Safety Assessment of *Portulaca oleracea*- Derived Ingredients as Used in Cosmetics

Status:	Final Report
Release Date:	October 11, 2022
Panel Meeting Date:	September 26-27, 2022

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ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 4 *Portulaca oleracea*-derived ingredients as used in cosmetic formulations. These ingredients are mostly reported to function as skin-conditioning agents. Because final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers; with *Portulaca oleracea*-derived ingredients, the Panel was concerned about the presence of terpenes as potential sensitizers in cosmetics. Industry should use current good manufacturing practices to minimize impurities. The Panel reviewed data relevant to the safety of these ingredients in cosmetic formulations, 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 is a safety assessment of the following 4 *Portulaca oleracea*-derived ingredients, as used in cosmetic formulations:

Portulaca Oleracea Extract	Portulaca Oleracea Juice
Portulaca Oleracea Flower/Leaf/Stem Extract	Portulaca Oleracea Water

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), all of these ingredients are reported to function as skin-conditioning agents in cosmetics (Table 1).[1] Additionally, Portulaca Oleracea Flower/Leaf/Stem Extract is reported to function as an antioxidant.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated.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 Expert Panel for Cosmetic Ingredient Safety (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 Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Botanicals, such as *Portulaca oleracea*-derived ingredients, may contain hundreds of constituents. Thus, in this assessment, the Panel will assess the safety of each of the *Portulaca oleracea*-derived ingredients as a whole, complex mixture; toxicity from single components may not predict the potential toxicity of botanical ingredients.

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 cosmetic ingredient. Therefore, if it is not known whether the test substance is the same as the cosmetic ingredient, the test substances will be identified by the standard scientific practice of using italics to identify genus and species (i.e., a *Portulaca oleracea* extract). However, if it is known that the substance is a cosmetic ingredient, the International Nomenclature Committee (INC) terminology "Portulaca Oleracea…" (e.g. Portulaca Oleracea Extract) will be used.

CHEMISTRY

Definition and Plant Identification

The definitions and functions for the 4 *Portulaca oleracea*-derived ingredients reviewed in this safety assessment are provided in Table 1.[1] The flower is the reproductive shoot in flowering plants, usually with sepals, petals, stamens, and pistil(s). The stem is defined as a slender or elongated structure which supports the plant, plant part, or plant organ, while the leaves are defined as the flattened photosynthetic organs, attached to the stems.

Portulaca oleracea is an annual herbaceous weed of the Portulacaceae family.[2] The genus *Portulaca* is thought to be derived from Latin 'porto,' to carry, and 'lac,' meaning milk, owing to the milky juice obtained upon expressing the plant.[3] It is commonly referred to as purslane, pigweed, Ma-Chi-Xian, and many other regionally specific names.[4] Although it is thought to originate from tropical and subtropical countries in Eastern Asia, it currently grows throughout the world, in unshaded areas. In spite of growing optimally in temperate climates, *Portulaca oleracea* also thrives under diverse geographic and climactic conditions due to its relatively low water and soil nutrient requirements, and tolerance to salt and drought.[5,6] As a dicotyledonous, C4 photosynthesis plant, displaying Kranz anatomy structure, *Portulaca oleracea* has high water efficiencies in conditions that promote carbon loss through photorespiration, such as high temperatures, high light intensities, and decreased water availability.[7,8]

The plant is a succulent, which usually grows close to the ground, and is up to 30 cm in height, with a cylindrical stem of 2 - 3 mm in diameter.[9] The leaves are oblong and grow in an alternate arrangement, broad at the apex and tapered at the base. The leaf apex is obtuse and smooth, with no teeth or lobes. The flowers are terminal in cluster, with 2 - 6 foliar involucres, and five bright yellow petals enclosed by two subequal lanceolate sepals. The fruit is a shell and the seed is kidney-shaped and flaky.[10] The stem is smooth, red, and circular, and consists of a distinct ~ 60 µm epidermis, 800 µm broad

cortex, and a pith consisting of cells similar to cortical parenchyma. The xylem elements are thick-walled and angular, and possess dense calcium oxalate crystals.

Chemical Properties

In an ultraviolet (UV) spectral analysis of crude, and methanol-soluble fractions of whole *Portulaca oleracea* extract, optical spectra maxima were recorded between 200 and 400 nm, in which phenolic compounds showed maximum absorbence.[11] The Fourier transform infrared spectroscopy (FTIR) spectrum of a chloroform extract of whole *Portulaca oleracea* showed peaks at 1019.52 and 1396.21/cm, corresponding to the wavenumber ranges for alcohols and phenols, amines, organic, and, possibly, nitrogen or oxygen-containing compounds.[12]

Method of Manufacture

An overview of 2 supplier-provided methods of manufacture for Portulaca Oleracea Extract, both using the whole plant,[13-15] is outlined in Figure 1.

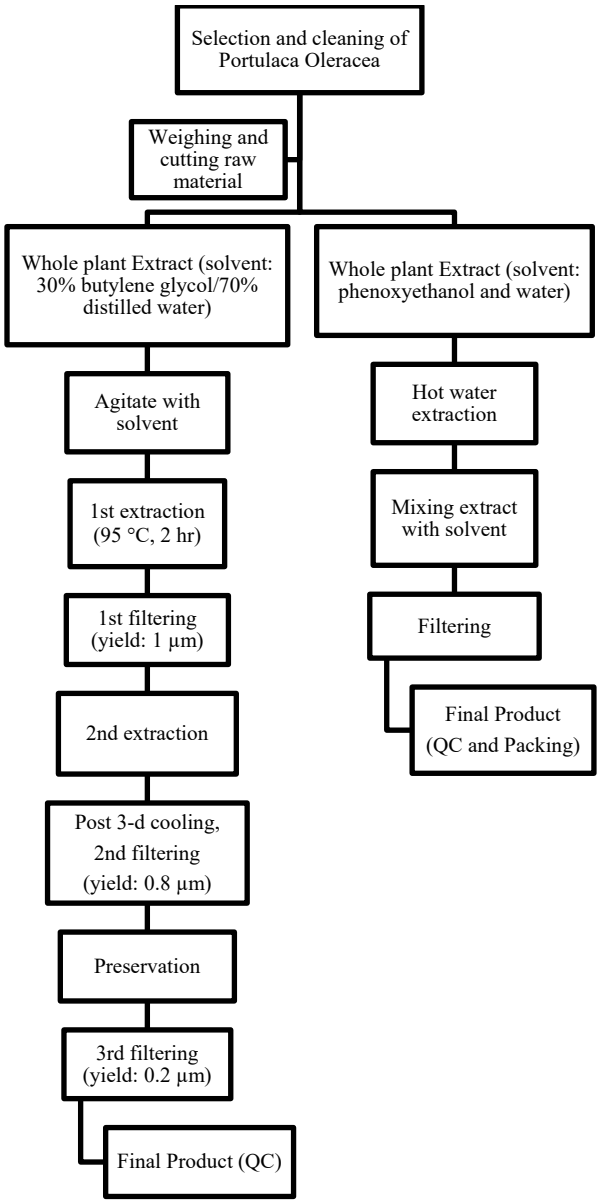

Figure 1. Overview of methods of manufacture for Portulaca Oleracea Extracts.[13-15]

Most of the methods below are general to the processing of *Portulaca oleracea*, and it is unknown if they apply to cosmetic ingredient manufacturing.

Portulaca Oleracea Extract

Extracts of *Portulaca oleracea* may be obtained through maceration of the fresh or dried plant in an alcoholic or aqueous solvent.[16] Most *Portulaca oleracea* extracts are obtained using ethanol or methanol solvents.[17] Methanol is preferred as a polar solvent which elutes the highest level of constituents from *Portulaca oleracea*, in turn affecting phenolic

compound content and potential antioxidant activity.[18-21] Levels of individual compounds detected in crude *Portulaca oleracea* extracts may be low (e.g., phenols), but may be isolated via various techniques such as reversed-phased separation.[11]

A method of preparing the aqueous extract of *Portulaca oleracea* (whole plant) is described as follows: distilled water (1500 ml) was added to 300 g of dried plant powder in a sealed glass container, set aside for 72 h, and then the filtrated extract was concentrated in a rotary evaporator under reduced pressure at 55 °C.[16] The resulting extract was dried in a warm water bath.

An alcoholic extract of *Portulaca oleracea* seeds was obtained by refluxing 500 g of powdered seeds with 2 l of rectified spirit for 10 h on a 100 °C water bath.[22] The initial filtrate was collected while hot, and the residual seeds were refluxed thrice more with 2 l of rectified spirit. Filtrates from the successive extractions were mixed and the rectified spirit was distilled off under reduced pressure, resulting in 50 g of an oily brown syrup. This syrup extract was suspended in 250 ml of sterile olive oil.

