion radical, and singlet oxygen were also greater compared to the methanolic extract ($IC_{50} = 0.64, 4.20, 1.42$ mg/ml vs. $IC_{50} = 1.25, 6.28, 2.40$ mg/ml). Although a dose-dependent release of LDH was observed for both the root extract and root juice, the observed cytotoxicity was relatively lower than in the reference antioxidants. The authors surmised that the higher L-ascorbic content of 1 mg of freeze-dried *Raphanus sativus* root juice compared to the root extract (24.11 μ g vs. 8.28 μ g), as well as higher phenolic content, may be responsible for greater anti-tyrosinase and radical scavenging activity, possibly leading to skin lightening.

Raphanus Sativus (Radish) Root Extract

Raphanus sativus radish root extracts were eluted using 3 solvents with varying polarities (hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar)) for 8, 16, and 24 h each, to determine which extract would have the highest phenolic or flavonoid content.⁸ The radish root extract extracted with ethyl acetate for 16 h was found to have the highest flavonoid content, and was used for further testing. The ethyl acetate radish root extract was tested for phenolic and flavonoid content stability based on changes in pH (4, 5, 6, and 7) and heating temperature (70, 80, 90 °C). In conjunction, the IC_{50} value of the ethyl acetate root extract was measured in a DPPH assay. Overall, decreases in total phenolic and flavonoid content, as well as antioxidant activity, were observed when the radish root extract was exposed to increasing heat and pH. Statistically significant interactions between change in pH and heating temperature with antioxidant activity were observed. The radish root extract with a pH of 4 at a temperature of 70° C had an IC_{50} value (1071.93 \pm 45.71 mg/l) closest to that of the control extract (770.78 \pm 99.91 mg/l) which was not exposed to pH or temperature changes).

Antimicrobial Activity

Leuconostoc/Radish Root Ferment Filtrate

According to specifications provided by a supplier, a sample of *Leuconostoc/Radish Root Ferment Filtrate* is expected to have a minimum inhibitory concentration (MIC) of 1- 4% against *Pseudomonas aeruginosa* , 0.50 – 4% against *Escherichia coli*, and 0.25 – 2% against *Aspergillus brasiliensis*, *Candida albicans*, and *Staphylococcus aeruginosa*.⁶

Raphanus Sativus (Radish) Root Juice

The antimicrobial potential of *Raphanus sativus* root juice was compared to that of ampicillin in strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*.¹⁰ Upon incubation with 0.078 - 2.5 mg/ml of the root juice for 24 h, the highest MIC values were against *P. aeruginosa* at 0.625 ± 0.4 mg/ml and *S.aureus* at 0.312 ± 0.2 mg/ml (significantly greater than the corresponding ampicillin MIC values of 0.156 ± 0.8 mg/ml and 0.156 ± 0.07 mg/ml) and the *Raphanus sativus* root juice MIC values against *E.coli* and *E. faecalis* were equivalent to ampicillin MIC values.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The dermal irritancy of *Leuconostoc/Radish Root Ferment Filtrate* was evaluated in a reconstructed three-dimensional human epidermis model (EpiDerm™).³⁴ A single application of 30 μ l of the test article was applied to the epidermis model (3 tissue samples). Sterile phosphate-buffered saline (PBS) was used as the negative control and chlorpromazine, ranging from concentrations of 0.001-0.1%, were used as positive controls. The tissues were washed with sterile PBS 1 h after the application, post-incubated under normal medium and culture conditions for 2 h, and then measured for cell viability via a 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) assay. Under the conditions of this study, the test article was considered non-irritating.

Sensitization

In Chemico/In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The sensitization potential of *Leuconostoc/Radish Root Ferment Filtrate* (in acetonitrile) was evaluated in a direct peptide reactivity assay (DPRA), in accordance with Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 442C.³⁵ This assay is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of test chemicals towards the model synthetic peptides containing cysteine and lysine. The mean percent depletion of cysteine and lysine was 2.89%, which was interpreted as minimal reactivity in the assay, with a prediction of no sensitization.

