Safety Assessment of *Paeonia suffruticosa*-Derived Ingredients as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date [i.e.,until **May 18, 2024**] to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific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.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

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

ABBREVIATIONS

CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMEM	Dulbecco's modified Eagle medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOPA	dihydroxyphenylalanine
ECVAM	European Centre for the Validation of Alternative Methods
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GLP	good laboratory practices
IC_{50}	half maximal inhibitory concentration
IL	interleukin
KFDA	Korea Food and Drug Administration
MDM2	mouse double minute 2 homolog
mLIF	murine leukemia inhibitory factor
α-MSH	α-melanocyte stimulating hormone
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NOEL	no-observed-effect-level
OECD	Organisation for Economic Cooperation and Development
p53	tumor protein p53
Panel	Expert Panel for Cosmetic Ingredient Safety
PARP	poly(adenosine diphosphate-ribose) polymerase
PBS	phosphate-buffered saline
Rac1	Ras-related C3 botulinum toxin substrate 1
RCF	relative centrifugal force
RhE	reconstructed human epidermis
RPMI	Roswell Park Memorial Institute
TG	test guideline
TNF-α	tumor necrosis factor alpha
US	United States
VCRP	Voluntary Cosmetic Registration Program
VEGFR-3	vascular endothelial growth factor receptor-3

INTRODUCTION

This assessment reviews the safety of 5 *Paeonia suffruticosa*-derived ingredients as used in cosmetic formulations:

Paeonia Suffruticosa Bark Extract
Paeonia Suffruticosa Extract
Paeonia Suffruticosa Root Extract
Paeonia Suffruticosa Seed Oil
Paeonia Suffruticosa (Tree Peony) Root Bark Extract

Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not included in the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*); however, it had reported uses in 2023 in the US Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP) database and thus is included in this review. According to the *Dictionary*, the other 4 ingredients are all reported to function in cosmetics as skin-conditioning agents; Paeonia Suffruticosa Seed Oil is also reported to function as a hair conditioning agent and a skin protectant (Table 1).[1]

Natural complex substances, such as *Paeonia suffruticosa*, may contain hundreds of constituents. Thus, in this assessment, the Expert Panel for Cosmetic Ingredient Safety (Panel) is evaluating the safety of each of the *Paeonia suffruticosa*-derived ingredients as a whole, complex substance; toxicity from single components may not predict the potential toxicity of botanical ingredients.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted in March 2024. 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. When referring to the plant from which these ingredients are derived, the standard scientific practice of using italics will be followed (i.e., *Paeonia suffruticosa*). Often in the published literature, a general name (e.g., *Paeonia suffruticosa* extract) is used. If it is not known whether the substance being discussed is equivalent to the cosmetic ingredient, the test substance will be identified by the name used in the publication that is being cited. However, if it is known that the substance is a cosmetic ingredient, the *Dictionary* nomenclature (e.g., Paeonia Suffruticosa Extract) will be used. For some studies, the genus and species of the test article is not indicated and it is referred to by the common name, peony; in these instances the common name is used (e.g., peony seed oil). Additionally, the root bark of *Paeonia suffruticosa* can be referred to as moutan cortex, or cortex moutan, in traditional Chinese medicine. However, this term may not be exclusive to the genus and species being reviewed in this report. Thus, test articles have been presented as described in the literature and data potentially referring to *Paeonia suffruticosa* root bark extract has been placed under the Paeonia Suffruticosa (Tree Peony) Root Bark Extract heading herein.

CHEMISTRY

Definition and Plant Identification

The definitions of 4 of the 5 *Paeonia suffruticosa*-derived ingredients reviewed in this assessment (Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not in the *Dictionary*) are presented in Table 1.[1] Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, Paeonia Suffruticosa Root Extract, and Paeonia Suffruticosa Seed Oil all share the generic CAS No. 223747-88-4.

Generally, the bark is the tough protective covering of the woody stems and roots of trees and other woody perennial plants, consisting of cells produced by a cork cambium.[1] The root is the organ of a plant that absorbs and transports water and nutrients, lacks leaves and nodes, and is usually underground. The seed is a propagating sexual structure resulting from the fertilization of an ovule, formed by embryo, endosperm, or seed coat; seeds can also result from non-sexual reproduction through apomixis and similar processes. Peony seeds are aggregate, oblong follicles with dense, yellowish-brown bristles that can be obtained after the peony follicles are cracked.[2] Peony seed is comprised of a hard shell and peony seed kernel.

Paeonia suffruticosa is commonly known as tree peony, moutan, or moutan peony, and has, historically, been cultivated in China.[3,4] It grows as a shrub, up to 4 m in height, has oval leaves, and its flowers are white, pink, red, or reddish-purple in color.[3] The root extends over 1 m into the ground and is 5 - 12 mm in diameter. The outer surface of the root is grayish or yellowish-brown, and pink when the bark falls off.[4]

Chemical Properties

Paeonia suffruticosa bark extract, *Paeonia suffruticosa* extract, *Paeonia suffruticosa* root bark extract, *Paeonia suffruticosa* root extract, and *Paeonia suffruticosa* seed oil are liquids.[5-9] Peony seed oil is semi-transparent and orange-yellow in color.[10] Further data on the chemical properties of the ingredients being reviewed were not found.

Method of Manufacture

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

Paeonia Suffruticosa Bark Extract

A methanolic *Paeonia suffruticosa* bark extract was prepared using 370 g of dried *Paeonia suffruticosa* bark.[5] The dried bark sample was pulverized and extracted with methanol under reflux.

Paeonia Suffruticosa Extract

Paeonia Suffruticosa Extract is the extract of the whole plant, *Paeonia suffruticosa*.[1] In one preparation of aqueous *Paeonia suffruticosa* extract, 100 g of dried *Paeonia suffruticosa* was boiled with 1500 ml of water at 100 °C for 30 min and filtered using a 100-mesh sieve.[6] The extract was concentrated to 100 ml, filtered through a 200-mesh sieve, dried by speed vacuum concentration, and stored at – 20 °C.

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

A *Paeonia suffruticosa* root bark extract was prepared by mixing cortex moutan powder with Roswell Park Memorial Institute (RPMI) 1640 medium and placing in an ultrasonic bath for 60 min.[11] The solution was filtered and concentrated resulting in a stock concentration of 50 mg/ml. An 80% ethanolic *Paeonia suffruticosa* root bark extract was prepared in an ultrasonic bath, filtered, concentrated under reduced pressure, and freeze-dried.[12] The concentrated extract was lyophilized, resulting in 20.5 g of powder which was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution of 200 mg/ml.

Paeonia Suffruticosa Root Extract

Paeonia Suffruticosa Root Extract is the extract of the roots of *Paeonia suffruticosa*.[1] A sample of *Paeonia suffruticosa* root extract was prepared by boiling 200 g of dried roots in 500 ml of distilled water for 1 h at 100 °C.[8] The root extract was then filtered through a 0.45 µm filter and lyophilized overnight. Aqueous *Paeonia suffruticosa* root extracts (ranging from 30 – 800 mg/ml) were reconstituted in 1 ml water.

Paeonia Suffruticosa Seed Oil

A sample of *Paeonia suffruticosa* seed oil was obtained via cold press extraction.[9] *Paeonia suffruticosa* seeds (1000 g) were pressed at room temperature, using a screw press. The expressed liquid was centrifuged at 8000 relative centrifugal force (RCF) for 10 min at 4°C, and the resulting *Paeonia suffruticosa* seed oil was collected and stored.