Portulaca Oleracea Flower/Leaf/Stem Extract

The aerial parts of *Portulaca oleracea* were used to prepare several extracts.[20] Four solvents (300 ml, each) of increasing polarity, namely, hexane, ethyl acetate, methanol, and water, were placed in the cartridge of separate Soxhlet extractors with 30 mg powdered aerial parts of *Portulaca oleracea*. The extractions took place over 24 h, after which the recovered extracts were conserved at 4 °C.

Aerial parts of the plant were washed with water, and the leaves along with the stems were stripped from the plant and divided into three equal batches.[23] The first batch was cut into small pieces and air dried at 45 °C. The second batch was boiled in water at 100 °C for 15 min in the ratio of 1:10 (w/v). The third batch was blanched in boiling water (at 100 °C) for 10 min in the ratio of 1:10 (w/v). After boiling and blanching, the remaining water was discarded and the three processed samples were cut into small pieces and dried at 45 °C. After drying, the samples were ground to a fine powder and extracted in aqueous acetone.

Portulaca Oleracea Juice

In another study, the aerial parts of *Portulaca oleracea* were washed with water, cut into small pieces, and blended.[24] The juice was obtained from the resultant puree by centrifugation (10,000 x g, 20 min, 4 °C) and was sterilized by filtration on 0.22 μm membrane filters.

Portulaca Oleracea Water

Portulaca Oleracea Water is the steam distillate obtained from the whole plant.[1]

Composition and Impurities

Water content is high in *Portulaca oleracea* (up to 92.32%).[10,11,25] Moisture migrates from the leaves to the stems as the plant matures.

Portulaca oleracea contains nutrients which are also found in major cultivated vegetables, and it contains a high amount of α-linolenic acid, an essential omega-3 fatty acid, compared to other leafy vegetables.[11,26] In a study comparing nutrients found in chamber and wild-grown *Portulaca oleracea* and spinach, although β-carotene levels were lower, ascorbic acid and glutathione levels were higher, and α-linolenic acid content and α-tocopherol levels were 7 times higher in both chamber and wild-grown *Portulaca oleracea*, than those found in spinach.[27] One serving of fresh chamber-grown *Portulaca oleracea* (100 g) was reported to contain 300 - 400 mg α-linolenic acid, 26.6 mg ascorbic acid, 12.2 mg α-tocopherol, 14.8 mg glutathione, and 1.9 mg β-carotene.

As a weed plant, the roots of *Portulaca oleracea* draw minerals from deeper layers of the soil, by degrading and absorbing residual solid parts of other plants.[10] The dry weight (mmol/kg DW) concentrations of calcium, magnesium, sodium, potassium, iron, and zinc monitored on day 15, 30, 45, and 60 of growth, were highest in the leaves of 60-d old *Portulaca oleracea* plants.[18] Varying climate and soil conditions among *Portulaca oleracea* plants grown in different locations also affect mineral composition, flavonoid, and carotenoid content.[28,29] Additionally, the composition and determination of individual constituents found in *Portulaca oleracea*-derived ingredients varies considerably depending on extraction solvent and method,[10,17] part of the plant,[25,30] and growth stage or time of harvest.[18,25] A list of constituents, isolated across different studies, by plant part, is presented in Table 2.

Oxalic acid, or oxalate, is found in a variety of plants, and is generally present in *Portulaca oleracea* at 1.3%.[31] Of the oxalate present in the *Portulaca oleracea* plant, it has been shown to be found in soluble (bound to potassium, sodium, and magnesium) and insoluble (bound to calcium and iron) forms; in one study, mean soluble oxalate values were 33% in the leaves, and 67% in the stems (each relative to the total mass balance of 1.3%).[32] Upon chemical analysis of oxalate content in *Portulaca oleracea*, the highest total concentration of soluble and insoluble oxalate was found in the leaves (23.45 g/kg fresh weight (fw)), and in lesser amounts in the buds (9.09 g/kg fw) and stems (5.58 g/kg fw).[32] In the same study, cooking the whole plant resulted in a 49% reduction of soluble oxalate content in plant buds, 33.5% reduction in the leaves, and 18%

reduction in the stems, while pickling the plant in white vinegar resulted in a 67% overall oxalate reduction. *Portulaca oleracea* is mentioned in the US FDA Poisonous Plant Database.[33] The potential for nitrate/nitrate poisoning in sheep and goats that consume *Portulaca oleracea*[34] and caution regarding the oxalate content in *Portulaca oleracea*[35] for dog, cat, and horse consumption has been documented.

Portulaca Oleracea Extract

Portulaca oleracea extract is composed of a wide range of constituents, of which flavonoids, alkaloids, terpenes, phenolic acids, and coumarins are preeminent.[2,19] Other notable constituents are omega-3-fatty acids, polysaccharides, vitamins, and amino acids.[30]

The phenolic and flavonoid content of hydrothermally processed *Portulaca oleracea* was evaluated.[23] The gallic acid equivalents of boiled, blanched, and raw *Portulaca oleracea* were determined to be 19.25, and 10.02 , and 22.94 g/extract, respectively. Boiling and blanching significantly increased the rutin equivalent to 85.14 and 81.57, respectively, compared to 64.99 mg/g extract in raw *Portulaca oleracea*.

Portulaca Oleracea Flower/Leaf/Stem Extract

The chemical composition and nutritional value of *Portulaca oleracea* plants was assessed, by plant part (leaves and stems) and stage of harvest, for up to 52 d after sowing.[25] The moisture content of leaves was the highest at day 29, while stems contained the most water on day 43. Higher macronutrient content and protein values were observed in the leaves at the last harvest, while the carbohydrate and α-linolenic acid content of leaves was highest at day 29. In a study of total flavonoid and total phenolic content in *Portulaca oleracea* flowers, leaves, and stems, total phenolic content was significantly higher in stems compared to leaves and flowers (1008.6 vs. 441.8 - 455.6 gallic acid equivalents), in spite of total flavonoid content not differing significantly.[36]

The impact of the dehydration method (100 W microwave, tray, vacuum, or low temperature, low humidity infrared) upon the retention of bioactive compounds in extracts made from dried *Portulaca oleracea* leaves and stems was evaluated.[21] Flavonoid content and fatty acid composition was highest in the extract of vacuum-dried leaves.

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, 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.

Portulaca Oleracea Extract is the only ingredient included in this report that is reported to be used in cosmetic formulations. According to 2022 VCRP survey data, Portulaca Oleracea Extract is reported to be used in 541 formulations (Table 3), of which 195 uses are in face and neck products, and 139 uses are in moisturizing products.[37] The results of the concentration of use survey, conducted by the Council in 2018 and updated in 2021, indicate that the reported maximum concentration of use for Portulaca Oleracea Extract is 0.5%, in non-spray face and neck formulations.[38,39] According to VCRP and Council survey data, the other 3 *Portulaca oleracea*-derived ingredients are not reported to be in use in cosmetic products (Table 4).

Portulaca Oleracea Extract is reported to be used in products which may allow exposure near the eye or mucous membranes. Concentration of use data were not reported for these categories of use. According to VCRP data, Portulaca Oleracea Extract is reportedly used in 2 face powder formulations,[37] and could possibly be inhaled; concentration of use data were not reported for this use.[38] In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most particles incidentally inhaled 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.

All of the ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[40]

Non-Cosmetic

Portulaca oleracea is consumed raw in salads, or is used as a potherb in cooked sauces, soups, and pickled dishes across many cultures.[4,41] Furthermore, the whole plant, and plant parts, of *Portulaca oleracea* are used as a food in various cultures.[26,42] Uses as an apotropaic agent and as a source of violet and gray dye for wool are also noted.[41]

Historically, *Portulaca oleracea* is reported to be widely used in traditional folk medicine. In Chinese traditional medicine, the plant is used for the treatment of dysentery with bloody stools, as a topical emollient, collyrium, and as an external muscle relaxant.[3,4] Native Americans use the plant to treat gout and headaches, and as a febrifuge.[4] In Africa, the *Portulaca oleracea* plant is considered to exhibit anti-inflammatory, analgesic, and antifungal activity; fresh juice is used in the treatment of dysuria, coughs, and as an anti-diabetic agent.[4,43] Additionally, it is used in religious ceremonies for purification, as an antiphlogistic substance, and for the treatment of skin diseases, erysipelas, insect and snake bites, abscesses, and eczema.[4,17] The World Health Organization (WHO) describes *Portulaca oleracea* as a medicinal plant, with antibacterial, anti-inflammatory and antihelminth properties; poultices of fresh leaves are used to treat mastitis, boils, and impetigo.[44]

TOXICOKINETIC STUDIES

No relevant toxicokinetic studies on *Portulaca oleracea*-derived ingredients were found in the public literature, and unpublished data were not submitted. In general, toxicokinetic data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute toxicity studies summarized below are described in Table 5.