Leuconostoc/Radish Root Ferment Filtrate was also evaluated for sensitization potential in an antioxidants response elements - transcription factor Nrf2 (ARE-Nrf2) luciferase assay utilizing the KeratinoSens™ cell line, in accordance with OECD TG 442D.³⁶ In this assay, transfection with the luciferase gene allows for measurement of the activation of the Keap1-Nrf2-ARE complex, a proxy for sensitization. Fifty μ l, each, of 12 concentrations of the test article prepared in DMSO

(ranging from 0.98 – 2000 µM) were added to human keratinocyte (HaCaT) cell lines seeded for 24 h, as per the KeratinoSens™ method, and were incubated for 48 h. No significant increases in luciferase expression were observed; the test article was predicted to be a non-sensitizer.

Human

Leuconostoc/Radish Root Ferment Filtrate

In a human repeated insult patch test (HRIPT), Leuconostoc/Radish Root Ferment Filtrate, as a 10% dilution in water, was tested in 50 subjects.³⁷ The test material was applied to the back via 9, occlusive, 24- h induction applications (0.2 g applied to an unspecified area), made over a 3-wk induction period. Induction sites were scored 24 or 48 h after patch removal. After a 2-wk non-treatment period, a 24-h challenge application was made to a previously untreated site in the same manner as the induction applications, and the reactions were scored on a scale of 0 - 4 at 24 and 48 h after application. No adverse reactions occurred during the course of the study; the researchers concluded that the test material was not an irritant or sensitizer.

An eyebrow gel formulation containing 0.04% Leuconostoc/Radish Root Ferment Filtrate was tested neat in an HRIPT in 105 subjects.³⁸ The test material was applied to the back via 9, occlusive, 24- h induction applications (0.2 g applied to approximately 0.75 in²), made over a 3-wk induction period. Induction sites were scored 24 or 48 h after patch removal. After a 2-wk non-treatment period, a 24-h challenge application was made to a previously untreated site in the same manner as the induction applications, and the reactions were scored on a scale of 0 - 4, at 24 and 72 h after application. No signs of irritation or sensitization were observed during induction or challenge; the researchers concluded that the test material did not cause dermal sensitization.

Phototoxicity

In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The phototoxicity of Leuconostoc/Radish Root Ferment Filtrate was tested using a reconstructed three-dimensional human epidermis model (EpiDerm™).³⁹ Five concentrations of the test article 0, 0.4%, 1.2%, 3.7%, and 11%, diluted in Dulbecco's modified Eagle medium were used. Sterile deionized water and 0.001- 0.1% chloropromazine were used as negative and positive controls, respectively. After the EpiDerm™ model was incubated in growth media for 1 h, 50 µl of each test article concentration was applied to tissue inserts and allowed to incubate overnight at 37 °C. The tissue inserts were either irradiated with 6 J/cm² UVA (ultraviolet), or incubated without irradiation, for 1 h at room temperature and were tested in an MTT assay. As per the definition of a potential photoirritant reducing cell viability by ≥ 20%, when comparing irradiated to non-irradiated controls, significant reduction was only seen in the 11% concentration (significantly higher than use levels in cosmetics), with and without radiation (51.1% and 72.6%, respectively). Leuconostoc/Radish Root Ferment Filtrate was therefore not considered a photoirritant at the 0.4, 1.2, or 3.7% concentrations.

OCULAR IRRITATION STUDIES

In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The ocular irritation potential of Leuconostoc/ Radish Root Ferment Filtrate to cause eye irritation was evaluated in a reconstructed human cornea-like epithelium test, using an EpiOcular™ three-dimensional human cornea model.³⁴ Fifty µl of the undiluted test article were applied to 2 tissue samples. The treated tissues were incubated for 90 min, washed out with PBS, post-incubated under normal medium and culture conditions for 2 h, and then measured for cell viability via an MTT assay. The negative control tissues received applications of de-ionized water. The test article was considered to be non-irritating.

