
Safety Assessment of *Centella asiatica*-derived Ingredients as Used in Cosmetics

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on this Scientific Literature Review and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst.

INTRODUCTION

The safety of the following ingredients in cosmetics is reviewed in this Scientific Literature Review: centella asiatica extract, centella asiatica callus culture, centella asiatica flower/leaf/stem extract, centella asiatica leaf cell culture extract, centella asiatica leaf extract, centella asiatica leaf water, centella asiatica meristem cell culture, centella asiatica meristem cell culture extract, and centella asiatica root extract. These ingredients function primarily as skin conditioning agents in cosmetic products. In addition to data on *Centella asiatica*-derived ingredients, data on the composition and biological activity/toxicity of *Centella asiatica* are included.

CHEMISTRY

Definition and Structure

Centella asiatica (hydrocotyle; Indian pennywort) is a herbaceous plant of the *Umbelliferae* family.¹ The definitions and functions of centella asiatica ingredients reviewed in this safety assessment are presented in Table 1.²

Method of Manufacture

Centella Asiatica Leaf Extract

Centella asiatica leaf extract has been prepared as follows:³ The *Centella asiatica* plant was cleaned with triple-distilled water and the leaves were separated and freeze-dried. The leaf aqueous extract was prepared by boiling the leaves in triple-distilled water and the extract was then lyophilized and stored at -80°C.

According to another method, the fresh leaves of the *Centella asiatica* plant are air-dried at 40°C and ground to powder, which is then subjected to exhaustive extraction using ethanol in a Soxhlet apparatus.⁴ The dark green liquid extract is concentrated under vacuum, and the resulting dried extract is lyophilized and preserved in a refrigerator at 4°C.

Composition/Impurities

Centella Asiatica Extract

Centella asiatica plant extract consists of the following:⁵

- plant sterols
- flavonoids
- tannins (20 to 25%)
- essential acid (0.1% with beta-chariophylen, trans-beta-pharnesen, and germachrene D)
- phytosterols (campesterol, sitosterol, stigmasterol)
- mucilages
- resins
- free amino acids (alanine, serine, aminobutyrate, aspartate, glutamate, lysine, and threonine)
- flavonoids (derivatives of chercetin and kempferol)
- an alkaloid (hydrochotine)
- vallerine
- fatty acids (linoleic, linolenic, oleic, palmitic, and stearic acids)

Centella asiatica

The composition of *Centella asiatica* has been described as follows, including triterpenoid saponins*:^{6,7}

- asiaticoside*
- centelloside*
- madecassoside*

- asiatic acid*
- volatile oils
- flavonoids
- tannins
- phytosterols
- amino acids
- sugars
- centellin (6-acetoxy-trideca-1,7-dien-4-yn-3-ol)
- asiaticin (p-benzoyloxy methyl-butyl benzoate)
- centellicin (1-(2',3'-dihydroxypropyl)-2-en-3-methyl-6-hydroxy-9-yn-undecanoate)

The most important constituents isolated from *Centella asiatica* were triterpenoid saponins known as centelloids (identified by an asterisk above). Saponins may account for 1% to 8% of all *Centella asiatica* constituents.⁸ The variable quantity of saponins mainly depends on the origin of the plant and can be established by HPLC-UV analysis. Other constituents of *Centella asiatica*, identified as centellosides, are primarily ursane- and oleanane-type pentacyclic triterpenoid saponins. The pharmacological activity of the centellosides is attributable to the compounds asiaticoside, madecassoside, asiatic acid, and madecassic acid. Other centellosides occurring in *C. asiatica* include triterpenic acids (e.g., brahmic acid, madasiatic acid, terminolic acid, centellic acid) as well as their glycosides, namely, brahminoside, madasiaticoside and centelloside. *Centella asiatica* also contains volatile oils (0.1%).

In the *Centella asiatica* plant, grown in Peninsular Malaysia, barium concentrations ($\mu\text{g/g}$ dry weight) ranged from 5.05 to 21.88 $\mu\text{g/g}$ for roots, 3.31 to 11.22 $\mu\text{g/g}$ for leaves, and 2.37 to 6.14 $\mu\text{g/g}$ for stems.⁹

The following levels of arsenic (mg/kg dry weight) were detected in *Centella asiatica* plants grown in arsenic-contaminated paddy fields of West Bengal, India: root (2.21 to 6.34), shoot (1.97 to 3.37), and leaves (0.91 to 2.17).¹⁰

USE

Cosmetic

Centella asiatica extract, *centella asiatica* flower/leaf/stem extract, *centella asiatica* leaf extract, *centella asiatica* leaf water, *centella asiatica* meristem cell culture extract, and *centella asiatica* root extract function as skin conditioning agents in cosmetic products.² Additionally, *centella asiatica* leaf cell culture extract and *centella asiatica* meristem cell culture function as antioxidants, and *centella asiatica* callus culture functions as an oxidant and skin conditioning agent in cosmetic products. A detailed list of *Centella asiatica*-derived ingredient functions is included in Table 1.