The dermal LD_{50} of an ethanolic extract of *Portulaca oleracea* (10%), in rabbits, was determined to be 1865 mg/kg bw.[45] The oral LD_{50} of an extract of whole *Portulaca oleracea* (water: ethanol; 1:1), in Swiss albino mice, was determined to be ≤ 500 mg/kg bw.[46,47] The oral LD_{50} of a petroleum ether *Portulaca oleracea* leaf extract, in Sprague-Dawley rats, was determined to be > 2000 mg/kg bw.[48] Maximum oral doses of 5000 mg/kg chloroform and methanolic *Portulaca oleracea* leaf extracts were well tolerated in rats.[49,50]

Short-Term Toxicity Studies

The short-term oral toxicity studies summarized below are described in Table 6.

Groups of 6 Swiss albino mice were administered an oral dose of 0, 200, or 400 mg/kg bw/d, ethanolic extract of whole *Portulaca oleracea* (water: ethanol; 1:1), via gavage, for 14 d.[46,47] No mortality occurred during observation; a statistically significant increase in hypoglycemic activity was observed in both treated groups, and to a greater extent in the 400 mg/kg bw group. Groups of 5 albino rats orally dosed at up to 75 mg/kg bw/d of aqueous or methanolic extract of whole *Portulaca oleracea* for 30 d showed a statistically significant decrease in white blood cell and neutrophil counts, and increase in lymphocyte counts in the 25 and 50 mg/kg bw/d aqueous extract groups.[51] Rats in the 25 mg/kg bw/d methanolic extract group showed a significant increase in mean corpuscular volume and mean corpuscular hemoglobin, while rats in the 75 mg/kg bw/d methanolic extract group had a significant decrease in total plasma protein and albumin levels. In a 14-d study, groups of 6 Sprague-Dawley rats were orally dosed with 0, 500, 1000, or 2000 mg/kg/d petroleum ether extract of *Portulaca oleracea* leaves.[48] No mortality occurred during observation and there was a non-significant increase in body weights. Hematological parameters were examined, and a significant, dose-dependent increase in hemoglobin, red blood cell count, packed cell volume, mean corpuscular volume, and total cholesterol levels was observed in all treated rats, compared to controls. Groups of 16 male albino Wistar rats were administered 0, 125, 250, or 500 mg/kg bw/d methanolic or chloroform extract of *Portulaca oleracea* leaves, via gavage, for 60 d.[49,50] The 500 mg/kg group showed a significant decrease in the mean hematocrit on day 28, which was considered incidental, and a significant increase in white blood cell count on day 42. Platelet count was increased in all treatment groups, and was significantly higher in the 125 and 500 mg/kg treated rats on day 60. Groups of 6 male albino Wistar rats were dosed with either distilled water or 1.5 ml/kg/d *Portulaca oleracea* juice extract, via gavage, for 12 d.[52] Blood samples in these rats showed a statistically significant increase in uric acid, and in glutathione, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase in the liver, kidney, and testes. A significant decrease in urea and creatine, reduction in malondialdehyde levels in the liver and kidney, and a significant reduction in aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), and bilirubin was observed; changes in ALT (alanine aminotransferase) were not significant.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Details of the oral developmental and reproductive studies summarized below are described in Table 7.

Male albino rats, that were orally administered either 75 mg/kg bw aqueous or methanolic *Portulaca oleracea* leaf and stem extract for 50 d, were cohabited with 3 female rats each for 4 wk.[53] No pregnancy (or sterile mating) occurred between males from either extract group and the untreated female rats. Body weight changes, blood samples, sperm, testes, and epididymis were analyzed in groups of 5 male Wistar rats orally dosed with 0, 400, or 800 mg/kg bw methanolic *Portulaca* oleracea leaf and stem extracts for 14 d.[54] Although no significant differences in luteinizing hormone and testosterone levels were seen in the animals treated with the methanolic extracts, significant increases in follicle-stimulating hormone and reduction in sperm count occurred in the 800 mg/kg group and a significant reduction in sperm motility was seen in both treatment groups, compared to controls. Groups of 5 male albino rats were orally dosed with 0, 25, 50, or 75 mg/kg/d aqueous, or methanolic, *Portulaca oleracea* leaf and stem extracts for 50 d, and had blood samples from day 51 analyzed for testosterone levels; the animals were sacrificed for semen and histological analyses of the testes.[55] A statistically significant decrease in testosterone levels was observed in rats in the aqueous 75 mg/kg group, and in all methanolic extract groups. Animals in all dose groups had significantly reduced sperm motility, sperm count, and increased sperm abnormalities. In another study, groups of 16 male albino rats were orally dosed with 0, 125, 250, or 500 mg/kg chloroform, or 80% aqueous methanolic extract, for 60 d; blood samples, testes, and epididymis were harvested from 4 animals in each treatment group on days 14, 28, 42, and 60.[56] A significant increase in sperm count was observed in the animals treated with both extracts in the 250 mg/kg groups on day 28 and a significant decrease in testosterone levels was observed in the animals treated with 125 and 500 mg/kg methanolic extract on days 28 and 60. Groups of 5 - 6 female Wistar albino rats were orally dosed with 0, 250, or 500 mg/kg bw/d, flavonoid-rich, *Portulaca oleracea* stem and leaf extract and were examined for potential effects on reproductive organ weight, estrous cycles, uterine characteristics, abortifacient activity, and implantation; significant uterine changes included larger diameter and endometrial thickness.[57] In two similarly completed, but separate studies, ovary and uterine weights were significantly lower in immature, bilaterally ovariectomized rats orally dosed with 250 or 500 mg/kg bw/d *Portulaca oleracea* stem and leaf extract (indicated as "total flavonoid extract") for 7 d, and, significantly higher in the mature rats orally dosed with 250 and 500 mg/kg bw/d of the same extract for 10 d; both effects were associated with significantly reduced protein and cholesterol uterine content, and suppression of follicular stimulating hormone, respectively.[57] The effect of orally administered 400 or 800 mg/kg bw/d *Portulaca oleracea* leaf and stem extract upon bilaterally ovariectomized rats, compared to control rats which only received distilled water, with or without ovariectomizing, was examined in a 14-d study.[58] Estrous cycle dysregulation and a statistically significant decrease in estradiol and testosterone was observed in all 3 ovariectomized groups, compared to non-ovariectomized controls, while a statistically significant increase in progesterone was only observed in the ovariectomized rats given 400 mg/kg bw and 800 mg/kg bw/d. In a 21-d study, female albino rats were first orally dosed with 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extracts, and then served as their own controls after an additional 21 d of no dosing to observe changes in estrous cycles.[59] Treatment for 21 d with either extract did not produce any significant changes in duration of estrous cycle phases. However, during the 21-day withdrawal of treatment, a significant decrease in the proestrus phase of both treated groups, increase in the estrous phase of the aqueous extract-treated rats, and increase in the metestrus phase of the methanolic extract group was observed. The effects of 0, 125, 250, or 500 mg/kg chloroform, or 80% aqueous methanolic, *Portulaca oleracea* leaf extracts upon estrous cycle, ovarian and uterine histology, and luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, and estrogen serum levels were examined in groups of 5 female albino rats for 21 d.[60] Significant decreases in luteinizing hormone levels in the 250 mg/kg chloroform extract group, and in follicle-stimulating hormone levels in the 250 and 500 mg/kg chloroform extract groups were observed. Hypertrophied ovarian follicles were observed in the 125 mg/kg methanolic extract group; no other significant effects were exhibited in estrous phase, hormone levels, or histology. Groups of 5 female albino rats were orally dosed with either 0.5 ml distilled water, or 75 mg/kg/d of aqueous or methanolic *Portulaca oleracea* leaf and stem extract for 25 d, to examine ovarian and uterine histopathology.[59] No significant pathological effects or changes in ovarian or uterine weights were observed. In another study, dams dosed with up to 500 mg/kg bw/d *Portulaca oleracea* leaf and stem extract (indicated as "total flavonoid extract") from day 7 to day 14 of gestation, via gavage, showed a statistically significant 30% abortion rate and 50% inhibition in implantation in the 250 mg/kg bw/d group, while animals in the 500 mg/kg bw/d group had a statistically significant 50% abortion rate and 70% inhibition in implantation, compared to controls (these dams were dosed from day 1 to day 7 of gestation).[57] In a teratology study of albino rats, animals were dosed with 0.5 ml distilled water or 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extract at three different time frames during 21 d of gestation.[53] No significant differences related to pregnancy stage, fetal development, or delivery were observed.