CLINICAL STUDIES

Occupational Exposures

A 46-yr-old kitchen porter, with metal allergy and no prior food allergies, presented to the emergency room with dizziness, generalized eruptions on the skin, and gastrointestinal upset.⁴⁰ During recent employment in a Korean kitchen, she had been exposed to *Raphanus sativus* roots while chopping fresh young radish, 1 and 3 d prior to her hospital treatment. Upon initial exposure, she experienced immediate urticaria with pruritus and burning sensation (which spontaneously disappeared); however, upon second exposure, the pruritus presented more severely with generalized erythematous eruption and dizziness. Systemic anaphylactic symptoms manifested within 12 h. Upon hospital admission, total serum immunoglobulin E (IgE) level was measured at 30 IU/l; she received subcutaneous epinephrine (0.3 ml) followed by intravenous saline and antihistamine. Three wk post-recovery, she tested positive to a skin prick test with young radish extract; 5 controls tested with a skin prick test using young radish extract and 55 common allergens did not exhibit positive reactions. The allergic reaction was attributed to biphasic, IgE-mediated anaphylaxis to physical contact with young radish.

A 38-yr-old waitress, with no prior history of dermatological illness, developed an acute vesiculo-bullous dermatitis of both palms, 3 wk after chopping tomatoes, cabbage, endive, and radishes for the salad bar.⁴¹ She sought medical attention 2 wk after the dermatitis appeared; findings were normal, with the exception of the sides of her fingers, which were more severely affected. Patch tests were performed with the neat application of *Raphanus sativus* root juice, cabbage leaf, tomato fruit, and endive leaf. Additionally, patch tests were performed with 0.1% allyl isothiocyanate, 0.1% benzyl isothiocyanate, 0.05% phenyl isothiocyanate, 1% sinigrin, and 1% myrosinase (all in petrolatum). Samples of the thioglucoside, sinigrin, which yields allyl isothiocyanate, and of the enzyme, myrosinase, were mixed together and either applied to the skin immediately after mixture or 1 wk later; a positive reaction to the previously mixed test article was observed. Positive reactions to allyl isothiocyanate, and benzyl isothiocyanate were also observed. There was no reaction to freshly mixed sinigrin and myrosinase. No further details were provided.

SUMMARY

This assessment reviews the safety of the following 7 radish root-derived ingredients. According to the *Dictionary*, various functions are reported for these ingredients, with hair and skin conditioning agents being the most common. Most of the other reported functions are not considered cosmetic in the US, and therefore, use as such does not fall under the purview of the Panel. Commercially-produced products of carbohydrates, such as glucose, sucrose, or lactose, which undergo lactic acid fermentation (fermentation organism not identified), are GRAS for their intended use in foods; *Leuconostoc* is an approved strain used as a butter starter distillate. *Leuconostoc*/Radish Root Ferment Filtrate is reported to have the greatest frequency of use, in 255 formulations, 104 of which are in leave-on moisturizing products. The highest reported concentration of use amongst these ingredients is for *Raphanus Sativus* (Radish) Root Extract, at up to 6% in lipstick formulations. *Raphanus sativus* roots are widely consumed in raw, cooked, and fermented forms; in the US, foods that are commercially produced using lactic acid fermentation are considered to have GRAS status.

Groups of albino rats were administered up to 550 mg/kg bw/d of methanolic *Daikon* (vegetable) extract, in the diet, for 90 d. Throughout the course of the experiment, no statistically significant differences were seen between controls and treated animals for mean body weights, organ weights, and hematological parameters such as red blood cell count, hemoglobin, white blood cell count, AST, ALT, ACP, urea, uric acid, and protein levels.

Leuconostoc/Radish Root Ferment Filtrate was not genotoxic when tested at concentrations up to 5000 µg/plate in an Ames test. In a study evaluating the genotoxic potential of several *Raphanus sativus* root juices against cancerous cell lines, 500 µl of the white tuber root juice caused the most DNA damage in all aberrant cell lines; the breast cancer adenoma cell line was the most highly affected.

Raphanus sativus root juice exhibited a higher potential for tyrosinase inhibition ($IC_{50} = 3.09$ mg/ml vs. 9.62 mg/ml), radical scavenging, and had a higher content of L-ascorbic acid than a methanolic *Raphanus sativus* root extract. In another study, ethyl acetate *Raphanus sativus* root extract exposed to pH and temperature changes exhibited an IC_{50} value that was closest to an unexposed control extract at a pH of 4 and temperature of 70 °C. A sample of *Leuconostoc*/Radish Root Ferment Filtrate exhibited MIC values of 1 - 4% against *P. aeruginosa*, 0.50 - 4% against *E. coli*, and 0.25 - 2% against *A. brasiliensis*, *C. albicans*, and *S. aeruginosa*. The highest MIC values for a *Raphanus sativus* root juice, which were greater than the corresponding ampicillin MIC values, were against *P. aeruginosa* and *S. aureus* at 0.625 ± 0.4 mg/ml and 0.312 ± 0.2 mg/ml, respectively.