In a solvent extraction method, 40 g of ground *Paeonia suffruticosa* seeds were extracted with 200 ml of either isopropanol, acetone, *n*-hexane/isopropanol (3:2 v/v), chloroform/methanol (1:1 v/v), ethyl acetate, *n*-hexane, or petroleum ether.[9] Each extract was homogenized with a magnetic stirrer at 7000 rpm for 8 min at room temperature. The homogenized substances were filtered through a Whatman filter paper and the filtrate was collected. The residue was extracted twice with 200 ml of the same solvent and filtrates from all 3 extractions were combined. A rotary evaporator and nitrogen were used to remove residual solvent from the extracts, and the *Paeonia suffruticosa* seed oil samples were stored.

Paeonia suffruticosa seed oil was also extracted from dried ground seed powder via supercritical carbon dioxide (CO_2) extraction, soxhlet extraction, and screw press expression methods.[13] For the CO_2 extraction, ground *Paeonia suffruticosa* seeds (100 g) were added to an extraction vessel. Liquid CO_2 was then transferred to the vessel via a high-pressure pump under optimized conditions (24 MPa, at a rate of 21 l/h, at 46 °C for 124 min). For the soxhlet extraction, *Paeonia suffruticosa* seed powder (20 g) was extracted with n-hexane at 80°C for 8 h; the solvent was removed in a rotary evaporator after extraction. In the screw press expression method, *Paeonia suffruticosa* seed powder (1000 g) was fed from the hopper to the screw press on demand by expeller and the oil was collected at the oil outlet. Oil samples obtained from each method were separated by centrifuging at 9000 rpm for 10 min and kept at 4°C.

Composition and Impurities

In a phytochemical analysis, flavonoids, tannins, terpenoids and steroids, paeonols, and phenols were identified as the main constituents present in the *Paeonia suffruticosa* plant.[14] The presence of various constituents by *Paeonia suffruticosa* plant part is outlined in Table 2.

Paeonia Suffruticosa Extract

Essential oil obtained from hydro-distilled *Paeonia suffruticosa* flowers was analyzed via gas chromatography-mass spectroscopy.[15] The main constituents in the sample of *Paeonia suffruticosa* flower oil were identified as alkanes, alkenes, terpenes, aliphatic alcohols, aliphatic aldehyde, benzoids, other oxygenated non-terpenes, terpene alcohols, and other oxygenated terpenes.

Paeonia Suffruticosa (Tree Peony) Root Bark Extract; Paeonia Suffruticosa Root Extract

The total phenolic content found in 8 extracts of *Paeonia suffruticosa* root bark ranged from 63.81 ± 3.96 to 112.95 ± 3.97 mg gallic acid equivalents/g extract.[16]

Paeonia Suffruticosa Seed Oil

The following were found in a nutritional analysis of peony seeds: crude oil (26.24 – 34.25%), crude protein (18.41 – 25.40%), starch (9.9 – 19.24%), soluble sugar (2.38 – 5.82%), crude fiber (7.8 – 12.29%), and water (8.54 – 10%).[2] The major constituent groups found in peony seed oil are phenolic acids, flavonoids, stilbenoids, monoterpene glycosides, and paenol and its derivatives.[17] In another sample of *Paeonia suffruticosa*, seed oil, fatty acids accounted for 98.46% of the total weight (of which 89.34% comprised of unsaturated fatty acids).[18] Polyunsaturated fatty acids were found in the following amounts: n-3 α-linolenic acid (38.86%), n-6 linoleic acid (26.74%), and oleic acid (23.74%). The fairly low ratio of n-3 to n-6 fatty acids (0.69), uncommonly higher levels of α-linolenic acid, and much higher levels of γ-tocopherol compared to other conventional seed oils were noted by the researchers. Stilbenes were identified as prominent secondary metabolites in a phytochemical analysis of *Paeonia suffruticosa* seeds.[14] As is often observed with botanical extracts, the percent yield and resulting phytochemical composition of *Paeonia suffruticosa* seed oil is affected by the utilized solvent and method of extraction.[9,13]

USE

Cosmetic

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

According to 2023 VCRP survey data, Paeonia Suffruticosa Root Extract is reported to be used in 213 formulations, 173 of which are leave-on formulations (Table 3).[19] The other ingredients have 18 or fewer reported uses. The results of the concentration of use survey conducted by the Council in 2022 indicate Paeonia Suffruticosa Root Extract also has the highest maximum reported concentration of use at up to 0.05% in face powders.[20]

Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, and Paeonia Suffruticosa Root Extract are reported to be used in products applied near the eye (concentrations of use not reported). Additionally, most of the ingredients are used in formulations that could come in contact with mucous membranes (e.g., Paeonia Suffruticosa Seed Oil at up to 0.0025% in bath soaps and detergents).

Some of these ingredients are used in cosmetic powders and possibly cosmetic sprays, and can possibly be inhaled; for example, Paeonia Suffruticosa Root Extract is reported to be used at 0.05% in face powders. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetics 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 *Paeonia suffruticosa*-derived ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[21]

Non-Cosmetic

The root bark of *Paeonia suffruticosa* is often referred to as moutan cortex, cortex moutan, mockdanpi, or mu dan pi, and is used in traditional Chinese medicine for its anti-inflammatory, antioxidant, anti-tumor, anti-diabetic, cardiovascular protective, neuroprotective, and hepatoprotective effects.[11,12,22-24] Fresh *Paeonia suffruticosa* flowers are also considered edible in China.[25] In 2011, the Chinese Ministry of Health acknowledged the high level of α-linolenic acid (≥ 38%) present in peony seed oil and approved the oil as a new resource food.[26]

TOXICOKINETIC STUDIES

No relevant toxicokinetics studies on *Paeonia suffruticosa*-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 natural complex substances because they are a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Paeonia Suffruticosa Seed Oil

Kunming mice (10/sex) were administered a single oral dose of 15,000 mg/kg bw peony seed oil, via gavage.[17,27] All of the animals survived and the acute LD_{50} was determined to be > 15,000 mg/kg bw. Further details could not be gleaned (original article is in Chinese).

In another acute oral toxicity study, ICR mice (10/sex/group) were given 0, 30, or 60 ml/kg peony seed oil in 2 doses, 6 h apart, via gavage.[10] Controls received water. On the first day of dosing, mice showed reduced food intake and decreased activity; oily feces and anal oil staining were more pronounced in the 60 ml/kg group. By the second and third day of dosing, activity levels in all groups normalized. No deaths occurred during the 7-d observation period and no statistically significant pathological changes occurred in the heart, liver, spleen, lungs, kidneys, and gastrointestinal organs of treated mice compared to controls. Further details were not provided (article is in Chinese).

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

In an acute oral toxicity study, the LD_{50} for an herbal mixture containing 14.29% moutan cortex was determined to be > 5000 mg/kg.[23] The mixture comprised a total of 2100 g, including 28.57% *Rehmannia radix preparata*, 14.29% moutan cortex, 14.29% *Schisandrae fructus*, 14.29% Asparagi tuber, 10.71% *Armeniacae semen*, 10.71% *Scutellariae radix*, and 7.14% *Stemonae radix*.

The acute oral toxicity of *Paeonia suffruticosa* tree peony bark extract was evaluated as part of a developmental toxicity study in mice.[24] The LD_{50} was determined to be 3400 mg/kg. No further details were provided for either study.