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), the following *Centella asiatica*-derived ingredients are being used in cosmetic products:¹¹ *centella asiatica* extract, *centella asiatica* flower/leaf/stem extract, *centella asiatica* leaf extract, and *centella asiatica* meristem cell culture. Results from a survey of ingredient use concentrations conducted by the Personal Care Products Council (Council) in 2014 indicate that *centella asiatica* extract, *centella asiatica* flower/leaf/stem extract, and *centella asiatica* meristem cell culture are being used in cosmetics.¹² The Council survey data also indicate that *Centella asiatica*-derived ingredients are being used in cosmetics at maximum ingredient use concentrations up to 0.5% (i.e., for *centella asiatica* extract in face and neck products [not spray]). Use frequency and concentration of use data on *Centella asiatica*-derived ingredients are included in Table 2.

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

Noncosmetic

Centella Asiatica Extract

The extract from the fresh and dried leaves and stems of *Centella asiatica* contains triterpenic derivatives (madecassic acid, asiatic acid, and asiaticoside), which have been shown to promote epithelialization and have anticellulitic and vasotonic activity.¹³ According to another source, the active principle of *Centella asiatica* is a triterpenic derivative, from

which a glycolic extract is obtained.¹ Reportedly, this glycolic extract is widely used topically in dermatology to promote the epithelialization of wounds and ulcers, and as an anticellulitic and vasotonic.

Centella asiatica

The herb *Centella asiatica* has been used in traditional Asian medicine for many years, especially to treat dermatological conditions, including small wounds, scratches, and burns, and as a hypertrophic wound healing agent and an anti-inflammatory agent, particularly in eczema.⁸ As mentioned above, this is because of the pharmacological activity of the *Centella asiatica* compounds (triterpenoid saponins) asiaticoside, madecassoside, asiatic acid, and madecassic acid.

TOXICOKINETICS

Animal

Centella asiatica

The disposition and metabolism of madecassoside, the main bioactive saponin in *Centella asiatica*, was evaluated using groups of 6 Sprague-Dawley rats.¹⁴ The test substance was administered orally at a single dose of 100 mg/kg. Plasma, heart, liver, spleen, lung, kidney, brain, bile, urine and feces were collected. Madecassoside concentrations in biological samples were determined using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). After oral dosing, madecassoside was widely distributed to the heart, liver, spleen, lung and kidney of rats, and the concentrations of madecassoside in liver and kidney were relatively higher than in other organs. Values for the excretion of madecassoside in bile, urine, and feces were 7.16% (0-12h), 0.25% (0-72h) and 24.68% (0-72h) of the administered dose, respectively. Madecassoside was metabolized by hydrolase isozymes produced by intestinal bacteria, and the following 3 deglycosylated metabolites identified in rat feces were consistent with the sequential cleavage of C-28 glycoside bonds: O-glucopyranosyl(1,6)-glucopyranosyl-2,3,6,23-tetrahydroxyurs-12-en-28-oate; O-glucopyranosyl-2,3,6,23-tetrahydroxyurs-12-en-28-oate, and madecassic acid.

Human

Centella Asiatica Extract

Following a single 30 mg and 60 mg oral dose of centella asiatica extract administered to 12 human subjects, maximum plasma levels of asiatic acid were attained in 4.5 h and 4.2 h, respectively.^{6,15} Plasma half-lives were 2.2 h (30 mg dose) and 3.4 h (60 mg dose), with no detectable levels of the saponin in plasma 24 h post-dosing. The same doses of centella asiatica extract administered orally for 7 days resulted in higher peak plasma concentrations, longer half-lives, and greater area-under-the-curve values.

TOXICOLOGY

Acute Toxicity

Oral

Animal

Centella Asiatica Leaf Extract

The acute oral toxicity of centella asiatica leaf extract was evaluated using groups of 8 adult Wistar albino male rats.⁴ The test substance was administered orally (intubation) at a single dose of 100, 500, 1000, or 2000 mg/kg. The LD₅₀ was 200 mg/kg (calculated value).

Repeated Dose Toxicity

Oral

Human

Centella Asiatica Extract

A study was performed to evaluate the clinical efficacy of centella asiatica extract (extraction method not specified) oral administration and identify any side effects.¹⁶ The study involved 84 diabetic wound patients receiving oral doses of the extract and a placebo group consisting of 86 patients. A mean age of 59 years was reported for patients involved in the study. Two centella asiatica extract capsules (50 mg of extracted asiaticoside/capsule) were taken after a meal 3 times per day for 21 days. Asiaticoside is one of the active components of centella asiatica extract in the wound healing process. No systemic side effects or complications were reported.

Skin Irritation and Skin Sensitization

Animal

Centella Asiatica Extract

The skin irritation threshold of centella asiatica extract, in emulsion prepared from Freund's complete adjuvant (FCA) and physiological saline, was determined using 10 guinea pigs (test protocol not included).¹⁷ Unprocessed dry leaves of *Centella asiatica* were extracted with diethyl ether and ethanol. The irritancy threshold of the extract was determined to be greater than 30%.