A single 30 µl application of *Leuconostoc*/Radish Root Ferment Filtrate did not cause irritation in a triplicate series of EpiDerm™ model epidermis tests. In a DPRA assay testing the sensitizing potential of 100 mM *Leuconostoc* Ferment Filtrate, the mean percent depletion for cysteine and lysine was 2.89%; the test article was predicted to be a non-sensitizer. *Leuconostoc*/Radish Root Ferment Filtrate, tested at concentrations of up to 2000 µM in DMSO (50 µl), was found to be non-sensitizing in an ARE-Nrf2 luciferase assay. *Leuconostoc*/Radish Root Ferment Filtrate, as a 10% dilution in water, did not cause sensitization in an occlusive HRIPT using 50 subjects. An eyebrow gel formulation containing 0.04% *Leuconostoc*/Radish Root Ferment Filtrate also was found to be non-sensitizing in an occlusive HRIPT using 105 subjects.

Leuconostoc/Radish Root Ferment Filtrate was tested at 0, 0.4, 1.2, 3.7, and 11% (in Dulbecco's modified Eagle medium) for phototoxicity in an irradiated EpiDerm™ reconstructed epidermis model. Significant reduction in cell viability (≥ 20 %) when compared to non-irradiated controls, was seen at the 11% concentration, both with and without radiation; the test article was not considered a photoirritant. *Leuconostoc*/Radish Root Ferment Filtrate was not considered an ocular irritant when tested in 2 EpiOcular™ human cornea-like epithelium tissue samples.

A 46-yr-old female kitchen porter, with pre-existing metal allergy, presented to the emergency room with dizziness, generalized eruptions on the skin, and gastrointestinal upset after chopping fresh young radish 1 and 3 d prior to hospitalization. Systemic anaphylactic symptoms manifested within 12 h. Three wk post-recovery the subject tested positive to a skin prick test with young radish extract, which was attributed to biphasic, IgE-mediated anaphylaxis upon physical contact. A 38-yr-old female waitress developed an acute vesiculo-bullous dermatitis of both palms 3 wk after chopping tomatoes, cabbage, endive, and radishes for the salad bar. Patch tests were performed with the neat application of all plant substances, plus, 0.1% each of allyl isothiocyanate, benzyl isothiocyanate, sinigrin, myrosinase, and 1% sinigrin, either mixed with 1% myrosinase 1 wk prior to application, or mixed with 1% myrosinase immediately prior to application. Positive

reactions were observed for *Raphanus sativus* root juice, allyl isothiocyanate, benzyl isothiocyanate, and to the sinigrin previously mixed with myrosinase.

DISCUSSION

The Panel reviewed the safety of 7 ingredients obtained from radish roots, all of which are derived from the *Raphanus sativus* species. The Panel concluded that the available data are sufficient for determining the safety of all 7 ingredients, as reportedly used in cosmetics. The Panel noted that the radish root-derived ingredients that are included in this safety assessment are consumed regularly as derivatives of food, and, therefore, these food exposures would likely result in much larger systemic exposure compared to that resulting from use in cosmetic products. Likewise, the fermentation of a few of these ingredients with lactic acid bacteria, including *Lactobacillus* and *Leuconostoc* strains, which have GRAS status, was not concerning to the Panel. The Panel discussed that although data from a wide variety of radishes is included in this report (i.e., various colors, sizes, etc.), most of these radishes are indicated to be consumed as food, mitigating any concerns for systemic toxicity. Additionally, the potential for systemic exposure from the absorption of these ingredients through the skin is expected to be much less than the potential for systemic exposure from absorption through oral exposures. These considerations, coupled with low reported use concentrations and negative findings in human dermal irritation and sensitization studies, led the Panel to determine that the radish root-derived ingredients are safe as used in cosmetic products.