Short-Term Toxicity Studies

Oral

Paonia Suffruticosa Seed Oil

Healthy rats (12/sex) were administered 1250, 2500, or 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d.[17,27] Vegetable oil (5000 mg/kg bw/d) was given to controls. No abnormal changes in health status, biochemical indexes, hematological and blood biochemical indexes or immune organ indexes were observed at the end of dosing. Based on these results, the maximum non-effective dosage, which is equivalent to the no-observed-effect-level (NOEL), was estimated to be > 5000 mg/kg bw. Further details could not be gleaned (articles in Chinese).

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

The short-term oral toxicity of an herbal mixture containing 14.29% (300 of 2100 g) moutan cortex was evaluated in accordance with Korea Food and Drug Administration (KFDA) Notification no. 2005-60 "The Standards of Toxicity Study for Medicinal Products" and KFDA Notification no. 2005-79 "Good Laboratory Practice (GLP)."[23] Other components of the herbal mixture included: 28.57% *Rehmannia radix preparata*, 14.29% *Schisandrae fructus*, 14.29% *Asparagi tuber*, 10.71% *Armeniacae semen*, 10.71% *Scutellariae radix*, and 7.14% *Stemonae radix*. In a 4-wk study, groups of rats were dosed with 800, 2000, or 5000 mg/kg/d of the herbal mixture, via gavage. A decrease of sodium in 5000 mg/kg/d females was considered test article-related. Increased liver weights were observed in the 2000 and 5000 mg/kg/d groups, although the statistical significance was not confirmed (no further details provided).

Subchronic Toxicity Studies

Oral

Paeonia Suffruticosa Seed Oil

Groups of Sprague-Dawley rats (10/sex/group) were administered 0, 5, or 10 ml/kg/d peony seed oil, via gavage, for 90 d.[10] Controls received water. Body weights were measured every 10 d. After 90 d, the heart, liver, spleen, lungs, kidneys, brain, adrenal glands, testes, uterus, and ovaries were removed, weighed, and organ:body weight ratios were calculated. Blood was collected and analyzed for hematological analyses (hemoglobin, red blood cell and white blood cell counts, neutrophils, lymphocytes, and platelets) and biochemical markers (serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea nitrogen, total protein, albumin, total cholesterol, total bilirubin, creatinine, blood sugar, triglycerides, and uric acid). Besides lower blood sugar levels in treated rats, no other statistically significant differences were observed been treated rats and controls. No significant histopathological findings, such as tissue degeneration, inflammation, bleeding, or necrosis, were observed upon necropsy. (No further details provided; article is in Chinese).

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

In a 13-wk oral toxicity study, groups of male and female Sprague-Dawley rats (10/sex/group) were administered 0, 750, 1500, or 3000 mg/kg of the previously described herbal mixture (containing 14.29% moutan cortex), dissolved in saline, via gavage.[23] No mortality, clinical changes related to test article administration, or statistically significant differences in body weight or food consumption between treated and control animals were observed. A statistically significant increase in white blood cell values was observed in both male and female rats in the 750 and 3000 mg/kg/d groups; a statistically significant decrease was observed in hematocrit and mean corpuscular hemoglobin values for 750 mg/kg/d female rats, compared to controls. Hemoglobin distribution width and hemoglobin concentrations were notably lower for 3000 mg/kg/d females, compared to controls. However, these values were within the normal range and were not considered to be test-article related. Similarly, notably increased alkaline phosphatase and total bilirubin levels in female rats from the 3000 mg/kg/d group and increased relative liver weight in males from the 3000 mg/kg/d treatment group were within the normal range and occurred in the absence of histopathological effects in the liver, indicating that these changes were not test article-related. No systemic or toxicologically significant changes related to the test article were observed. The no-observed-adverse-effect-level (NOAEL) of the herbal mixture was determined to be 3000 mg/kg/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Vitro

Paeonia Suffruticosa Bark Extract

The embryotoxic potential of an aqueous *Paeonia suffruticosa* tree peony bark extract was evaluated in an embryonic stem cell test, consisting of differentiation and cytotoxicity experiments, validated by the European Centre for Validation of Alternative Methods (ECVAM).[24,28] For the cardiomyocyte differentiation experiment, undifferentiated mouse embryonic stem cell line was maintained in complete medium containing Dulbecco's modified Eagle medium (DMEM) with 20% fetal bovine serum, 2 mM L-glutamine, 0.5% penicillin/streptomycin, 1% non-essential amino acids, 0.1 mM β-mercaptoethanol, and 103 U/ml murine leukemia inhibitory factor (mLIF). For generation of mouse embryonic stem cell line embryoid bodies, cells were cultured in DMEM without mLIF, and were seeded in the complete medium as hanging drops (20 µl each) in the presence of the aqueous extract at concentrations of 0.01, 0.1, 1, 10, 100, 1000, or 10,000 µg/ml for 3 d. Subsequently, embryoid bodies formed at each concentration were plated onto a non-adhesive petri dish for 2 d and then transferred to 24-well plates (1 embryoid body/well) for 5 d. The beat rate of cardiomyocytes from treated-cells was compared with that from untreated cells. These ratio values and corresponding concentrations were used to calculate ID_{50} values, expressed as the concentration of test materials that inhibited differentiation of cardiomyocytes in comparison to the DMEM solvent control. The cytotoxicity of test materials (ranging from $1 \times 10^{-1} - 1 \times 10^{6}$ µg/ml) were determined using mouse embryonic stem cells and mouse fibroblast cell lines in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 10 d of treatment. In cells treated with the *Paeonia suffruticosa* bark extract, mouse fibroblast cytotoxicity was observed before stem cell cytotoxicity or inhibition of differentiation, suggesting a lack of embryotoxicity. These results were confirmed by an in vitro prediction model and *Paeonia suffruticosa* bark extract was classified as non-embryotoxic.

Animal

Paeonia Suffruticosa Seed Oil

The effect of peony seed oil on sperm abnormality was evaluated in male rats.[17,27] Sexually mature male mice were administered 1250, 2500, or 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d. Vegetable oil (5000 mg/kg bw) was given to negative controls and cyclophosphamide (40 mg/kg bw) was given to positive controls. On day 35, animals were killed and both epididymides were collected, sperm specimens were prepared, and eosin staining was performed. Sperm deformity rates were in the normal range (0.8 – 3.4%) and no significant difference in the abnormality rate was observed between each dose group and the negative controls. In an embryonic development study, pregnant rats were orally administered 0.55, 0.75, or 1.1 ml/kg bw/d peony seed oil for 20 d.[17] No significant differences in maternal weight gain, early embryonic development, live fetal mouse development, live fetal bone development, or organ development were observed, compared to controls, suggested that peony seed oil did not have embryotoxic or teratogenic effects on maternal and fetal rats. No further details were provided or could be gleaned (articles are in Chinese).

GENOTOXICITY STUDIES

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

CARCINOGENICITY STUDIES

In Vitro Cell Transformation

Paeonia Suffruticosa Extract

The antimigration and antiproliferative effects of an aqueous *Paeonia suffruticosa* extract upon 786O renal carcinoma cells were evaluated in several tests.[6] In MTT and cell migration assays, the aqueous *Paeonia suffruticosa* extract exhibited an inhibitory effect on cancer cell growth (IC_{50} growth = 1.5 mg/ml) and a cancer cell proliferation and migration ratio that indicated the same effect on (IC_{50} growth/$IC_{50\ migration}$ = 5.0). Polymerization of the actin filament was suppressed and the

ratio of F-actin to G-actin was significantly reduced in *Paeonia suffruticosa* extract-treated cells, compared to controls. Cells treated with *Paeonia suffruticosa* extract had inhibited expression of vascular endothelial growth factor receptor-3 (VEGFR-3) and remarkably reduced phosphorylation of focal adhesion kinase, both of which are involved in the activation of Ras-related C3 botulinum toxin substrate 1 (Rac -1), a modulator of cytoskeletal dynamics.