GENOTOXICITY STUDIES

Genotoxicity data on *Portulaca oleracea*-derived ingredients were not found in the published literature, and unpublished data were not submitted.

CARCINOGENICITY STUDIES

Carcinogenicity data on *Portulaca oleracea*-derived ingredients were not found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Anti-Inflammatory and Antioxidant Studies

Portulaca oleracea extracts were shown to significantly reduce lipopolysaccharide (LPS)-induced synthesis of nitric oxide, the production of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and the expression levels of various transcription factors, in murine macrophage cells.[61] Luteolin, kaempferol, and quercitrin components identified in the extracts were postulated to account for these anti-inflammatory effects.

Three aqueous extracts of *Portulaca oleracea* flowers, leaves, and stems were prepared using distilled water.[36] *Escherichia coli* DNA interjected with pBR322 plasmid, exposed to hydrogen peroxide in a DNA nicking assay, was incubated with 5 µl (80 µg/ml) of each extract for 10 min and measured for plasmid DNA damage. Aqueous extracts from each plant part showed a protective effect against DNA damage, through the inhibition of Fenton reaction free radicals; the highest effect was observed with the stem extract, and the lowest effect was observed in the flower extract.

Cytotoxicity

Portulaca Oleracea Extract

A 70% ethanolic crude extract of whole *Portulaca oleracea* was tested at concentrations of 0.2, 0.4, 0.8, 1.6, 3.2, or 6.4 mg/ml on human peripheral lymphocytes for the effect on mitotic index (MI) and blast index (BI).[62] Increased MI and BI values were observed, but were not significantly different when compared with those in the positive control group, which were treated with phytohemagglutinin.

The cytotoxic potential of the chloroform extract of whole *Portulaca oleracea* against human colon adenocarcinoma (HCT-15) and normal (Vero) cell lines was examined in a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, with doxorubicin as a reference.[12] The 50% cell growth inhibition concentration (IC_{50}) for the chloroform extract was 1132.02 µg/ml in HCT-15 cells and 767.60 µg/ml in Vero cells, while the IC_{50} for doxorubicin was 460.13 µg/ml in HCT-15 cells and 2392.71 µg/ml in Vero cells. The chloroform extract was not considered cytotoxic to HCT-15 cells, but was considered possibly toxic to Vero cells. Cell viability was recorded to be 67%, 31%, 21%, and 17% in human hepatocellular carcinoma cells (HepG2) exposed to 50, 100, 250, and 500 µg/ml *Portulaca oleracea* seed extracts, respectively.[63]

An aqueous extract of whole *Portulaca oleracea* was tested in an MTT assay for its cytotoxic potential against pancreatic carcinoma cell line (PANC-1), with human umbilical vein endothelia cell (HUVEC) as a reference cell line.[64] The *Portulaca oleracea* extract was administered at concentrations of up to 1000 µg/ml. The IC_{50} for the aqueous extract was 174.5 µg/ml in HUVEC and 500 µg/ml in PANC-1 cells. Additionally, treatment with the aqueous *Portulaca oleracea* extract at the highest IC_{50} level of 500 µg/ml for 24 h showed an increase in the percentage of PANC-1 cells in late apoptosis, compared to untreated controls.

Portulaca Oleracea Flower/Leaf/Stem Extract

The antiproliferative potential of aqueous and methanolic extracts of *Portulaca oleracea* leaves was examined in murine mammary adenocarcinoma (AMN3) cells, human rhabdomyosarcoma (RD) cells, and normal kidney epithelium cells of the African green monkey, at concentrations up to 10,000 µg/ml, over 72 h.[65] Both extracts exhibited time-dependent antiproliferative effects against both cancer cell lines, with more sensitivity in the AMN3 cells. The normal cells showed resistance towards all concentrations of both extracts, except the 10,000 µg/ml dose. Similarly, significantly reduced cell viability was seen in HeLa cervical cancer cells exposed to concentrations of 0, 300, 500, 700, 1000, 1200, or 1500 µg/ml of *Portulaca oleracea* stems and leaf extracts for up to 48 h.[66]

DERMAL IRRITATION AND SENSITIZATION STUDIES

The dermal irritation and sensitization studies are summarized below and described in Table 8.

A 24-h, single-insult occlusive patch test (SIOPT) was performed in 22 subjects with a body lotion containing 0.1% Portulaca Oleracea Extract.[67] Another lotion was used as the reference control. No significant differences were observed in the irritation response of subjects exposed to the test material and the reference control, and the primary irritation index (PII) was 0.0 for both materials. The skin sensitization potential of a body moisturizer containing 0.1% Portulaca Oleracea Extract was evaluated in a maximization study completed in 26 subjects.[68] A total of 5, 48-h occlusive induction applications (0.05 ml of the test material, applied neat) were made, after inducing irritation with a 1-h, occlusive application of 0.05 ml 0.25% aqueous sodium lauryl sulfate. After a 10-d non-treatment period, an occlusive 48-h challenge application was made to an induced, but previously untreated, site. The challenge site was graded 15 - 30 min and 24 h after patch removal. Scores for all 26 subjects who completed the study were 0 at both readings (on a 0 - 3 scoring scale). The test substance was considered non-sensitizing. Two additional maximization studies, performed in an identical fashion, tested the sensitization potential of a face lotion and face treatment product, both containing 0.5% Portulaca Oleracea Extract, and were completed in 27 and 26 subjects, respectively.[69,70] No signs of sensitization were observed for either product during the induction or challenge phase; both test articles were considered non-sensitizing.

OCULAR IRRITATION STUDIES

Data on the ocular irritation potential of *Portulaca oleracea*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were they submitted.

CLINICAL STUDIES

Clinical Use

A 3-wk use study of a formulation containing 0.1% Portulaca Oleracea Extract was performed in 46 subjects.[71] Dermatologist-assessed facial exams were conducted at the test center during the initial and final visit. Thirty-three (72%) of subjects were assessed as having sensitive skin, based on test center results for various skin conditions, as well as self-reported sensitivity to sun, allergies, and eczema at the end of the 3-wk use period. Subjects were instructed to apply the test product over their entire face (including the eye area, but avoiding contact with the eyes), at least twice a day. Subjects were also allowed to apply their own moisturizer following use of the test material, if desired. No product-related irritation was observed. Changes in scaling/flaking and conditions of acne, including papules and pustules which occurred, were determined to be within expected fluctuation in the general population. No irritation was observed.

SUMMARY

The safety of the following 4 *Portulaca oleracea*-derived ingredients, as used in cosmetics, is reviewed in this safety assessment: Portulaca Oleracea Extract, Portulaca Oleracea Flower/Leaf/Stem Extract, Portulaca Oleracea Juice, and Portulaca Oleracea Water. These ingredients are all reported to function as skin-conditioning agents in cosmetics.

Portulaca Oleracea Extract is the only ingredient included in this report that is reported to be used in cosmetic formulations. According to 2022 VCRP survey data, Portulaca Oleracea Extract is reported to be used in 541 formulations, of which 195 uses are in face and neck products and 139 are in moisturizing products. The results of the concentration of use survey conducted by the Council indicate Portulaca Oleracea Extract is used at a maximum concentration of 0.5% (in non – spray face and neck products).

The dermal LD_{50} of a 10% ethanolic *Portulaca oleracea* extract was determined to be 1865 mg/kg in rabbits. The oral LD_{50} of an ethanolic extract of whole *Portulaca oleracea* was determined to be ≤ 500 mg/kg bw in Swiss albino mice, while the oral LD_{50} of a petroleum ether *Portulaca oleracea* leaf extract was determined to be > 2000 mg/kg bw in Sprague-Dawley rats. Maximum oral doses of 5000 mg/kg methanolic and chloroform *Portulaca oleracea* leaf extracts were well tolerated in rats.