An in vitro study investigated the potential for a freeze-dried juice and methanolic extract of white *Raphanus sativus* root to have an inhibitory effect on tyrosinase activity, which can be associated with skin-lightening. Upon review of the paper by the Panel, it was noted that very low potency for inhibiting tyrosinase was actually demonstrated in the study. Nevertheless, the Panel stated that skin lightening is considered to be a drug effect and should not occur during the use of cosmetic products.

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

Some radish root-derived ingredients were reported to be used in spray products that could possibly be inhaled. For example, *Leuconostoc*/Radish Root Ferment Filtrate is reported to be used at up to 0.03% in spray face and neck products. Inhalation toxicity data were not available; the Panel reiterated that radish root-derived ingredients are used as foods, mitigating concerns of systemic toxicity. Also, the Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns for these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at <https://www.cir-safety.org/cir-findings>.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 7 radish root-derived ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing:

Lactobacillus/Radish Root Ferment Extract Filtrate*	Raphanus Sativus (Radish) Root Extract
Lactobacillus/Radish Root Ferment Filtrate	Raphanus Sativus (Radish) Root Juice*
Leuconostoc/Radish Root Ferment Filtrate	Raphanus Sativus (Radish) Root Powder*
Leuconostoc/Radish Root Ferment Lysate Filtrate*	

**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 product categories and at concentrations comparable to others in this group.*

TABLES

Table 1. INCI names, definitions, and functions of *Raphanus sativus* (root)-derived ingredients in this safety assessment¹

Ingredient/ CAS Number	Definition	Function(s)
Lactobacillus/Radish Root Ferment Extract Filtrate	is a filtrate of the extract of the product obtained by the fermentation of the roots of <i>Raphanus sativus</i> (radish) by the microorganism, <i>Lactobacillus</i> .	Preservative
Lactobacillus/Radish Root Ferment Filtrate	is a filtrate of the product obtained by the fermentation of the roots of <i>Raphanus sativus</i> (radish) by the microorganism, <i>Lactobacillus</i> .	Antimicrobial agent; hair conditioning agent; skin- conditioning agent - miscellaneous
Leuconostoc/Radish Root Ferment Filtrate	is a filtrate of the product obtained by the fermentation of <i>Raphanus sativus</i> roots by the microorganism, <i>Leuconostoc</i> .	Anti-dandruff agent; antifungal agent; antimicrobial agent; hair conditioning agent; skin-conditioning agent - miscellaneous
Leuconostoc/Radish Root Ferment Lysate Filtrate 1686112-10-6	is a filtrate of a lysate of the product obtained by the fermentation of the roots of <i>Raphanus sativus</i> (radish) by the microorganism, <i>Leuconostoc</i> .	Hair conditioning agent; skin-conditioning agent- miscellaneous
Raphanus Sativus (Radish) Root Extract 84775-94-0 (generic)	is the extract of the roots of <i>Raphanus sativus</i> .	Antioxidant; skin-conditioning agents - miscellaneous
Raphanus Sativus (Radish) Root Juice	is the juice expressed from the roots of <i>Raphanus sativus</i> .	Skin-conditioning agents - miscellaneous
Raphanus Sativus (Radish) Root Powder	is the powder obtained from the dried, ground roots of <i>Raphanus sativus</i> .	Skin – conditioning agents – emollient; skin – conditioning agents – humectant

Table 2. Chemical properties of Leuconostoc/Radish Root Ferment Filtrate

Property	Value	Reference
Leuconostoc/Radish Root Ferment Filtrate		
Physical Form	Clear to slightly hazy liquid	6
Color	Yellow to light amber	6
Odor	Characteristic	6
Specific Gravity (@ 25 °C)	1.140 – 1.180	6
pH	4.0 - 6.0	6
log K _{OW} ; K _{OW}	-1.92; 0.013	7

Table 3. Frequency (2021)¹⁹ and concentration (2020)²⁰ of use according to duration and exposure