Paeonia Suffruticosa Root Extract

The oncolytic activity of an aqueous *Paeonia suffruticosa* root extract was investigated in a triple negative breast cancer cell line, MDA-MB-231.[8] Human keratinocyte cells and MDA-MB-231 cells were treated with 0.6, 2.5, or 4 mg/ml aqueous *Paeonia suffruticosa* root extract for 48 h. Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. A biphasic dose-response with cell proliferation at low concentrations (0.6 mg/ml) and reduced cell viability at concentrations greater than 2 mg/ml was observed. Notably, for human keratinocyte cells, 2.5 and 4 mg/ml aqueous *Paeonia suffruticosa* root extracts did not reduce cell viability, which was indicative of a selective oncolytic effect. Cytokine production in MDA-MB-321 cells after 48-h treatment with aqueous *Paeonia suffruticosa* root extracts was examined in an enzyme-linked immunosorbent assay (ELISA). A statistically significant decrease in interleukin-6 (IL-6), interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-α) levels were observed in cells treated with 0.6 mg/ml aqueous extract , but subsequently increased at concentrations greater than 2.5 mg/ml. Levels of interleukin-24 (IL-24) were notably increased at the 2.5 and 4 mg/ml concentrations, when measured by an indirect ELISA, compared to controls; this increase of IL-24 was considered an up-regulation caused by increased IL-2 production. Caspase-Glo assays were performed to measure caspase 3/7, 8, and 9 and to analyze anti-apoptotic effects of the *Paeonia suffruticosa* root extracts. Caspase 3/7 and 9 activity decreased at the 0.6 mg/ml concentration but increased in a dose-dependent fashion in cells treated with 2.5 and 4 mg/ml aqueous extracts; caspase-8 activity was observed to decrease or remain at vehicle-control levels at every concentration. The increase in caspase-9 activity coupled with a decrease in caspase-8 activity indicated a mechanism of action of apoptosis that is intrinsic and possibly mediated through IL-24.

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

The ability of a *Paeonia suffruticosa* root bark extract (root bark powder extracted with RPMI 1640 medium to affect cell viability, cell cycle stage, apoptosis, and cell invasion in human bladder papillary transitional cell carcinoma 5637 cells and mouse bladder carcinoma MB49 cells was examined.[11] MB49, 5637, and SV-HUC1 (human normal epithelium) cells were incubated with 0, 0.5, 1, 2, 3, or 3.5 mg/ml *Paeonia suffruticosa* root bark extract for 24 and 48 h. The IC_{50} values of *Paeonia suffruticosa* root bark extract were 1.6 mg/ml at 24 h and 1.3 mg/ml at 48 h in mouse bladder cancer cells, and 2.0 mg/ml at 24 h and 1.4 mg/ml at 48 h in human bladder cancer cells, which was higher than the IC_{50} value in human normal epithelium at 24 h (3.5 mg/ml). In the cell cycle analysis, exposure to *Paeonia suffruticosa* root bark extract increased the number of cells in the G1 and S phase in mouse bladder cells and human bladder carcinoma cells, showing that the *Paeonia suffruticosa* root bark extract induced the activation of caspase-3, and -8 (via extrinsic apoptosis) in a dose-dependent manner. The invasive activity of the *Paeonia suffruticosa* root bark extract was examined in 5637 cells in the cell assay. The *Paeonia suffruticosa* root bark extract inhibited cell invasion in a dose dependent manner; the inhibition percentage was higher than that of cell growth at the same dose, suggesting anti-invasive activity.

Several tests were performed to investigate whether an ethanolic *Paeonia suffruticosa* root bark extract displays growth suppressive activity and induces apoptosis in human gastric cancer cells.[12] The viability of human gastric cancer cells treated with 0, 0.01, 0.05, 0.1, 0.25, or 0.5 mg/ml *Paeonia suffruticosa* root bark extract for 48 or 72 h, was tested in a MTT assay. Untreated human gastric cancer cells served as negative controls. The *Paeonia suffruticosa* root bark extract inhibited cell growth in both a dose- and time-dependent manner; compared to controls, the IC_{50} values of *Paeonia suffruticosa* root bark extract were approximately 220 and 200 µg/ml at 48 and 72 h, respectively. The lethal concentration (LC_{50}) values of human gastric cancer cells treated with 0, 0.01, 0.05, 0.1, 0.25, or 0.5 mg/ml ethanolic *Paeonia suffruticosa* root bark extract for 48 or 72 h, in a cell cytotoxicity test, were approximately 140 and 190 µg/ml at each time point. To further study the cytotoxic effects of the extract, human gastric cancer cells were treated with 200 µg/ml ethanolic *Paeonia suffruticosa* root bark extract for 12 - 36 h and then analyzed for cell cycle stage and deoxyribonucleic acid (DNA) content using flow cytometry. At this concentration, the *Paeonia suffruticosa* root bark extract increased the sub-G1 apoptotic fraction from 3.81% at 12 h to 18.75% at 36 h in a time-dependent manner; neither untreated controls or positive controls (DMSO-treated cells) showed statistically significant changes in apoptotic fractions. Furthermore, results from a DNA fragmentation ladder analysis showed that ethanolic *Paeonia suffruticosa* root bark extract decreased monolayer cell growth and changed cell morphology in a similar manner to cells treated with cisplatin, an anti-cancer agent. Additionally, the ethanolic *Paeonia suffruticosa* root bark extract was found to cause apoptotic cell death via the extrinsic caspase-dependent apoptosis pathway, due to its activation of the Fas death receptor protein and cleaving of caspase-8, caspase-3, and poly(adenosine diphosphate-ribose) polymerase (PARP). The extract was also shown to increase the expression of the active, phosphorylated form of tumor protein p53 (p53), and to decrease the expression of the active form of phosphorylated mouse double minute 2 homolog (MDM2), a negative regulator of p53. To confirm that p53 is implicated in the apoptosis induced by the *Paeonia suffruticosa* root bark extract, cells were treated with p53 inhibitor, pifithrin-α, and Western blot analysis was performed. Cleavage of caspase-8, caspase-3, and PARP were inhibited by the p53 inhibitor, suggesting that the ethanolic *Paeonia suffruticosa* root bark extract induced apoptosis via the MDM2-p53-dependent pathway in human gastric cancer cells.

Tumor Promotion

Paeonia Suffruticosa Extract

The effects of an aqueous *Paeonia suffruticosa* extract upon tumor growth was evaluated using renal carcinoma cells in a mouse model.[6] Mice were subcutaneously inoculated with 786O renal carcinoma cells in the flank; 2 days after injection, mice (4/group) were orally administered either water or aqueous *Paeonia suffruticosa* extract (290 mg/kg) 5 d/wk and tumors were measured every 5 d till necropsy at 45 d. Statistically significant lower tumor weights were observed in treated mice compared to controls (234.8 vs. 437.5 mg; $p < 0.05$). For pulmonary tumor metastasis experiments, 8 female NOD-SCID mice were intravenously inoculated with 786O renal carcinoma cells (2×10^6) in the lateral tail vein. Two days after injection, mice were randomly divided into 2 groups (4/group) and orally administered water or aqueous *Paeonia suffruticosa* extract (290 mg/kg) 5 d/wk and body weight was measured every 5 d, for 48 d. Lungs of the mice were excised and metastatic nodules were counted to evaluate the approximate pulmonary tumor content. There were a statistically significant lower number of pulmonary nodules in treated mice compared to controls (10 ± 1.2 vs 18 ± 3.3 nodules/lung; $p < 0.01$). No statistically significant effect on the body weight of the mice was observed, suggesting low oral toxicity of the *Paeonia suffruticosa* extract.