The skin sensitization potential of centella asiatica extract (extracted with diethyl ether and ethanol) was evaluated in a sensitization test involving groups of 10 female guinea pigs.¹⁷ The extract (30 mg in FCA and saline) was injected intradermally into the shoulder area. Following an 11-day non-treatment period, the animals were challenged on day 20. Additionally, centella asiatica extract was dissolved in a mixture of acetone/ethanol (1:1) and applied epicutaneously (open) to skin of the right flank. Closed epicutaneous elicitation with centella asiatica extract in petrolatum was performed in accordance with the guinea pig maximization test procedure. Reactions were scored at intervals up to 72 h in accordance with International Contact Dermatitis Research Group criteria. Centella asiatica extract was classified as a weak sensitizer. Using a 30% concentration of the extract for elicitation yielded a + reaction in 1 animal at 24 h and 40 h readings.

Case Reports

Centella Asiatica Extract (from leaf and stem)

Erythema and mild eczematous lesions were observed on keloid skin of a 33-year-old woman after application of Madecassol® ointment (composition described below).¹⁸ Patch test results were positive (++) for Madecassol.

- Titrated extract of *Centella asiatica* (TECA, 1g)
- Asiaticoside (0.4 g in TECA)
- Asiatic acid (0.3 g in TECA)
- Madecassic acid (0.3 g in TECA)
- Glycol stearate (15 g)
- Propylene glycol (30 g)
- White Vaseline (5 g)
- Lavender oil (0.143 ml)
- Geranium oil (0.143 ml)

Negative patch test results were reported for TECA at 1% and 10%. In a second case report, a 23-year-old woman applied Madecassol® ointment on the donor site of her skin graft and itchy, oozing erythematous lesions were observed. Patch test results were positive for the following materials: Madecassol, 10% TECA, propylene glycol (20%, 30%, 40%, and 50%), and geranium oil (20%).¹⁸

A case of allergic contact dermatitis in a 54-year-old woman, with no history of atopic or allergic contact dermatitis, after application of an ointment containing centella asiatica extract was reported.¹³ The patient was patch tested with centella asiatica extract (1% and 10% in petrolatum; and 2% in ethanol 70° [70° = alcohol proof]). Patch testing with the 1% centella asiatica extract resulted in a + reaction at 48 h and a ++ reaction at 72 h and 96 h. A +++ reaction to 2% and 10% centella asiatica extract was observed at 48 h, 72 h, and 96 h.

Centella Asiatica Extract

A 42-year-old woman, with no history of atopy, developed severe dermatitis of the legs after application of a vasotonic cream containing centella asiatica extract (2% in alcohol 70°).¹ The extraction method was not specified. The patient was patch tested using Finn Chambers® and the True Test®. Reactions were scored at 2 and 4 days. A +++ reaction to centella asiatica extract was reported.

A red vesicular reaction, with exudation and intense itching, was observed in a 39-year-old woman after applying a cream containing centella asiatica extract (powder).¹⁹ The extraction method was not specified. Patch testing with the powder yielded a +++ reaction on days 2 and 3. However, negative reactions were observed in 50 control subjects patch tested with 1% centella asiatica extract (same powder) in petrolatum.

An eczematous reaction on both knees of a healthy 38-year-old man was observed after topical application of a cream for joint pain that contained centella asiatica extract.²⁰ The extraction method was not specified. Patch test results for centella asiatica extract were positive (+++ reaction).

Localized, severe eczema on the neck and upper chest was observed in a 42-year-old non-atopic woman after treatment of a scar with Madecassol® ointment (composition described at beginning of section).²¹ Patch test results were positive for the ointment and centella asiatica extract (extraction method not specified) at concentrations of 1% and 5% in petrolatum. Negative patch test results were reported for 20 control subjects patch tested with centella asiatica extract (1% and 5% in petrolatum).

Centella asiatica

Three women (61, 52, and 49 years old) developed jaundice after taking *Centella asiatica* tablets (method of manufacture not specified) as a slimming remedy (i.e., weight loss) for 30, 20, and 60 days, respectively.²² The respective diagnoses were: granulomatous hepatitis with marked necrosis and apoptosis; chronic hepatitis with cirrhotic transformation and intense necroinflammatory activity; and granulomatous hepatitis. All three cases recovered after discontinuing use of *Centella asiatica*.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Centella Asiatica Leaf Extract

The reproductive toxicity of centella asiatica leaf extract (in distilled water) was evaluated using 5 groups of 8 Wistar adult male rats.⁴ Four groups received oral doses (gavage; dose volume = 1 ml) of 10, 50, 80, and 100 mg/kg/day, respectively, for 8 weeks. The fifth group was given distilled water and served as the control. Animals were killed on the last day of dosing (day 60). When compared to the control group, statistically significant ($p < 0.01$ or $p < 0.001$) reductions in sperm viability and motility were noted in each group dosed with centella asiatica leaf extract. In each experimental group, histopathological examination of the testis revealed a significant (p value not stated) decrease in the number of spermatogenic cells (spermatogonia, spermatocyte, spermatid, and sperm) in the seminiferous tubules. Also, when compared to the control group, intertubular spaces and venous congestion were increased in experimental groups. The authors noted that the reported loss in testicular weight likely corresponded to a dose-dependent decrease in mean spermatogenic cells in seminiferous tubules. Additionally, degeneration of seminiferous tubules and a significant (p value not stated) reduction in sperm density were reported. It was concluded that centella asiatica leaf extract was toxic to the reproductive system of male rats.