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Lactobacillus/Radish Root Ferment Filtrate		Leuconostoc/Radish Root Ferment Filtrate		Raphanus Sativus (Radish) Root Extract	
Totals*	2	NR	255	0.0001 – 1.1	2	6
Duration of Use						
<i>Leave-On</i>	2	NR	185	0.0001 - 0.03	2	6
<i>Rinse-Off</i>	NR	NR	70	0.0001 – 1.1	NR	NR
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	NR	NR	3	0-0.002 – 0.01	NR	NR
Incidental Ingestion	NR	NR	9	0.0002	NR	6
Incidental Inhalation-Spray	2 ^a	NR	5; 111 ^a ; 40 ^b	0.0001-0.03; 0.001 ^a	NR	NR
Incidental Inhalation-Powder	NR	NR	40 ^b ; 1 ^c	0.0002 - 0.002 ^c	2 ^b	NR
Dermal Contact	2	NR	220	0.0002 – 1.1	2	NR
Deodorant (underarm)	NR	NR	1 ^a	NR	NR	NR
Hair - Non-Coloring	NR	NR	26	0.0001 – 0.002	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.0022 – 0.01	NR	NR
Mucous Membrane	NR	NR	39	0.0002	NR	6
Baby Products	NR	NR	1	NR	NR	NR

*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.

^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

NR – not reported

Table 4. Raphanus sativus – derived ingredients not reported to be in use^{19,20}

Lactobacillus/Radish Root Ferment Extract Filtrate
Leuconostoc/Radish Root Ferment Lysate Filtrate
Raphanus Sativus (Radish) Root Juice
Raphanus Sativus (Root) Powder

REFERENCES

1. Nikitakis J., Kowcz A. Web-based International Cosmetic Ingredient Dictionary and Handbook (wINCI Dictionary). <http://webdictionary.personalcarecouncil.org/jsp/IngredientSearchPage.jsp>. Last Updated 2021. Accessed October 20, 2021.
2. Sabishruthi S, K Rajan A, Sai C, Arshath A, Benita S. A disquisition on *Raphanus sativus* Linn- a propitious medicinal plant. *Int J Chemtech Res.* 2018;11:48-55.
3. Gutiérrez R, Perez R. *Raphanus sativus* (Radish): Their Chemistry and Biology. *ScientificWorldJournal.* 2004;4:811-837.
4. Adams M, Moss M. *Food Microbiology*. 3rd edition ed. Cambridge, United Kingdom: Royal Society of Chemistry; 2008.
5. Puebla-Barragan S, Reid G. Probiotics in cosmetic and personal care products: Trends and challenges. *Molecules.* 2021;26(5):1249.
6. Active Micro Technologies. 2020. Specifications Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).
7. Active Micro Technologies. 2017. K_{ow} statement Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).
8. Eveline E, Pasau R. Antioxidant activity and stability of radish bulbs (*Raphanus sativus* L.) crude extract. *IOP Conference Series: Earth and Environmental Science.* 2019;292:012036.
9. Enkhtuya E, Tsend M. The effect of peeling on antioxidant capacity of Black Radish Root. *Ital J Food Sci.* 2020;32:701-711.
10. Shukla S, Chatterji S, Yadav DK, Watal G. Antimicrobial efficacy of *Raphanus sativus* root juice. *Int J Pharm Pharm Sci.* 2011;3:89-92.
11. Duy H, Ngoc P, Anh L, Dong D, Nguyen DC, Than VT. In vitro antifungal efficacy of white radish (*Raphanus sativus* L.) root extract and application as a natural preservative in sponge cake. *Processes.* 2019;7:549.
12. Active Micro Technologies. 2021. Composition Leucidal® Liquid (Leuconotoc/Radish Root Ferment Filtrate).
13. Goyeneche R, Roura S, Ponce AG, et al. Chemical characterization and antioxidant capacity of red radish (*Raphanus sativus* L.) leaves and roots. *J Func Foods.* 2015;16:256-264.
14. Ragasa C, Ebajo Jr V, Tan MC, Brkljača R, Urban S. Chemical constituents of *Raphanus sativus*. *Der Pharma Chemica.* 2015;7:354-357.
15. Baranidharan B, Shamina S. Subacute toxicity study of Daikon (vegetable) extract on albino rats. *World J Pharm Res.* 2018;7(6):725-731.
16. Hanlon PR, Barnes DM. Phytochemical composition and biological activity of 8 varieties of radish (*Raphanus sativus* L.) sprouts and mature taproots. *J Food Sci.* 2011;76(1):C185-192.
17. Lee MY, Kim SS. Characteristics of six isoperoxidases from Korean radish root. *Phytochemistry.* 1994;35(2):287-290.
18. Wada K, Onda M, Matsubara H. Amino acid sequences of ferredoxin isoproteins from radish roots. *J Biochem.* 1989;105(4):619-625.
19. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2021. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients.
20. Personal Care Products Council. 2020. Concentration of Use by FDA Product Category: Leuconostoc/Radish Root Ferment Filtrate and Related Ingredients.
21. Johnsen M. The influence of particle size. *Spray Technol Marketing.* 2004;14(11):24-27.
22. Rothe H. 2011 2011. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment.