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

In a tumor promotion study, MB49 mouse bladder cancer cells were implanted in female C57BL/6 mice (age 6 wk).[11] After MB49 inoculation, mice were randomly assigned to 2 groups (8 mice/group). One group was intravesically treated with RPMI 1640 medium, and the other group received 2.5 mg/mouse *Paeonia suffruticosa* root bark extract intravesically every other day from day 16 to 24. On day 26, the mice were killed and bladder volumes were measured before formalin fixation. After cutting the paraffin-embedded bladder tissues into 4 μm sections, slides of each mouse bladder were examined under a microscope in histological analysis by hematoxylin and eosin staining. No statistically significant differences between the body weights of control and treated mice were observed. Treatment with *Paeonia suffruticosa* root bark extract caused a statistically significant decrease in bladder volume and retarded the invasion of tumor tissue into the muscle layer. No notable differences in the blood urea nitrogen, serum creatinine, serum glutamic-oxaloacetic transaminase, or serum glutamic pyruvic aminotransferase levels were observed between both groups. The researchers considered that these results may suggest that intravesical treatment with the *Paeonia suffruticosa* root bark extract decreased bladder tumor size without adversely affecting the liver or kidney.

OTHER RELEVANT STUDIES

Tyrosinase Inhibition

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

The anti-melanogenesis properties of several *Paeonia suffruticosa* root cortex extracts were tested in murine melanoma B16 cells.[29] Plant material was extracted with 95% ethanol (extract 1) and the resulting extract was partitioned between ethyl acetate (extract 2) and water (extract 3). The ethyl acetate layer was partitioned with n-hexane (extract 4) and 90% methanol (extract 5). Subsequently, the 90% methanol layer was subjected to a Sephadex LH-20 column and eluted with methanol to obtain three fractions (extract 6, extract 7, and extract 8). Based on results from an MTT assay, extract 1, extract 3, extract 4, and extract 6 did not induce observable morphological changes in human skin fibroblast Hs68 and B16 cells and were chosen for further anti-melanogenesis analyses. To measure cellular tyrosinase activity, B16 cells were treated with 1 μM α-melanocyte-stimulating hormone (α-MSH) alone and with 50 or 100 μg/ml of the extracts, arbutin, or ascorbic acid for 72 h. Extract 1 and extract 6 inhibited cellular tyrosinase activity by 79.6 and 65%, respectively, compared to controls. Extract 1 and extract 6 also decreased dihydroxyphenylalanine (DOPA)quinone and melanin content in melanoma B16 cells as compared to controls. Notably, extract 6 had an inhibitory effect on melanin formation similar to that of arbutin and ascorbic acid, but with lower cytotoxicity. Extract 3 and extract 4 did not reduce tyrosinase activity, DOPAquinone content, or melanin formation, and were, thus, not included in further tests.

In a fluorescence staining quantitative analysis, melanoma B16 cells were treated with α-MSH alone or with 100 μg/ml of extract 1 or extract 6 for 72 h to determine melanogenesis-related protein expression and nuclei content.[29] Both extracts did not reduce the percentage DNA content or change cell nuclear morphology. Cells treated with 100 μg/ml of either extract showed markedly lower expressions of melanocortin-1 receptor, microphthalmia-associated transcription factor, tyrosinase, and tyrosinase-related protein-1 (tyrosinase-related protein-2 levels were not affected). The researchers surmised that extract 1 and extract 6 may inhibit melanin synthesis through the downregulation of these associated enzymes.

The inhibitory effect of 2 *Paeonia suffruticosa* root bark extracts (aqueous and ethanolic) upon tyrosinase activity was evaluated in A2058 human melanoma cells.[7] First, cells were incubated with 0.5, 1, 2, 2.5, or 5 mg/ml of the extracts, paeonol (a bioactive component of the extract), or arbutin (positive control) for 24 h and followed by ultraviolet (UV) irradiation, in a cellular tyrosinase assay. The ethanolic *Paeonia* suffruticosa root bark extract and paeonol were both found to be noncompetitive inhibitors in a kinetic analysis of tyrosinase inhibition. Furthermore, the ethanolic *Paeonia suffruticosa* root bark extract exhibited a greater tyrosinase inhibition rate compared to the aqueous extract ($p < 0.01$) and was used for additional studies. The ethanolic extract (6.25, 12.5, 25, or 50 μg/ml) showed a moderate and consistent reduction in the melanin content of A2058 melanoma cells when incubated for 24 h in a melanin synthesis assay; no statistically significant

difference in melanin content was observed when compared to paeonol and arbutin-treated cells. In an L-DOPA oxidation assay, cells were treated with 6.25, 12.5, or 25 µg/ml of the ethanolic *Paeonia suffruticosa* root bark extract, paeonol, or arbutin for 24 h; paeonol exhibited the greatest tyrosinase inhibition compared to the ethanol extract and arbutin, but these differences were not statistically significant. Tyrosinase activity was downregulated in a dose-dependent manner by the ethanolic *Paeonia suffruticosa* root bark extract.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Paeonia Suffruticosa Bark Extract

The skin irritation potential of an aqueous *Paeonia suffruticosa* bark extract was predicted in an EpiDerm™ skin irritation test, as outlined by the European Centre for Validation of Alternative Methods (ECVAM) and Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 439.[24] A previously incubated reconstructed human epidermis (RhE) tissue sample was moistened with 25 µl of sterile Dulbecco's phosphate-buffered saline (PBS), followed by application of 100 ml aqueous *Paeonia suffruticosa* bark extract. Two separate solutions containing 1% (v/v) sodium dodecyl sulfate in either sesame seed oil or saline solution were used as positive controls and Dulbecco's PBS-treated epidermis was used as the negative control, respectively. The tissue sample was incubated for 3 h in an MTT reduction assay. Compared to the negative control, cell viability of the skin tissue sample exposed to *Paeonia suffruticosa* bark extract was within the range of 87.5 – 101.1% (> 50%) indicating that the tested extract did not produce irritation.

OCULAR IRRITATION STUDIES

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

SUMMARY

The safety of the following 5 *Paeonia suffruticosa*-derived ingredients as used in cosmetics is reviewed in this safety assessment: Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, Paeonia Suffruticosa Root Extract, Paeonia Suffruticosa Seed Oil, and Paeonia Suffruticosa (Tree Peony) Root Bark Extract. Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not included in the *Dictionary*; however, it has reported uses in the 2023 VCRP database. Thus, it is included in this review. According to the *Dictionary*, the other 4 ingredients are reported to function as skin-conditioning agents in cosmetics. Paeonia Suffruticosa Seed Oil is also reported to function as a hair conditioning agent and a skin protectant.

Paeonia Suffruticosa Root Extract is reported to have the greatest frequency of use, in 213 formulations, 173 of which are leave-on formulations; all other ingredients have 18 or fewer reported uses. Results from a 2022 concentration of use survey conducted by the Council indicate that Paeonia Suffruticosa Root Extract also has the highest reported concentration of use at up to 0.05% in face powders.