No mortality occurred, and a significant increase in hypoglycemic activity was observed, in groups of 6 Swiss albino mice orally dosed with up to 400 mg/kg bw/d of a whole ethanolic *Portulaca oleracea* extract for 14 d. Albino rats dosed, orally, at up to 75 mg/kg bw/d of aqueous or methanolic extract of whole *Portulaca oleracea* for 30 d showed a significant decrease in white blood cell and neutrophil count in the 25 and 50 mg/kg bw/d aqueous extract groups, as well as a significant increase in mean corpuscular volume and mean corpuscular hemoglobin in the 25 and 75 mg/kg bw/d methanolic extract groups. No mortality occurred and a significant, dose-dependent increase in hematological parameters and cholesterol levels was observed in all Sprague-Dawley rats orally dosed with 500, 1000, or 2000 mg/kg bw/d petroleum ether extract of *Portulaca oleracea* leaves for 14 d. Groups of 16 male albino Wistar rats were orally administered 125, 250, or 500 mg/kg bw/d of methanolic or chloroform extract of *Portulaca oleracea* leaves, for 60 d; a significant decrease in the mean hematocrit on day 28 and a significant increase in white blood cell count on day 42 was observed in the 500 mg/kg group. Platelet count was increased in all treatment groups, and was significantly higher in the 125 and 500 mg/kg treated rats on day 60. Blood samples of male albino Wistar rats orally dosed with 1.5 ml/kg/d *Portulaca oleracea* juice extract for 12 d exhibited significant variability in serum levels of urea, creatine, glutathione, and bilirubin.

No pregnancies resulted from mating between male albino rats dosed, via gavage, with 75 mg/kg bw aqueous or methanolic *Portulaca oleracea* leaf extract for 50 d, and untreated female rats. Groups of 5 male Wistar rats were orally dosed with 0, 400, or 800 mg/kg bw methanolic *Portulaca oleracea* leaf and stem extract for 14 d. Significant increases in follicle-stimulating hormone and reduction in sperm counts were seen in the 800 mg/kg group, and sperm motility was significantly reduced in both 400 and 800 mg/kg groups, compared to controls; differences in luteinizing hormone and testosterone levels were not significant. Groups of 5 male albino rats orally dosed with 0, 25, 50, or 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extracts for 50 d exhibited significantly decreased testosterone levels at the maximum aqueous extract dose, and in all methanolic extract dose groups. Animals in all dose groups had significantly reduced sperm motility, sperm count, and increased sperm abnormalities. Groups of 16 male albino rats were orally dosed with 0, 125, 250, or 500 mg/kg/d chloroform or methanolic extract for 60 d; significant increases in sperm count were seen in the 250 mg/kg groups for both extracts on day 28, and testosterone levels were significantly decreased in the 125 and 500 mg/kg methanolic extracts groups on days 28 and 60. In two separate studies of groups of 5 - 6 female Wistar albino rats, ovary and uterine weights were significantly higher in mature rats, and significantly lower in immature bilaterally ovariectomized rats orally dosed with 0, 250, or 500 mg/kg bw/d *Portulaca oleracea* stem and leaf extract (indicated as “total flavonoid extract”). In a 14-d study examining the effect of orally administered 0, 400 mg/kg bw/d, or 800 mg/kg bw/d *Portulaca oleracea* extract upon ovariectomized rats, estrous cycle dysregulation and a statistically significant decrease in

estradiol and testosterone was observed in ovariectomized controls and both dose groups, and a significant increase in progesterone was only observed in the ovariectomized 400 mg/kg bw/d and 800 mg/kg bw d groups. In a 21-d study of female albino rats dosed, via gavage with either 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extracts, no significant changes in duration of estrous cycle phases were observed, however, upon withdrawal of both treatments in a 21-d follow-up period, a significant decrease in the proestrus phase was observed in both treated groups, as well as a significant increase in the estrous phase of the aqueous-treated rats, and increase in the metestrus phase of the methanolic extract group. Groups of 5 female albino rats orally dosed with 0, 125, 250, or 500 chloroform, or 80% aqueous methanolic extract for 21 d exhibited significant decreases in LH, from the 250 mg/kg chloroform extract group, and FSH from the 250 and 500 mg/kg chloroform extract groups. No other significant effects upon estrous cycle, ovarian and uterine histology, or LH, FSH, estrogen, or progesterone levels were observed. No significant pathological effects or changes in ovarian and uterine weights were observed in rats orally dosed with 75 mg/kg/d of aqueous or methanolic *Portulaca oleracea* leaf and stem extract for 25 d. In another study, dams orally dosed with up to 500 mg/kg bw/d of *Portulaca oleracea* leaf and stem extracts (indicated as "total flavonoid extracts") had a statistically significant 30% abortion rate and 50% inhibition in implantation in the 250 mg/kg bw/d group, while animals in the 500 mg/kg bw/d group had a statistically significant 50% abortion rate and 70% inhibition in implantation, compared to controls. No significant differences in pregnancy stage, fetal development, or delivery were observed in albino rats dosed with 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extract, via gavage, during either day 1 to day 5 (implantation), day 6 to day 15 (mid-pregnancy), or day 16 to day 21 (late pregnancy).

Portulaca oleracea extracts were shown to significantly reduce LPS-induced synthesis of nitric oxide, the production of TNF-α, IL-6, and the expression levels of various transcription factors, in murine macrophage cells. An aqueous extract (80 µg/ml) of *Portulaca oleracea* stems had the most protective effect against *E. coli* plasmid DNA damage in a DNA nicking assay, compared to leaf and flower extracts. A 70% ethanolic crude extract of whole *Portulaca oleracea*, tested at doses of up to 6.4 mg/ml on human peripheral lymphocytes, produced a non-significant increase in MI and BI values compared to the positive controls treated with phytohemagglutinin. In an MTT assay, the chloroform extract of whole *Portulaca oleracea* exhibited an IC_{50} of 1132.02 µg/ml in HCT-15 cells and 767.60 µg/ml in Vero cells, compared to 460.13 µg/ml and 2392.71 µg/ml, for doxorubicin, respectively. The chloroform extract was not considered cytotoxic to HCT-15 cells, but was considered possibly toxic to Vero cells. Cell viability was recorded to be 67%, 31%, 21%, and 17% in HepG2 cells exposed to increasing doses of up to 500 µg/ml *Portulaca oleracea* seed extracts. An aqueous *Portulaca oleracea* extract tested for cytotoxic potential against PANC-1 pancreatic cell lines exhibited an IC_{50} of 500 µg/ml in an MTT assay. The antiproliferative potential of aqueous and methanolic extracts of *Portulaca oleracea* leaves was examined in AMN3 cells, RD cells, and normal kidney epithelium cells of the African green monkey, at concentrations up to 10,000 µg/ml, over 72 h. Both extracts exhibited time-dependent antiproliferative effects against both cancer cell lines, with more sensitivity in the AMN3 cells. Similarly, significantly reduced cell viability was seen in HeLa cervical cancer cells exposed to up to 1500 µg/ml *Portulaca oleracea* stems and leaf extracts for up to 48 h.

No dermal irritation responses were seen in an SIOPT of a body lotion containing 0.1% Portulaca Oleracea Extract, in 22 subjects. The skin sensitization potential of a body moisturizer containing 0.1% Portulaca Oleracea Extract was tested in a maximization study involving 26 subjects; the test substance was deemed non-sensitizing. A face lotion containing 0.5% Portulaca Oleracea Extract and a face treatment product containing 0.5% Portulaca Oleracea Extract were not considered sensitizing when tested in a maximization study involving 27 and 26 subjects, respectively.

In a 3-wk use study, 46 subjects were instructed to apply a formulation containing 0.1% Portulaca Oleracea Extract at least two times a day to the entire face. Dermatological changes in skin texture and acne were determined to be within expected ranges; no irritation was observed.

DISCUSSION

This assessment reviews the safety of 4 *Portulaca oleracea*-derived ingredients as used in cosmetic formulations. 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 the safety assessment when formulated to be non-sensitizing.

Based on information from peer-reviewed literature, the Panel acknowledged that *Portulaca oleracea* leaves and stems, as well as the whole plant, are consumed as food, thereby mitigating potential concerns regarding systemic toxicity; food use also mitigated concerns about the lack of carcinogenicity and genotoxicity data. Adverse and contradictory effects in the reported developmental and reproductive toxicity studies were noted by the Panel, including: no changes in testosterone levels in one study, decreases in testosterone in 2 other studies, reduced sperm quality for male rats, and abortifacient activity for dams. However, the Panel noted that the effects seen in the developmental and reproductive toxicity studies occurred at high doses, and resulted from oral administration, thus, resulting in much higher blood concentrations of the extract components than would occur from topical cosmetic use.

Because final product formulations may contain multiple botanicals, each possibly containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *Portulaca oleracea*-derived ingredients, the Panel was concerned about the presence of terpenes as potential

sensitizers in cosmetics. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

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.