23. Bremmer HJ, Prud'homme de Lodder L, van Engelen J. Cosmetics Fact Sheet: To assess the risks for the consumer, Updated version for ConsExpo4. 2006. Pages 1-77. <http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf>. Accessed June 25, 2019.
24. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.
25. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. <http://ec.europa.eu/growth/tools-databases/cosing/>. Last Updated 2020. Accessed 04/21/2021.
26. Manivannan A, Kim J-H, Kim D-S, Lee E-S, Lee H-E. Deciphering the nutraceutical potential of *Raphanus sativus*-A comprehensive overview. *Nutrients*. 2019;11(2):402.
27. Tamang J, Sarkar P. Sinki: A traditional lactic acid fermented radish tap root product. *J Gen Appl Microbiology*. 1993;39:395-408.
28. Patra JK, Das G, Paramithiotis S, Shin H-S. Kimchi and other widely consumed traditional fermented foods of Korea: a review. *Front Microbiol*. 2016;7(1493).
29. Vedomuthu ER. The dairy *Leuconostoc*: Use in dairy products. *J Dairy Sci*. 1994;77(9):2725-2737.
30. Ashaolu TJ, Reale A. A holistic review on Euro-Asian Lactic acid bacteria fermented cereals and vegetables. *Microorganisms*. 2020;8(8).
31. Active Micro Technologies. 2018. Bacterial reverse mutation test Leucidal® Liquid (*Leuconostoc*/Radish Root Ferment Filtrate).
32. Tan MCS, Enriquez MLD, Arcilla RG, Noel MG. Determining the apoptotic-inducing property of isothiocyanates extracted from three cultivars of *Raphanus sativus* Linn. Using the comet assay. *J Appl Pharm Sci*. 2017;7(09):044-051.
33. Jakmatakul R, Suttisri R, Tengamnuay P. Evaluation of antityrosinase and antioxidant activities of *Raphanus sativus* root: Comparison between freeze-dried juice and methanolic extract. *Thai J Pharm Sci*. 2009;33:22-30.
34. Active Micro Technologies. 2017. Dermal and ocular irritation tests Leucidal® Liquid (*Leuconostoc*/Radish Root Ferment Filtrate).
35. Active Micro Technologies. 2017. OECD TG 442C: *In Chemico* skin sensitization Leucidal® Liquid (*Leuconostoc*/Radish Root Ferment Filtrate).
36. Active Micro Technologies. 2017. OECD TG 442D: *In Vitro* skin sensitization Leucidal® Liquid (*Leuconostoc*/Radish Root Ferment Filtrate).
37. AMA Laboratories. 2008. 50 Human subject repeat insult patch test skin irritation/sensitization evaluation (occlusive patch) Leucidal® Liquid (*Leuconostoc*/Radish Root Ferment Filtrate).
38. Personal Care Products Council. 2021. Repeated insult patch test (eyebrow gel containing 0.04% *Leuconostoc*/Radish Root Ferment Filtrate)
39. Active Micro Technologies. 2017. Phototoxicity Assay Analysis Leucidal® Liquid (*Leuconostoc*/Radish Root Ferment Filtrate).
40. Lee YH, Lee JH, Kang HR, Ha JH, Lee BH, Kim SH. A case of anaphylaxis induced by contact with young radish (*Raphanus sativus* L). *Allergy Asthma Immunol Res*. 2015;7(1):95-97.
41. Mitchell JC, Jordan WP. Allergic contact dermatitis from the radish, *Raphanus sativus*. *Br J Dermatol*. 1974;91(2):183-189.