Centella Asiatica Extract

A study was performed to evaluate the effects of centella asiatica extract (ethanol extract) on the rat testis.²³ *Centella asiatica* was obtained from a local market in Malaysia. The following groups of 8 male Sprague-Dawley rats (dosed orally) were used in the study: low dose group (100 mg/kg body weight), medium dose group (200 mg/kg body weight), high dose group (300 mg/kg body weight), and control group (distilled water). The groups were force fed for 42 consecutive days,

after which the animals were killed and the testis removed for histological examination. Animals of all dose groups had some degeneration of spermatogenic cells and reduction of spermatozoa in the lumen of the seminiferous tubules. When compared to the control group, the serum testosterone level decreased in a dose-dependent manner and there was a significant decrease in cauda epididymal sperm count. A statistically significant reduction ($p < 0.05$) in sperm count was observed in the 200 mg/kg and 300 mg/kg dose groups, but not in the 100 mg/kg dose group. Differences in sperm motility were also observed. Slow or sluggish progressive sperm motility was reported for the control and 100 mg/kg dose group. Non-progressive motility ($< 5 \mu\text{m}/\text{second}$) was reported for both the 200 mg/kg and 300 mg/kg dose groups. In control animals, the testis had normal features, with successive stages of transformation of the seminiferous epithelium into spermatozoa. However, abnormalities in seminiferous tubules were observed in all dose groups. Complete arrest of the seminiferous tubules was observed only in the 300 mg/kg dose group. It was concluded that centella asiatica extract (ethanol extract) caused antispermatogenic and antifertility effects on the reproductive system of male rats.

GENOTOXICITY

In Vitro Assays

Bacterial and Mammalian Cells

Centella Asiatica Leaf Extract

The genotoxicity of centella asiatica leaf extract (acetone extract) was evaluated in the chromosomal aberration assay using human peripheral blood lymphocytes.²⁴ Results were negative over the range of test concentrations (1.075×10^{-4} to 4.17×10^{-4} g/ml). The negative control (dimethyl sulfoxide [DMSO], 5 $\mu\text{l}/\text{ml}$) also produced negative results. The sister chromatid exchange assay was also used to evaluate the genotoxicity of the same test substance using human peripheral blood lymphocytes. Results were also negative over the same concentration range. Again, results for the DMSO control were negative.

The genotoxicity of centella asiatica leaf extract (acetone extract) was evaluated in a chromosomal aberration assay using human peripheral blood lymphocytes.²⁴ Results were negative over the range of test concentrations (1.075×10^{-4} to 4.17×10^{-4} g/ml). The negative control (DMSO, 5 $\mu\text{l}/\text{ml}$) also produced negative results. A sister chromatid exchange assay was also used to evaluate the genotoxicity of the same test substance using human peripheral blood lymphocytes. Results were negative over the same concentration range tested in the chromosomal aberration assay. Again, results for the DMSO control were negative.

Centella Asiatica Extract

The genotoxicity of centella asiatica extract (aqueous extract of edible plant parts) was evaluated in the Ames test using *Salmonella typhimurium* strains TA98 and TA100.²⁵ The extract was tested, with and without metabolic activation, at concentrations of 2 and 5 $\mu\text{g}/\text{plate}$. Results were uniformly negative.

CARCINOGENICITY

Data on the carcinogenicity of *Centella asiatica*-derived ingredients were not found in the published literature.

Anticarcinogenicity

Centella Asiatica Leaf Extract

The cytotoxic activity of centella asiatica leaf extract (aqueous extract) against four cancer cell lines and one normal cell line was studied using the MTT assay, a colorimetric assay for assessing cell viability.²⁶ MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a colorimetric dye. Cultures were incubated with centella asiatica leaf extract at concentrations ranging from 0.1 to 1000 $\mu\text{g}/\text{ml}$. The 50% inhibitory concentrations (IC_{50}) were calculated by linear regression over the range of test concentrations. Centella asiatica leaf extract was cytotoxic to the following cancer cell lines: human breast cancer MDA-MB 231 ($\text{IC}_{50} = 648 \mu\text{g}/\text{ml}$), mouse melanoma B16F₁ ($\text{IC}_{50} = 698 \mu\text{g}/\text{ml}$), and rat glioma C6 ($\text{IC}_{50} = 1,000 \mu\text{g}/\text{ml}$). The leaf extract was not cytotoxic at concentrations up to 1,000 $\mu\text{g}/\text{ml}$ to the human lung carcinoma (A549) and normal hamster kidney (BHK-21) cell lines.