Kunming mice (10/sex) were administered a single oral dose of 15,000 mg/kg bw peony seed oil, via gavage. The acute oral LD_{50} was determined to be > 15,000 mg/kg bw. No mortality or statistically significant pathological changes occurred in ICR mice (10/sex/group) administered an oral dose of up to 60 ml/kg peony seed oil. In another acute oral toxicity study, the LD_{50} for a herbal mixture (2100 mg) containing 14.29% moutan cortex (300 g) was determined to be > 5000 mg/kg. The acute oral LD_{50} of a *Paeonia suffruticosa* tree peony bark extract was determined to be 3400 mg/kg in mice.

Healthy rats (12/sex) were administered up to 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d. No abnormal changes in health status, biochemical indexes, hematological and blood biochemical indexes or immune organ indexes were observed; the maximum non-effective dosage, which is equivalent to the NOEL was estimated to be > 5,000 mg/kg bw.

The oral toxicity of an herbal mixture containing 14.29% moutan cortex (300 g of total 2100 g) was evaluated in 4-wk and 13-wk studies in rats, in accordance with KFDA standards for a toxicity study and GLP practices. In the 4-wk study, rats were dosed with 800, 2000, or 5000 mg/kg/d of the herbal mixture; a decrease of sodium in females from the 5000 mg/kg/d group was considered test article-related. The statistical significance of increased liver weights in the 2000 and 5000 mg/kg/d groups was not confirmed. In the 13-wk study, male and female Sprague-Dawley rats (10/sex/group) were administered 0, 750, 1500, or 3000 mg/kg/d of the herbal mixture, dissolved in saline, via gavage. No clinical abnormalities related to the test article administration were observed. A statistically significant increase in both male and female rats in the 750 and 3000 mg/kg/d groups and a statistically significant decrease in hematocrit and mean corpuscular hemoglobin values for female rats in the 750 mg/kg/d group was observed. Hemoglobin distribution width and hemoglobin concentrations were notably lower in female rats from the 3000 mg/kg/d group. However, these values, in addition to notable increases in alkaline phosphatase and total bilirubin levels in the female rats from the 3000 mg/kg/d group and in relative liver weight in males from the 3000 mg/kg/d group, were within the normal range and were not considered to be test article-related. The NOAEL of the herbal mixture was determined to be 3000 mg/kg/d. Groups of Sprague-Dawley rats (10/sex/group) were administered 0, 5, or 10 ml/kg/d peony seed oil, via gavage, for 90 d. Besides lower blood sugar levels in treated rats, no other statistically significant differences were observed between treated rats and controls.

An embryonic stem cell test, validated by ECVAM, was used to evaluate the developmental toxicity of an aqueous *Paeonia suffruticosa* bark extract. Cultured, undifferentiated mouse embryonic stem cells were treated with the aqueous extract at concentrations of 0.01 0.1, 10, 100, 1000, or 10,000 µg/ml for 3 d. The beat rate of cardiomyocytes from the resultant embryoid bodies in treated embryonic stem cells was compared to those in untreated cells and these ratio values and corresponding concentrations were used to calculate differentiation ID_{50} values. In the cytotoxicity portion of the test, mouse embryonic stem cell and mouse fibroblast cell lines were treated with the test materials (in concentrations ranging from 1 x 10^{-1} – 1 x 10^{6} µg/ml) and evaluated in an MTT assay after 10 d of treatment. For cells treated with the aqueous *Paeonia suffruticosa* bark extract, cytotoxicity was observed in mouse fibroblast cell lines prior to stem cell cytotoxicity or inhibition of differentiation, suggesting a lack of embryotoxicity. These results were confirmed by an in vitro prediction model and *Paeonia suffruticosa* bark extract was classified as non-embryotoxic. Sperm deformity rates were within a normal range for male rats administered up to 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d; no significant differences in sperm abnormality rates were observed between each dose group and the negative controls. No embryotoxic or teratogenic effects were seen in an embryonic development study in which pregnant dams were orally dosed with up to 1.1 ml/kg bw/d peony seed oil for 20 d.

An aqueous extract of *Paeonia suffruticosa* exhibited an inhibitory effect on 786O renal carcinoma cell growth ($IC_{50\ growth}$ = 1.5 mg/ml), which was reflected in the ratio between inhibitory effects on cancer cell proliferation and migration ($IC_{50\ growth}/IC_{50\ migration}$ = 5.0). Cells treated with aqueous *Paeonia suffruticosa* extract had inhibited expression of VEGFR-3 and remarkably reduced phosphorylation of focal adhesion kinase, both of which are involved in the activation of Rac -1.

The oncolytic activity of an aqueous *Paeonia suffruticosa* root extract was investigated using multiple tests in a triple negative breast cancer line, MDA-MB-231. In a MTS assay, a biphasic dose-response with cell proliferation at low concentrations and reduced cell viability at concentrations greater than 2 mg/ml was observed in triple negative breast cancer cells treated with up to 4 mg/ml aqueous *Paeonia suffruticosa* root extract. Notably, for human keratinocyte cells, 2.5 and 4 mg/ml aqueous *Paeonia suffruticosa* root extracts did not reduce cell viability, which was indicative of a selective oncolytic effect. A statistically significant decrease in IL-6, IL-2, and TNF-α levels occurred at the 0.6 mg/ml concentration, but subsequently increased at concentrations greater than 2.5 mg/ml in an ELISA assay. IL-24 levels were notably increased in cells treated with 2.5 and 4 mg/ml aqueous *Paeonia suffruticosa* root extracts, compared to controls; this increase of IL-24 was considered an up-regulation caused by increased IL-2 production. In Caspase-Glo assays, caspase 3/7 and 9 activity increased in a dose-dependent fashion in cells treated with 2.5 and 4 mg/ml aqueous extracts; caspase-8 activity was observed to decrease or remain at vehicle-control levels at every concentration. The increase in caspase-9 activity coupled with a decrease in caspase-8 activity indicated a mechanism of action of apoptosis that is intrinsic and possibly mediated through IL-24.

The IC_{50} values of a *Paeonia suffruticosa* root bark extract were higher in mouse bladder and human bladder cancer cells than the IC_{50} value in human normal epithelium at 24 h. Exposure to *Paeonia suffruticosa* root bark extract increased the number of cells in the G1 and S phase in MB49 mouse bladder carcinoma and 5637 human bladder papillary transitional cell carcinoma cells, showing that *Paeonia suffruticosa* root bark extract induced the activation of caspase-3, and -8 (via extrinsic apoptosis) in a dose-dependent manner. *Paeonia suffruticosa* root bark extract inhibited cell invasion in a dose-dependent manner and a higher percentage than that of cell growth at the same dose, suggesting anti-invasive activity.

An ethanolic *Paeonia suffruticosa* root bark extract inhibited cell growth in human gastric cancer cells in both a dose- and time-dependent manner; compared to controls, the IC_{50} values of the *Paeonia suffruticosa* root bark extract were approximately 220 and 200 µg/ml at 48 and 72 h, respectively. In a cell cytotoxicity test, the LC_{50} values of human gastric cancer cells treated with up to 0.5 mg/ml ethanolic *Paeonia suffruticosa* root bark extract, were approximately 140 and 190 µg/ml at 48 or 72 h, respectively. In a cell cycle stage and DNA fragmentation analysis, 200 µg/ml *Paeonia suffruticosa* root bark extract increased the sub-G1 apoptotic fraction from 3.81% at 12 h to 18.75% at 36 h in a time-dependent manner. The extract also decreased monolayer cell growth and changed cell morphology, similar to cells treated with cisplatin, an anti-cancer agent. Additionally, the ethanolic *Paeonia suffruticosa* root bark extract was suggested to induce apoptosis via the MDM2-p53-dependent pathway, an extrinsic caspase-dependent apoptosis pathway, in human gastric cancer cells.