The Panel discussed the issue of incidental inhalation exposure from use of these ingredients in products that could possibly be inhaled. For example, Portulaca Oleracea Extract has reported use in face powders (concentration of use is not available). 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 physiological activity of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which the 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.

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 *Portulaca oleracea*-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.

Portulaca Oleracea Extract
Portulaca Oleracea Flower/Leaf/Stem Extract*
Portulaca Oleracea Juice*
Portulaca Oleracea 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 product categories and at concentrations comparable to Portulaca Oleracea Extract.*

TABLES

Table 1. Definitions and functions of *Portulaca oleracea*-derived ingredients in this safety assesment[1]

Ingredient/CAS No.	Definition	Function
Portulaca Oleracea Extract 90083-07-1	Portulaca Oleracea Extract is the extract of the whole plant, *Portulaca oleracea.*	Skin- conditioning agent - humectant
Portulaca Oleracea Flower/Leaf/Stem Extract	Portulaca Oleracea Flower/Leaf/Stem Extract is the extract of the flowers, leaves and stems of *Portulaca oleracea.*	Antioxidants; skin-conditioning agent – misc.
Portulaca Oleracea Juice	Portulaca Oleracea Juice is the liquid expressed from the whole plant, *Portulaca oleracea.*	Skin-conditioning agent – misc.
Portulaca Oleracea Water	Portulaca Oleracea Water is the steam distillate obtained from the whole plant, *Portulaca oleracea.*	Skin-conditioning agent – misc.

Table 2. Constituents found in *Portulaca oleracea*, by plant part*[30]

Classification	Whole plant	Flower, Leaf, and Stem**	Leaf and Stem***	Leaf	Stem
Flavonoids	genistein genistin luteolin myricetin quercetin	portulacanones a portulacanones b portulacanones c portulacanones d 2,2'-dihydroxy-4',6'-dimethoxychalcone	apigenin kaempferol		
Alkaloids	adenosine oleraceins A oleraceins B oleraceins C oleraceins D oleraceins E	aurantiamide aurantiamide acetate cyclo(L-tyrosinyl-L-tyrosinyl *N-trans*-feruloyltyramine (7*R*)-*N*-feruloylnormetanephrine *N-cis*-feruloyltryramine *N-trans*-feruloyloctopamine *N-cis*-feruloyloctopamine Thymine trollisine uracil 1,5- dimethyl-6-phenyl-1,2-dihydro-1,2,4-triazin-3(2H)-one 1,5-dimethyl-6-phenyl-1,6,3,4-tetrahydro-1,2,4-2(1H)-triazin (3*R*)-3,5-bis(3-methoxy-4-hydroxyphenyl)-2,3-dihydro-2(1H)-pyridinone	dopamine noradrenalin		oleraceins I oleraceins II
Terpenoids		friedelane lupeol portuloside A portuloside B portulene (2α, 3α)-3-((4-*O*-(β-D-glucopyranosyl)-β-D-xylopyranosyl)oxy)-2,23-dihyroxy-30-methoxy-30-oxoolean-12-en-28-oic acid (2α, 3α)-2,23,30-trihydroxy-3-((β-D-xylopyranosyl)oxy)olean-12-en-28-oic acid (3*S*)-3-*O*-(β-D-glucopyranosyl)-3,7-dimethylocta-1,6-dien-3-ol (3*S*)-3-*O*-(β-D-glucopyranosyl)-3,7-dimethylocta-1,5-dien-3,7-diol			
Organic Acids	*p*-Coumaric acid Ferulic acid	caffeic acid indole-3-carboxylic acid 3-quinolinecarboxylic acid		α- linoleic acid linoleic acid oleic acid oxalic acid palmitic acid stearic acid	docosapentaenoic acid
Vitamins				α-tocopherol folates hesperidin niacin pantothenic acid pyridoxine riboflavin thiamin vitamin A vitamin C	
Minerals			calcium copper iron phosphorus manganese	magnesium selenium zinc	

Table 2. Constituents found in *Portulaca oleracea*, by plant part*[30]

Classification	Whole plant	Flower, Leaf, and Stem**	Leaf and Stem***	Leaf	Stem
Other compounds		β-sitosterol daucosterol portulacerebroside A		β-carotene chlorophyll glutathione melatonin proline tannin 1,4-di-*O*-acetyl-2,3,5-tri-*O*- methyl-L-arabinitol 1,4,5-tri-*O*-acetyl-2,3-di-*O*- methyl-L-arabinitol 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-galactitol	

*the solvent used for extraction determines total constituent content
**defined as aerial part(s) in primary reference
***sometimes includes root or seed

Table 3. Frequency (2022)[37] and concentration of use (2019)[38,39] data for Portulaca Oleracea Extract

	# of Uses	*Max Conc of Use (%)*
Totals*	**541**	**0.001-0.5**
Duration of Use		
Leave-On	*461*	*0.001 – 0.5*
Rinse-Off	*80*	*0.002*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type		
Eye Area	19	NR
Incidental Ingestion	1	NR
Incidental Inhalation-Spray	155[a]; 205[b]	NR
Incidental Inhalation-Powder	2; 205[b]; 7[c]	0.002 – 0.5[c]
Dermal Contact	527	0.001 – 0.5
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	13	NR
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	13	NR
Baby Products	10	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 that these products are sprays or powders, 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. Ingredients not reported to be in use[37-39]

Portulaca Oleracea Flower/Leaf/Stem Extract
Portulaca Oleracea Juice
Portulaca Oleracea Water

Table 5. Acute toxicity studies

Ingredient/ Extraction Method	Animals	No./Group	Vehicle/Control	Concentration/Dose/Protocol	LD_{50}/Results	Reference
DERMAL						
Portulaca oleracea extract Ethanolic (10%)	Rabbits	strain and # not specified	90% ethanol	A 0.05 ml dose of the test article was administered via intradermal injection, after which the test article was applied to the shaved backs of the animals.	LD_{50} was determined to be 1865 mg/kg bw. No sensitivity was observed.	45
ORAL						
Portulaca oleracea extract (water:ethanol; 1:1)	Swiss albino mice (sex not specified)	2/group	2 % gum acacia	0, 500, 1000, 1500, or 2000 mg/kg bw, via gavage; Performed in accordance with OECD TG 423. The animals were observed 72 h for behavioral changes and mortality.	$LD_{50} \leq 500$ mg/kg bw. After 48 h, half of the animals in the 500 mg/kg group, and all the animals in the 1000, 1500, and 2000 mg/kg bw groups showed sedation, respiratory arrest, convulsions, decreased motor activity, and mortality.	46,47
Portulaca oleracea leaf extract, Petroleum ether	Sprague-Dawley Rats (sex not specified)	6/group	10 ml/kg saline	0, 500, 1000, or 2000 mg/kg bw; The rats were observed up to 24 h for general changes in behavior, physiological function, and mortality.	LD > 2000 mg/kg bw. No mortality occurred, and no signs of toxicity were observed in the control and 500 mg /kg bw dose groups. The animals in the 1000 and 2000 mg /kg bw dose groups exhibited heightened asthenia, defecation, salivation, and urination compared to the control group.	48
Portulaca oleracea leaf extract, Chloroform/Methanolic extract	Rats (sex not specified)	strain and # not specified	80% aqueous methanol	NR	Well tolerated at the maximum dose of 5000 mg/kg. Not toxic.	49,50,56,60