Centella Asiatica Extract

The potential for centella asiatica extract (methanolic extract) to induce apoptosis was evaluated in the following cancer cell lines: MCF-7, HeLa, HepG2, and SW 480.²⁷ Cells grown in 96-well microtitre plates (7000 cells/well) were incubated for 48 h with and without centella asiatica extract (10.5 to 82 µg/100 µL). The MCF-7 cell line was found to be most sensitive to *in vitro* growth inhibitory activity. Centella asiatica extract inhibited proliferation of the MCF-7 cell line in a concentration-dependent manner (LD₅₀ = 66 mg). The highest amount of the extract (82 µg) inhibited MCF-7 cell growth to an extent almost equivalent to that induced by 10 mM tamoxifen. Centella asiatica extract induced apoptosis in MCF-7 cells, which was consistent with the observed nuclear condensation, increased annexin staining, loss of mitochondrial membrane potential, and DNA breaks.

OTHER EFFECTS

Wound Healing

Centella Asiatica Extract

The active ingredients of centella asiatica extract in the process of wound healing are the triterpenoid compounds asiatic acid, madecassic acid, asiaticoside, and madecassodie.¹⁶

Centella asiatica extract has a history of use in keloid management (i.e., anti-scar activity), and asiatic acid is one of the extract's main components.²⁸ Keloids are fibroproliferative disorders characterized by exuberant extracellular matrix deposition. The transforming growth factor (TGF)-β/Smad pathway plays a pivotal role in keloid pathogenesis. In an *in vitro* assay, asiatic acid inhibited TGF-β1-induced collagen and plasminogen activator inhibitor-1 (PAI-1) expression in keloid fibroblasts through peroxisome proliferator-activated receptor-γ (PPAR-γ) activation. The authors noted that this finding suggests that asiatic acid was one of the active constituents of *Centella asiatica* responsible for the successful use of the extract in keloid management.

Human foreskin fibroblast cells were incubated with centella asiatica extract (100 µg/ml) for 48 h.²⁹ The extraction method was not specified. Centella asiatica extract stimulated collagen and fibronectin synthesis in fibroblasts. When compared to control cultures, collagen synthesis was statistically significantly increased (p < 0.05) and fibronectin synthesis was elevated by approximately 1.5-fold.

Centella asiatica

Following oral and topical administration of *Centella asiatica* in rats, increased cellular hyperplasia and collagen production were observed at the site of injury in a wound healing assay. The following served as measurements of increased cellular hyperplasia and collagen production: increased levels of DNA, protein, total collagen, and hexosamine in granulation tissue. More rapid maturation and cross-linking of collagen were observed in animals treated with centella asiatica extract, as determined by elevated stability of acid-soluble collagen and increases in aldehyde content and tensile strength. When compared to control wounds, rats treated with *Centella asiatica* had a higher degree of epithelialization and a higher rate of wound contraction.^{6,30,31}

Effect on Mucopolysaccharide Metabolism

Centella asiatica

Individuals with varicosities receiving 30 mg total triterpenoid fraction of *Centella asiatica* twice daily for 3 months had significantly reduced levels of serum enzymes involved in mucopolysaccharide metabolism (beta-glucuronidase, beta-N-acetylglucosaminidase, and arylsulfatase), compared to baseline values (p < 0.01).^{6,32} The authors noted that these results suggest an effect on the connective tissue of varicosities.

Effect on Nerve Regeneration

Centella Asiatica Extract

Rats given centella asiatica extract (extraction method not specified) in drinking water recovered more quickly after nerve damage, compared to controls. Increased axonal regeneration and more rapid functional recovery were observed.^{6,33} The authors noted that the capacity to regenerate axons is an important component of healing after nerve damage.

Modulation of α -Synuclein Aggregation

Centella Asiatica Leaf Extract

The modulation of α -synuclein aggregation by centella asiatica leaf extract (aqueous extract) was studied.³ α -synuclein aggregation is one of the major etiological factors implicated in Parkinson's disease. The anti-aggregating potential of centella asiatica extract in 3 phases of the aggregation pathway was evaluated. α -synuclein (50 μ M) was dissolved in 20 mM Tris-HCl (pH 8.4) and 100 μ g of freshly dissolved centella asiatica leaf aqueous extract was added. Results indicated that centella asiatica leaf extract completely inhibited the α -synuclein aggregation from monomers. Furthermore, the extract significantly inhibited the formation of oligomer to aggregates and favored disintegration of the preformed fibrils.