To investigate the effects of aqueous *Paeonia suffruticosa* extract on tumor growth, female NOD-SCID mice were subcutaneously injected with 786O renal carcinoma cells; the animals (4/group) were orally administered either water or *Paeonia suffruticosa* extract (0.29 g/kg) 5 d/wk, and tumors were measured every 5 d till necropsy at 45 d. Tumor weights of the *Paeonia suffruticosa* extract-treated mice were remarkably lower than that of the control group (234.8 mg vs. 437.5 mg). In a pulmonary metastasis test, there were a statistically lower number of pulmonary nodules found in the mice intravenously inoculated with aqueous *Paeonia suffruticosa* extract compared to controls.

MB49 mouse bladder cancer cells were implanted in female C57BL/6 mice and mice (8/group) that were intravesically treated with either RPMI 1640 medium or 2.5 mg/mouse *Paeonia suffruticosa* root bark extract every other day from day 16 to day 24. Mice were killed and bladder volumes were measured on day 26. Treatment with *Paeonia suffruticosa* root bark extract caused a statistically significant decrease in bladder volume and retarded the invasion of tumor tissue into the muscle layer. No statistically significant differences in the blood urea nitrogen, serum creatinine, serum glutamic-oxaloacetic transaminase, or serum glutamic pyruvic aminotransferase levels were observed between both groups. The researchers

considered that intravesical treatment with the *Paeonia suffruticosa* root bark extract may decrease bladder tumor size without adversely affecting the liver or kidney.

The anti-melanogenesis properties of 8 *Paeonia suffruticosa* root cortex extracts (including sequential subfractions) were tested in murine melanoma B16 cells. Cells were treated with 1 μM α-MSH, alone, and with 50 or 100 μg/ml of the extracts, arbutin, or ascorbic acid for 72. The extract obtained with 95% ethanol (extract 1) and a methanolic subfraction obtained from the ethyl acetate layer of the ethanolic extract (extract 6) inhibited cellular tyrosinase activity by 79.6 and 65%, respectively, and decreased DOPAquinone and melanin content in B16 cells compared to controls. Notably, extract 6 had an inhibitory effect on melanin formation similar to that of arbutin and ascorbic acid, but with lower cytotoxicity. In a fluorescence staining quantitative analysis, DNA content or nuclear morphology were not altered in B16 cells treated with 100 μg/ml of extract 1 or extract 6, in the presence of α-MSH; treated cells showed markedly lower expressions of melanocortin-1 receptor, microphthalmia-associated transcription factor, tyrosinase, and tyrosinase-related protein-1 (tyrosinase-related protein-2 levels were not affected). Thus, the researchers surmised that extract 1 and 6 may inhibit melanin synthesis through downregulation of these associated enzymes.

The inhibitory effects of aqueous and ethanolic extracts of *Paeonia suffruticosa* root bark were evaluated in A2058 human melanoma cells in a tyrosinase assay. The ethanolic *Paeonia suffruticosa* root bark extract exhibited a greater tyrosinase inhibition rate compared to the aqueous extract. In subsequent studies, the ethanolic extract (tested at 6.25, 12.5, 25, or 50 μg/ml) showed a moderate and consistent reduction in the melanin content of human melanoma cells; no statistically significant difference in melanin content was observed when compared to cells treated with paeonol or arbutin. In an L-DOPA oxidation assay, paeonol exhibited the greatest tyrosinase inhibition compared to the ethanol extract and arbutin, but these differences were not statistically significant. Tyrosinase activity was downregulated in a dose-dependent manner by the ethanolic *Paeonia suffruticosa* root bark extract.

A reconstructed human epidermis tissue sample was treated with 100 ml of an aqueous *Paeonia suffruticosa* bark extract in an EpiDerm™ skin irritation test (measured as percent viability in the MTT reduction assay), in accordance with OECD TG 439. Compared to negative controls, cell viability of skin tissue samples exposed to aqueous *Paeonia suffruticosa* bark extract was within the range of 87.5 – 101.1% (> 50%); the tested extract was not considered irritating.

INFORMATION SOUGHT

The following information on these ingredients is being sought, with specific application to cosmetics, for use in the resulting safety assessment:

1. Method of manufacture, composition, and impurities data for these ingredients as used in cosmetics
2. Dermal irritation and sensitization data, at or above the maximum reported concentration of use
3. Toxicological data, and any other information that would inform this safety assessment

TABLES

Table 1. Definitions and functions of *Paeonia suffruticosa*–derived ingredients[1*]

Ingredient/CAS No.	Definition	Function
Paeonia Suffruticosa Bark Extract 223747-88-4 (generic)	Paeonia Suffruticosa Bark Extract is the extract of the bark of *Paeonia suffruticosa*.	Skin-conditioning agents - miscellaneous
Paeonia Suffruticosa Extract 223747-88-4 (generic)	Paeonia Suffruticosa Extract is the extract of the whole plant, *Paeonia suffruticosa*.	Skin-conditioning agents - miscellaneous
Paeonia Suffruticosa Root Extract 223747-88-4 (generic)	Paeonia Suffruticosa Root Extract is the extract of the roots of *Paeonia suffruticosa*.	Skin-conditioning agents - miscellaneous
Paeonia Suffruticosa Seed Oil 223747-88-4 (generic)	Paeonia Suffruticosa Seed Oil is the fixed oil expressed from the seeds of *Paeonia suffruticosa*.	Hair conditioning agent Skin protectants Skin-conditioning agents – emollient Skin conditioning agents – humectant Skin conditioning agents - miscellaneous

*Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not included in this table because it is not an INCI ingredient

Table 2. Constituents in *Paeonia suffruticosa*, by plant part[14]

Constituent*	Flower	Fresh leaves	Root	Root Cortex	Seed
Monoterpenoid Glycosides					
α-(benzolyloxy)paeoniflorin				•	
β-(benzoyloxy)paeoniflorin			•	•	
(-)-paeonisuffrone				•	
(galloyloxy)paeoniflorin				•	
6-*o*-vanillyloxypaeoniflorin				♦	
albiflorin			•	•	
benzoylpaeoniflorin			•	•	
deoxypaeonisuffrone				•	
galloylpaeoniflorin			•	•	
isopaeonisuffral				•	
mudanpioside A				•	
mudanpioside B				•	
mudanpioside C				•	
mudanpioside D				•	
mudanpioside E				•	
mudanpioside F				•	
mudanpioside G				•	
mudanpioside H				•	
mudanpioside I				•	
mudanpioside I				•	
mudanpioside J				•	
oxypaeoniflorin			•	•	
paeoniflorigenone				•	
paeoniflorin			•	•	•
paeonisothujone				•	
paeonisuffral			•		
paeonisuffrone			•		
Flavonoids					
5,6,4'-trihydroxy-7,3'-dimethoxyflavone					•
apigenin 7-neohesperidoside	•				
apigenin 7-rhamnoside	•				
astragalin	•				
catechin				•	•
chalcone (flower)	•				
cosmosin	•				
cyanidine 3,5-glucoside	•				
cyanidine-3-glucoside	•				
kaempferol				•	
kaempferol 3,7-*β*-D-diglucoside	•				
kaempferol 7-rhamnoglucoside	•				
luteolin					•
luteolin 7-glucoside					
pelargonin	•				
peonidin 3,5-di-*O*-*β*-D-glucopyranoside	•				
peonin chloride	•				
populnin	•				
quercetin				•	