NR- not reported

Table 6. Short-term oral toxicity studies

Ingredient Extraction method	Animals/Group	Study Duration	Vehicle/Control	Dose/Concentration	Results	Reference
			ORAL			
Portulaca oleracea extract (water: ethanol; 1:1)	Swiss albino mice; 6/group	14 d	2% gum acacia	0, 200, or 400 mg/kg bw/d, via gavage	No mortality occurred during observation. Biochemical evaluations were performed on day 15. A statistically significant increase in hypoglycemic activity was observed in both treated groups, and to a greater extent in the 400 mg/kg bw group. The hepatotoxic potential of *Portulaca oleracea* extract was assessed by fixing and examining liver tissue. Histopathology results in treated mice showed no abnormalities and were comparable to control mice.	[46,47]
Portulaca oleracea extract Aqueous extract or 70% Methanolic extract	Albino rats; 5/group/sex	30 d	0.5 ml distilled water	25, 50, or 75 mg/kg bw; aqueous and methanolic extracts	Red blood cell production was not affected by oral administration of aqueous and methanolic extracts. Rats treated with 25 and 50 mg/kg bw of an aqueous extract for 15 d showed a statistically significant decrease in white blood cell and neutrophil counts, and significant increase in lymphocyte counts, relative to controls. Rats dosed with 25 mg/kg bw of a methanolic extract showed a significant increase in mean corpuscular volume and mean corpuscular hemoglobin relative to their respective controls. Thirty-day treatment with 25 mg/kg bw aqueous extract and 75 mg/kg bw methanolic extract produced a significant decrease in total plasma protein and albumin levels.	[51]
Portulaca oleracea leaf extract Petroleum ether extract	Sprague-Dawley rats; 6/group	14 d	10 ml/kg normal saline	500, 1000, or 2000 mg/kg/d, via gavage	Rats dosed with 2000 mg/kg *Portulaca oleracea* leaf extract exhibited decreased motor activity. Body weights were increased in the treatment groups, but the increase was not statistically significant. No mortality occurred during observation. Animals were sacrificed on the 15th day, during which blood samples were collected for hematological assay, and liver, kidney, spleen, and stomach tissue were fixed and stained for examination. A significant, dose-dependent increase in hemoglobin, red blood cell count, packed cell volume, and mean corpuscular hemoglobin was observed, and total cholesterol levels were slightly increased, in all treated rats, compared to controls. Although renal weights had increased, and epithelial inflammation, oxalate stones, and hemorrhagic spots were observed in the 1000 and 2000 mg/kg groups, statistically relevant weight difference in the organ weights were not observed, compared to controls.	[48]
Portulaca oleracea leaf extract Chloroform/methanolic extract	Male albino Wistar rats; 16/group	60 d	0.5 ml/kg bw, 20% Tween 80	0, 125, 250, or 500 mg/kg bw/d, via gavage	Blood samples were collected on day 14, 28, 42, and 60 of treatment. The 500 mg/kg group showed a significant decrease in the mean hematocrit level on day 28, which was considered incidental, while a significant increase in white blood cell count was observed on day 42. Platelet count was increased in all treatment groups, and was significantly higher in the 125 and 500 mg/kg treated rats on day 60. No significant differences were observed in leukocyte (white blood cell) or erythrocyte (red blood cell) counts.	[49,50]
Portulaca oleracea juice Aqueous extract, 1.5 w/v	Male albino Wistar rats; 6/group	12 d	Distilled water	0.2 ml saline water (control) or 1.5 ml/kg/d extract/d, via gavage	Blood samples were obtained, prior to animal sacrifice, and analyzed to assess the effect of the extract upon liver and kidney function. Samples from rats treated with *Portulaca oleracea* juice showed a statistically significant increase in uric acid (28%), decrease in urea and creatine (33.2 and 28%), reduction in malondialdehyde of liver and kidney (30.9 and 8.7%), and an increase in glutathione, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase in the liver, kidney, and testes (up to 94.1%). A significant reduction in AST, γ-GT, ALP, and bilirubin was observed (-7.4, -10.1, -31, and -13.3%), while the change in ALT was not significant.	[52]

Abbreviations: γ- GT- γ-glutamyl transpeptidase; AST – aspartate aminotransferase; ALP- alkaline phosphatase; ALT- alanine aminotransferase

Table 7. Oral reproductive and developmental toxicity studies

Test Article/ Extraction Solvent	Animals/Group	Vehicle	Dose/Concentration	Type of Study/Procedure	Results	Reference
			ORAL			
Portulaca oleracea leaf and stem extract AEPO and MEPO	Male albino rats; 3/group	distilled water	0, 75 mg/kg bw AEPO or MEPO, via gavage	Fertility study in male albino rats (mating experiment). Three male albino rats were orally administered either 100 ml distilled water, 75 mg/kg bw AEPO, or 75 mg/kg bw MEPO for 50 d. Three untreated, fertile female rats were cohabitated with each of the treated male rats for 4 wk. Vaginal lavages were obtained from these females daily to identify the presence of sperm.	No pregnancy (or sterile mating) occurred between males from either extract group and the untreated female rats. Cohabitation of the control male rat with the untreated female rats resulted in pregnancy.	[53]
Portulaca oleracea leaf and stem extract MEPO	Male Wistar rats; 5/group	20 ml distilled water	0, 400, or 800 mg/kg bw MEPO, via gavage	Reproductive parameters in male Wistar rats. Animals were orally dosed for 14 d, fasted overnight after the last dosing, and then killed. Body weight was measured before and after administration of the test substance. After sacrifice, blood samples, sperm, testes, and epididymis were collected for serum hormones, sperm, and histological analyses.	Body weight significantly increased in both the control and 800 mg/kg MEPO group. No significant changes in serum LH and testosterone levels were observed in either MEPO group, compared to the controls. However, the 800 mg/kg bw group had a significant increase in FSH levels and reduction in sperm count, when compared to controls. Significant reduction in sperm motility was seen in both MEPO- treated groups compared to the controls. While the testis showed no abnormalities in its histology across groups, the epididymis showed some blood congestion in MEPO-treated groups.	[54]
Portulaca oleracea leaf and stem extract AEPO and MEPO	Male albino rats; 5/group	100 ml distilled water	0, 25, 50, 75 mg/kg AEPO or MEPO, via gavage	Reproductive parameters in male albino rats. Animals were orally dosed for 50 d. Body weight was monitored on a weekly basis. One day after the last dose (day 51), blood samples were collected to measure testosterone levels using ELISA and animals were sacrificed to collect semen and prepare testes for histological analysis.	Exposure to either *Portulaca oleracea* extract did not produce any significant changes in body weight, relative to controls. A statistically significant decrease in testosterone levels was observed in rats in the 75 mg/kg AEPO group, and in all MEPO groups. Testosterone decline may explain the concurrently observed acellular seminiferous tubules and Leydig cell hyperplasia in all-treated animals, which was most pronounced in the highest dosage group (75mg/kg). All animals dosed with the extracts had significantly reduced sperm motility, sperm count, and increased % of sperm abnormalities. These differences were mostly dose-dependent. A non-significant reduction in % of viable sperm was observed.	[55]
Portulaca oleracea leaf extract Chloroform and 80% aqueous methanol	Male albino rats; 16/group	0.5 ml 20% Tween 80	0, 125, 250, or 500 mg/kg chloroform or methanolic extract, via gavage	Reproductive parameters in male albino rats. Animals were orally dosed for 60 d. Blood samples, testes, and epididymis were harvested from 4 animals from each of the experimental groups on days 14, 28, 42, and 60.	Neither extract had a significant effect on the testicular weights, or sperm motility, viability, and morphology of treated rats, relative to the controls. A significant increase in sperm count was observed in the animals treated with the 250 mg/kg chloroform extract on days 14 and 28 and in the 250 mg/kg methanolic extract group on day 28, compared to controls. No effects on testosterone levels were observed in animals treated with the chloroform extract; a significant decrease in testosterone levels was observed in the animals treated with 125 and 500 mg/kg methanolic extract on days 28 and 60. No significant changes in testes histology were observed in animals from either treatment groups.	[56]