Effect on Enzyme Activity

Centella Asiatica Leaf Extract

The effects of centella asiatica leaf extract (aqueous extract) on the activity of subtypes of phospholipase A₂ (PLA₂) in primary cultures of rat cortical neurons were investigated.³⁴ The authors noted that the cholinergic and glutamatergic systems are closely connected to a group of key enzymes in membrane lipid metabolism, the PLA₂ family. It was also noted that there is increasing evidence that free radical damage to brain phospholipids, carbohydrates, proteins, and DNA is involved in neuron death in Alzheimer's disease, and that PLA₂ may play a role in memory impairment and neurodegeneration in this disease by generating free radicals. A dose-dependent inhibitory effect of centella asiatica leaf extract (water extract, 125 to 500 μ g/ml) on cytosolic Ca²⁺-dependent PLA₂ (cPLA₂) activity in rat cortical neurons was observed. At a concentration of 500 μ g/ml, inhibitory activity was maximal (56%) when compared to control cultures. Inhibitory activity at a concentration of 125 μ g/ml was 31%. The extract also caused maximal inhibition (52%) of secretory Ca²⁺-dependent PLA₂ (sPLA₂) activity at a concentration of 500 μ g/ml. However, no significant change in Ca²⁺-independent PLA₂ (iPLA₂) activity was observed.

Effect on Neurotoxicity

Centella Asiatica Extract

Centella asiatica extract (aqueous extract) (100 μ g/mL) mitigated amyloid- β -induced cell death in the MC65 and SH-SY5Y neuroblastoma cell lines.³⁵ The attenuation of amyloid- β -induced alterations in tau expression and phosphorylation in both cell lines was also noted. The authors noted that the accumulation of amyloid- β is a hall mark of Alzheimer's disease, and is known to result in neurotoxicity both *in vivo* and *in vitro*.

Immunomodulatory Activity

Centella Asiatica Extract

The effects of centella asiatica extract (aqueous and ethanol extracts, whole plant) on cell-mediated and humoral immune responses was evaluated.³⁶ In human peripheral blood mononuclear cells (PBMCs), centella asiatica extract (aqueous extract) significantly increased proliferation and the production of IL-2 and TNF- α . Thus, the aqueous extract exerted immunostimulating activity on mitogen-stimulated proliferation of human PBMCs. In contrast, centella asiatica extract (ethanol extract) inhibited human PBMC mitogenesis and the production of IL-2 and TNF- α (i.e., exhibited immunosuppressive activity).

In another experiment, 6 male BALB/c mice were fed centella asiatica extract (aqueous extract, 100 mg/kg body weight) and immunized with bovine serum albumin (BSA). The control group (6 mice) received distilled water. The experimental group had greater responses to both primary and secondary antibodies against BSA when compared to the non-treated group. It was concluded that centella asiatica extracts (aqueous and ethanol) had immunomodulating activity with respect to both non-specific cellular and humoral immune responses.³⁶

SUMMARY

Centella asiatica, the plant source of ingredients reviewed in this safety assessment, is a herbaceous plant of the *Umbelliferae* family. Reportedly, the most important components of *Centella asiatica* (triterpenoid saponins), because of their pharmacological activity, are asiaticoside, madecassoside, asiatic acid, and madecassic acid.

Barium has been detected in the roots, leaves, and stems of *Centella asiatica* plants grown in Malaysia. The highest concentration (22 µg/g dry weight) was detected in the root. In *Centella asiatica* plants grown in arsenic-contaminated paddy fields of India, arsenic has been detected in roots, shoots, and leaves (highest concentration = 6.34 mg/kg dry weight in roots).

Collectively, information supplied to FDA by industry as part of the VCRP and a survey of ingredient use concentrations conducted by the Council indicate that the following *Centella asiatica*-derived ingredients are being used in cosmetic products: centella asiatica extract, centella asiatica flower/leaf/stem extract, centella asiatica leaf extract, and centella asiatica meristem cell culture. The Council survey data also indicate that *Centella asiatica*-derived ingredients are being used in cosmetics at maximum ingredient use concentrations up to 0.5% (i.e., for centella asiatica extract in face and neck products [not spray]).

The glycolic extract of *Centella asiatica* is widely used topically in dermatology to promote the epithelialization of wounds and ulcers and as an anticellulitic and vasotonic agent.

Oral dosing with centella asiatica extract in human subjects yielded plasma half-lives of 2.2 h (30-mg dose) and 3.4 h (60-mg dose) for asiatic acid, with no detectable levels of the saponin remaining at 24 h post-dosing. After oral dosing of rats with madecassoside, this saponin component of *Centella asiatica* was widely distributed to the heart, liver, spleen, lung and kidney, and the levels of madecassoside in the liver and kidneys were relatively higher than in other organs. Values for the excretion of madecassoside in bile, urine, and feces were 7.16% (0-12h), 0.25% (0-72h) and 24.68% (0-72h) of the administered dose, respectively.

In an acute oral toxicity study of centella asiatica leaf extract involving rats, an LD₅₀ of 200 mg/kg was calculated. No systemic toxicity or complications were reported in a study in which 84 diabetic wound patients received oral doses of centella asiatica extract (50 mg extracted asiaticoside/capsule) 3 times per day for 21 days.

The skin irritancy threshold of centella asiatica extract was determined to be > 30% in a test involving 10 guinea pigs. In the skin sensitization phase of this study, centella asiatica extract was classified as a weak sensitizer. Using a 30% concentration of the extract for elicitation yielded a + reaction in 1 guinea pig at 24 h and 40 h readings.