Table 2. Constituents in *Paeonia suffruticosa*, by plant part[14]

Constituent*	Flower	Fresh leaves	Root	Root Cortex	Seed
Paeonols					
apiopaeonoside				•	
paenol				•	
paeonolide				•	
paeonoside				•	
suffruticoside A				•	
suffruticoside B				•	
suffruticoside C				•	
suffruticoside D				•	
suffruticoside E				•	
Phenols					
2,3-dihydroxy-4-methoxyacetophenone				•	
2,5-dihydroxy-4-methoxyacetophenone				•	
3-hydroxy-4-methoxyacetophenone				•	
3-hydroxy-4-methoxybenzoic acid				•	
4-hydroxyacetophenone				•	
4-hydroxybenzoic acid				•	
acetovanillone				•	
gallacetophenone				•	
gallic acid				•	
methyl 3-hydroxy-4-methoxybenzoate				•	
methyl gallate				•	
mudanoside A				•	
resacetophenone				•	
trans-caffeic acid stearyl ester				•	
Tannins					
mudanoside B				•	
1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose				•	
1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose		•			
6-*O*-(*m*-galloyl)galloyl-1,2,3,4-tetra-*O*-galloyl-*β*-D-glucose		•			
(-)-epigallochatechin gallate				•	
Stilbenes					
(*Z*)-resveratrol					•
suffruticosol A					•
suffruticosol B					•
suffruticosol C					•
Terpenoids and Steroids					
β-sitosterol				•	
betulinic acid				•	
campesterol				•	
daucosterol				•	
oleanolic acid				•	
Others					
adenosine				•	

*quantities of chemicals not provided; ♦referred to as root bark

Table 3. Frequency (2023)[19] and concentration (2022)[20] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Paeonia Suffruticosa Bark Extract		Paeonia Suffruticosa Extract		Paeonia Suffruticosa Root Extract	
Totals*	**8**	**NR**	**18**	**NR**	**213**	**0.000029 – 0.05**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*6*	*NR*	*14*	*NR*	*173*	*0.00009 - 0.05*
Rinse-Off	*2*	*NR*	*4*	*NR*	*40*	*0.000029 - 0.0025*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	1	NR	3	NR	9	NR
Incidental Ingestion	NR	NR	NR	NR	2	NR
Incidental Inhalation-Spray	4[a]	NR	4[a]; 5[b]	NR	84[a]; 46[b]	0.0011[b]
Incidental Inhalation-Powder	4[a]	NR	4[a]	NR	84[a]; 2[c]	0.05; 0.0014 - 0.005[c]
Dermal Contact	8	NR	16	NR	193	0.000029 - 0.05
Deodorant (underarm)	NR	NR	NR	NR	1[b]	NR
Hair - Non-Coloring	NR	NR	2	NR	12	0.00009 - 0.0011
Hair-Coloring	NR	NR	NR	NR	2	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	2	NR	1	NR	14	0.0025
Baby Products	NR	NR	NR	NR	3	NR
as reported by product category						
Baby Products						
Baby Shampoos					1	NR
Baby Lotions/Oils/Powders/Creams					2	NR
Bath Preparations (diluted for use)						
Other Bath Preparations						
Eye Makeup Preparations						
Eyebrow Pencil					1	NR
Eye Shadow			1	NR	1	NR
Eye Lotion					2	NR
Mascara					2	NR
Other Eye Makeup Preparations	1	NR	2	NR	3	NR
Hair Preparations (non-coloring)						
Hair Conditioner					3	0.00009
Rinses (non-coloring)					3	NR
Shampoos (non-coloring)					5	0.0009
Tonics, Dressings, and Other Hair Grooming Aids					NR	0.0011
Other Hair Preparations			2	NR	NR	0.00009
Hair Coloring Preparations						
Hair Dyes/Colors (all types requiring caution statements and patch tests)					2	NR
Makeup Preparations						
Face Powders					NR	0.05
Makeup Bases					3	NR
Makeup Fixatives					1	NR
Other Makeup Preparations					1	NR
Oral Hygiene Products						
Other Oral Hygiene Products					2	NR
Personal Cleanliness Products						
Bath Soaps and Detergents					7	0.0025
Deodorants (underarm)					1	NR
Douches					2	NR
Other Personal Cleanliness Products	2	NR	1	NR	3	NR
Skin Care Preparations						
Cleansing			2	NR	9	NR
Face and Neck (exc shave)	4	NR	4	NR	55	not spray: 0.0014
Body and Hand (exc shave)					29	not spray: 0.005
Moisturizing			4	NR	42	NR
Night			1	NR	3	NR
Paste Masks (mud packs)			1	NR	3	0.000029
Skin Fresheners					1	NR
Other Skin Care Preparations	1	NR			26	NR

Table 3. Frequency (2023)[19] and concentration (2022)[20] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Paeonia Suffruticosa Seed Oil		Paeonia Suffruticosa (Tree Peony) Root Bark Extract			
Totals*	**4**	**0.0025**	**2**	**NR**		
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*NR*	*NR*	*1*	*NR*		
Rinse-Off	*1*	*0.0025*	*1*	*NR*		
Diluted for (Bath) Use	*3*	*NR*	*NR*	*NR*		
Exposure Type						
Eye Area	NR	NR	NR	NR		
Incidental Ingestion	NR	NR	NR	NR		
Incidental Inhalation-Spray	NR	NR	1[b]	NR		
Incidental Inhalation-Powder	NR	NR	NR	NR		
Dermal Contact	4	0.0025	2	NR		
Deodorant (underarm)	NR	NR	NR	NR		
Hair - Non-Coloring	NR	NR	NR	NR		
Hair-Coloring	NR	NR	NR	NR		
Nail	NR	NR	NR	NR		
Mucous Membrane	4	0.0025	NR	NR		
Baby Products	NR	NR	NR	NR		
as reported by product category						
Baby Products						
Baby Shampoos						
Baby Lotions/Oils/Powders/Creams						
Bath Preparations (diluted for use)						
Other Bath Preparations	3	NR				
Eye Makeup Preparations						
Eyebrow Pencil						
Eye Shadow						
Eye Lotion						
Mascara						
Other Eye Makeup Preparations						
Hair Preparations (non-coloring)						
Hair Conditioner						
Rinses (non-coloring)						
Shampoos (non-coloring)						
Tonics, Dressings, and Other Hair Grooming Aids						
Other Hair Preparations						
Hair Coloring Preparations						
Hair Dyes/Colors (all types requiring caution statements and patch tests)						
Makeup Preparations						
Face Powders						
Makeup Bases						
Makeup Fixatives						
Other Makeup Preparations						
Oral Hygiene Products						
Other Oral Hygiene Products						
Personal Cleanliness Products						
Bath Soaps and Detergents	1	0.0025				
Deodorants (underarm)						
Douches						
Other Personal Cleanliness Products						
Skin Care Preparations						
Cleansing						
Face and Neck (exc shave)						
Body and Hand (exc shave)						
Moisturizing			1	NR		
Night						
Paste Masks (mud packs)			1	NR		
Skin Fresheners						
Other Skin Care Preparations						

NR – not reported

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

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] 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

[b] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

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