Table 7. Oral reproductive and developmental toxicity studies

Test Article/ Extraction Solvent	Animals/Group	Vehicle	Dose/Concentration	Type of Study/Procedure	Results	Reference
Portulaca oleracea leaf and stem extract "total flavonoid extract"*	Female Wistar albino rats; 5-6/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Estrogenic/anti-estrogenic activity. Bilaterally ovariectomized, immature female rats received "total flavonoid extract" of *Portulaca oleracea* leaves and stems for 7 d. On day 8, all animals were sacrificed, uteri were fixed in Bouin's fluid and dissected. Biochemical analysis of the adrenal glands and uteri of treated rats was also performed.	Administration of the "total flavonoids extract" at both doses caused a significant decrease in the uterine weight of the immature rats, and produced estrous cycles characterized by significantly longer diestrus phases. Protein and cholesterol (a precursor for steroidal hormone) content of the uterus was also significantly reduced in both doses, by 50% and 30%, respectively. Significant uterine changes included larger diameter and endometrial thickness.	[57]
Portulaca oleracea leaf and stem extract "total flavonoid extract"*	Female Wistar albino rats; 5/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Flavonoid (estrogenic) effect on reproductive organ and body weight. All three groups were dosed for 10 d. On day 11, all animals were weighed and sacrificed. The ovaries and uteri were freed from surrounding tissue, weighed, and dissected.	The ovary and uterine weights were significantly higher in both extract-treated groups. The increase in the wet weight of the ovary was postulated to indicate inhibition of ovulation through suppression of follicular stimulating hormone.	[57]
Portulaca oleracea leaf and stem extract MEPO	Female Wistar rats; 5/group	Distilled water	0, 400, 800 mg/kg bw/d, MEPO, via gavage	Estrous cycle effects after bilateral ovariectomization. All four groups were dosed for 14 d. Negative controls were not ovariectomized, and received distilled water, while the positive controls were ovariectomized, but had no further treatment. Two test groups were ovariectomized and received either 400 or 800 mg/kg bw/d of the test article. Body weight changes, estrous cycle-phase, and blood samples were collected after dosing and analyzed for LH, FSH, E2, TT, and PG.	Significant body weight gain was observed in the positive control group (18.1%) and the 800 mg/kg bw/d group (44.5%), compared to the negative controls. A significant decrease in E2 and TT, and estrous cycle dysregulation, was observed in all 3 ovariectomized groups, while a statistically significant increase in PG levels was only observed in the 400 mg/kg bw/d and 800 mg/kg bw/d groups.	[58]
Portulaca oleracea leaf and stem extract AEPO and MEPO	Female albino rats; 5/group	100 ml distilled water	75 mg/kg/d AEPO or MEPO, via gavage	Estrous cycle effects. Animals were dosed for 21 d and vaginal smears were microscopically examined daily to classify rats into estrous cycle phase and determine cycle length. Vaginal smears were also evaluated for 21 d after cessation of dosing with the extracts; the experimental animals served as their own controls.	Rats were examined for changes in the estrous cycle, both during the 21 d of dosing with either extract, and for 21 d after termination of dosing. No significant changes in duration of estrous cycle phases were observed during dosing, relative to pre-treatment. However, during the 21-d withdrawal of treatment with both extracts, a statistically significant decrease occurred in the proestrus phase. A significant increase in the estrous phase was seen when the AEPO group ceased treatment, and a significant increase in the metestrus phase was seen when the MEPO group ceased treatment, relative to the pre-treatment period.	[59]
Portulaca oleracea leaf extract Chloroform and 80% aqueous methanol	Female albino rats; 5/group	0.5 ml 20% Tween 80	0, 125, 250, or 500 mg/kg chloroform or methanolic extract, via gavage	Estrous cycle and ovarian/uterine histology effects. Animals were dosed for 21 d, which began at the start of the estrous cycle. Vaginal smears were examined daily to assess the phase of the estrous cycle and blood samples were collected on day 21 for hormonal analysis of LH, FSH, progesterone, and estrogen serum levels. After the last dose, animals were killed and the uterine horns and ovaries were harvested for histological analyses (the estrous cycle phase at which the samples were collected was not stated).	No obvious or significant effects were observed on the estrous cycle and ovarian and uterine histology for animals treated with either extract, compared to controls. However, the ovarian sections from 125 mg/kg methanolic extract group showed hypertrophied ovarian follicles. Treatment with both extracts resulted in a decline in the mean serum levels of LH in the proestrus phase, which was not entirely significant. A significant decrease in LH was observed in the 250 mg/kg chloroform extract group; significant decreases in mean serum levels of FSH were also observed in the 250 and 500 mg/kg chloroform extract groups. No other significant effects were seen in LH, FSH, progesterone or estrogen serum levels in the estrus, mestrus, or diestrus phases.	[60]

Table 7. Oral reproductive and developmental toxicity studies

Test Article/ Extraction Solvent	Animals/Group	Vehicle	Dose/Concentration	Type of Study/Procedure	Results	Reference
Portulaca oleracea leaf and stem extract AEPO and MEPO	Female albino rats; 5/group	100 ml distilled water	0.5 ml distilled water, 75 mg/kg/d of AEPO or MEPO, via gavage	Ovarian and uterine histology. Rats showing at least 3 regular 4 - 5-d estrous cycles received either the control, AEPO, or MEPO extract for 25 d. On day 26, all rats were sacrificed and ovaries and uteri were weighed, fixed with Bouin's fluid, and dissected.	Changes in ovarian and uterine weights were not considered significant. No significant pathologic effects on the ovaries or uterus were observed. Both AEPO and MEPO were considered non-toxic to female rat reproductive function.	[59]
Portulaca oleracea leaf and stem extract "total flavonoid extract"*	Female Wistar albino rats; 6/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Abortifacient activity. Female rats of estrous phase were kept with male rats of proven fertility in a ratio of 2:1. Upon evidence of mating, they were separated from the male partner and divided into groups of 6. These rats received *Portulaca oleracea* extract, from day 7 to day 14 of gestation. On day 15, all animals were sacrificed and uterine horns were examined for aborted embryos.	Dams in the 250 mg/kg bw/d group had a 30% abortion rate, while animals in the 500 mg/kg bw/d group had a statistically significant 50% abortion rate.	[57]
Portulaca oleracea leaf and stem extract "total flavonoid extract"*	Female Wistar albino rats; 6/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Implantation study. Same mating strategy and female selection as above study. These rats were dosed from day 1 to day 7 of gestation. On day 10, all animals were sacrificed and uterine horns were examined for number of implants.	A 50% inhibition in implantation was seen at the 250 mg/kg dose, while a statistically significant, 70% inhibition in implantation was seen in the 500 mg/kg dose group (3.22 ± 0.02 vs. 8.12 ± 0.44, in controls). The anti-implantation of the extract was observed after 24 h of the last administered dose.	[57]
Portulaca oleracea leaf and stem extract AEPO and MEPO	Female albino rats; 5/group	100 ml distilled water	0.5 distilled water, or 75 mg/kg/d AEPO or MEPO, via gavage	Teratology study. Adult female rats exhibiting 4 - 5-d estrous cycles, found in the estrous phase, were caged, with virile males, in a 2:1 ratio. Pregnant rats were exposed to control or AEPO/MEPO from: - day 1 to day 5 (implantation/early pregnancy study); - day 6 to day 15 (mid-pregnancy/organogenesis study); or - day 16 to day 21 (late pregnancy study)	A non-significant increase in implantations occurred in rats treated from day 1 to day 5 of gestation with AEPO and MEPO. Treatment of rats from day 6 to 15 with AEPO and MEPO caused a decrease in fetal size for the pups of AEPO-treated dams, and an increase in fetal size for the pups of MEPO-treated dams, relative to controls. Changes in fetal size were not statistically significant. No premature births or abortions occurred, and pups were delivered normally. Treatment of rats from day 16 to 20 caused no significant increase in delivery litter size, and litter weights relative to controls. No resorption or gross malformations were observed in treated and control rats in mid or late pregnancy.	[53]

Abbreviations: AEPO- aqueous extract *Portulaca* oleracea; ELISA- enzyme-linked immunosorbent assay; E2- estradiol; FSH-follicle-stimulating hormone; LH- luteinizing hormone; MEPO – methanolic extract *Portulaca oleracea*; NMRI- nuclear magnetic resonance imaging; PG – progesterone; TT - testosterone

*Methanol, ethanol, ethyl acetate, petroleum ether, diethyl ether, sulfuric acid, chloroform, HCL, potassium hydroxide, hexane, silica Gel 60-120 mesh, Tween 80 phosphate buffer saline, Folin- Ciocalteu reagent, are named as used chemicals, but are not specified as extract solvents.

Table 8. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Portulaca Oleracea Extract (body lotion containing 0.1%)	N/A	applied neat	22 subjects	24-h SIOPT; reference lotion used as control	Not irritating; PII = 0	[67]
Portulaca Oleracea Extract (body moisturizer containing 0.1%)	N/A	0.5 ml; applied neat	26 subjects	Maximization study. Irritation was induced prior to each induction application (total of 5), with an occlusive, 24-h application of 0.05 ml 0.25% aqueous SLS. A 48-h (72 h over the weekend) occlusive application of the test article was applied to the pre-treated sites. After a 10-d non-treatment period, a 1-h occlusive application of 0.05 ml 5.0% aqueous SLS was made to a previously untreated site to induce irritation, after which a 48-h, occlusive challenge application of the test article was made. The challenge site was graded (on a 0-3 scoring scale) 15 - 30 min and 24 h after patch removal.	Not sensitizing; scores were 0 for both readings	[68]
Portulaca Oleracea Extract (face lotion containing 0.5%)	N/A	applied neat	27 subjects	Maximization study. Identical to procedure described above	Not sensitizing	[69]
Portulaca Oleracea Extract (face treatment product containing 0.5%)	N/A	applied neat	26 subjects	Maximization study. Identical to procedure described above	Not sensitizing	[70]

Abbreviations: N/A – not applicable; PII – primary irritation index; SIOPT – single insult occlusive patch test; SLS – sodium lauryl sulfate

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