In case reports, patch test results for centella asiatica extract were positive at concentrations as low as 1%. It is important to note that 10% centella asiatica extract (from leaf and stem) yielded a positive reaction and a negative reaction in separate case reports.

In case reports, patch test results for centella asiatica extract were positive at concentrations as low as 1%. It is important to note that 10% centella asiatica extract (from leaf and stem) yielded a positive reaction in one case report and a negative reaction in another case report.

Degeneration of seminiferous tubules and a significant dose-dependent reduction in sperm density were reported in male rats dosed orally with centella asiatica leaf extract (up to 100 mg/kg/day). Centella asiatica extract caused antispermatogenic and antifertility effects on the reproductive system of male rats. A significant reduction ($p < 0.05$) in sperm count was observed in the 200 mg/kg/day and 300 mg/kg/day dose groups, but not in the 100 mg/kg/day dose group.

Centella asiatica leaf extract was not genotoxic in a chromosomal aberration assay involving human peripheral blood lymphocytes. Negative results were also reported for centella asiatica extract in the Ames test with and without metabolic activation.

Carcinogenicity data on *Centella asiatica*-derived ingredients were not identified in the published literature. *In vitro* studies on the anticarcinogenic activity of centella asiatica extract and centella asiatica leaf extract show that these botanicals are cytotoxic to various cancer cell lines.

Table 1. Definitions and functions of the ingredients in this safety assessment.²

Ingredient/CAS No.	Definition	Function
Centella asiatica extract 84696-21-9 84776-24-9	Centella asiatica extract is the extract of the whole plant, <i>Centella asiatica</i> .	<u>Skin-Conditioning Agents - Miscellaneous</u>
Centella asiatica callus culture	Centella asiatica callus culture is a suspension of the cultured callus cells of <i>Centella asiatica</i> .	<u>Antioxidants; Skin-Conditioning Agents - Miscellaneous</u>
Centella asiatica flower/leaf/stem extract	Centella asiatica flower/leaf/stem extract is the extract of the flowers, leaves and stems of <i>Centella asiatica</i> .	<u>Skin-Conditioning Agents - Miscellaneous</u>
Centella asiatica leaf cell culture extract	Centella asiatica leaf cell culture extract is the extract of a culture of the leaf cells of <i>Centella asiatica</i> .	<u>Antioxidants; Skin Protectants</u>
Centella asiatica leaf extract	Centella asiatica leaf extract is the extract of the leaves of <i>Centella asiatica</i> .	<u>Skin-Conditioning Agents - Miscellaneous</u>
Centella asiatica leaf water	Centella asiatica leaf water is an aqueous solution of the steam distillate obtained from the leaves of <i>Centella asiatica</i> .	<u>Skin-Conditioning Agents - Miscellaneous</u>
Centella asiatica meristem cell culture	Centella asiatica meristem cell culture is a suspension of the cultured meristem cells of <i>Centella asiatica</i> .	<u>Antioxidants; Skin Protectants</u>
Centella asiatica meristem cell culture extract	Centella asiatica meristem cell culture extract is the extract of centella asiatica meristem cell culture.	<u>Skin-Conditioning Agents - Emollient</u>
Centella asiatica root extract	Centella asiatica Root Extract is the extract of the roots of <i>Centella asiatica</i> .	<u>Skin-Conditioning Agents - Miscellaneous</u>

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{11,12}

	Centella Asiatica Extract		Centella Asiatica Flower/Leaf/Stem Extract		Centella Asiatica Leaf Extract	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	442	0.00002-0.5	1	0.001	56	NR
Duration of Use						
<i>Leave-On</i>	258	0.00002-0.5	NR	NR	53	NR
<i>Rinse off</i>	62	0.00002-0.082	1	0.001	3	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	43	0.1-0.3	NR	NR	7	NR
<i>Incidental Ingestion</i>	6	0.0003-0.01	NR	NR	7	NR
<i>Incidental Inhalation- Sprays</i>	123	0.0033*	NR	NR	34*	NR
<i>Incidental Inhalation- Powders</i>	105	0.0033**	NR	NR	31**	NR
<i>Dermal Contact</i>	359	0.00002-0.5	1	0.001	47	NR
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	38	0.0005-0.003	NR	NR	2	NR
<i>Hair-Coloring</i>	3	0.028	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	17	0.0001-0.01	NR	NR	8	NR
<i>Baby Products</i>	3	NR	NR	NR	NR	NR
	Centella Asiatica Meristem Cell Culture					
	# of Uses	Conc. (%)				
Totals/Conc. Range	4	0.05-0.1				
Duration of Use						
<i>Leave-On</i>	4	0.05-0.1				
<i>Rinse off</i>	NR	NR				
<i>Diluted for (bath) Use</i>	NR	NR				
Exposure Type						
<i>Eye Area</i>	1	0.05				
<i>Incidental Ingestion</i>	NR	NR				
<i>Incidental Inhalation- Sprays</i>	3*	NR				
<i>Incidental Inhalation- Powders</i>	3	0.05-0.1**				
<i>Dermal Contact</i>	4	0.05-0.1				
<i>Deodorant (underarm)</i>	NR	NR				
<i>Hair - Non-Coloring</i>	NR	NR				
<i>Hair-Coloring</i>	NR	NR				
<i>Nail</i>	NR	NR				
<i>Mucous Membrane</i>	NR	NR				
<i>Baby Products</i>	NR	NR				

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.

*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

**It is possible that these products may be powders, but it is not specified whether the reported uses are powders.

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

References

1. Izu, R., Aguirre, A., Gil, N., and Diaz Perez, J. L. Allergic contact dermatitis from a cream containing *Centella asiatica* extract. *Contact Dermatitis*. 1992;26(3):192-193.
2. Nikitakis, J. and Breslawec H. P. International Cosmetic Ingredient Dictionary and Handbook. 14 ed. Washington, DC: Personal Care Products Council, 2014.
3. Berrocal, R. Vasudevaraju P. Indi S. S. Rao K. R. S. S. an Rao K. S. *In vitro* evidence that an aqueous extract of *Centella asiatica* modulates a-synuclein aggregation dynamics. *Journal of Alzheimer's Disease*. 2014;39:457-465.
4. Heidari, M., Heidari-Vala, H., Sadeghi, M. R., and Akhondi, M. M. The inductive effects of *Centella asiatica* on rat spermatogenic cell apoptosis in vivo. *J Nat Med*. 2012;66(2):271-278.
5. Gohil, K. J. Patel J. A. and Gajjar A. K. Pharmacological review on *Centella asiatica*: A potential herbal cure-all. *Indian J.Pharm.Sci*. 2010;72(5):546-556.
6. Anonymous. Monograph. *Centella asiatica*. *Alternative Medicine Review*. 2007;12(1):69-72.
7. Siddiqui, B. S. Aslam H. Ali S. T. Khan S. and Begum S. Chemical constituents of *Centella asiatica*. *Journal of Asian Natural Products Research*. 2007;9(4):407-414.
8. Bylka, W. Znajdek-Awizen P. Studzinska-Sroka E. and Brezezinska M. Review article. *Centella asiatica* in cosmetology. *Postep.Derm.Alergol*. 2013;1:46-49.
9. Ong, G. H. Yap C. K. Mahmood M. Tan S. G. and Hamzah S. Barium levels in soils in *Centella asiatica*. *Tropical Life Sciences Research*. 2013;24(1):55-70.
10. Tripathi, P., Dwivedi, S., Mishra, A., Kumar, A., Dave, R., Srivastava, S., Shukla, M. K., Srivastava, P. K., Chakrabarty, D., Trivedi, P. K., and Tripathi, R. D. Arsenic accumulation in native plants of West Bengal, India: prospects for phytoremediation but concerns with the use of medicinal plants. *Environ Monit Assess*. 2012;184(5):2617-2631.
11. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2014. Washington, D.C.: FDA.
12. Personal Care Products Council. Concentration of use by FDA product category: *Centella asiatica*-derived ingredients. Unpublished data submitted by the Personal Care Products Council on 4-21-2014. 2014. pp.1
13. Gonzalo Garijo, M. A., Revenga, Arranz F., and Bobadilla, Gonz lez P. Allergic contact dermatitis due to *Centella asiatica*: a new case. *Allergol Immunopathol.(Madr.)*. 1996;24(3):132-134.
14. Leng, D. Han W. Rui Y. Dai Y. and Xia Y. *In vivo* disposition and metabolism of madecassoside, a major bioactive constituent in *Cetella asiatica* (L.) Urb. *Journal of Ethnopharmacology*. 2013;150:601-608.
15. Grimaldi, R., De, Ponti F., D'Angelo, L., Caravaggi, M., and Crema, A. Pharmacokinetics of the total triterpenic fraction of *Centella asiatica* after single and multiple administrations to healthy volunteers: new assay for asiatic acid. *J.Ethnopharmacol*. 1990;28(2):235-241.
16. Paocharoen, V. The efficacy and side effects of oral *Centella asiatica* extract for wound healing promotion in diabetic wound patients. *J.Med.Assoc*. 2010;93(7):S166-S170.
17. Hausen, B. M. *Centella asiatica* (Indian pennywort), an effective therapeutic but a weak sensitizer. *Contact Dermatitis*. 1993;29:175-179.
18. Eun, H. C. and Lee, A. Y. Contact dermatitis due to madecassol. *Contact Dermatitis*. 1985;13(5):310-313.

19. Danese, P. Carnevali C. and Bertazzoni M. G. Allergic contact dermatitis due to *Centella asiatica* extract. *Contact Dermatitis*. 1994;31:201.
20. Bilbao, I., Aguirre, A., Zabala, R., Gonzalez, R., Raton, J., and Diaz Perez, J. L. Allergic contact dermatitis from butoxyethyl nicotinic acid and *Centella asiatica* extract. *Contact Dermatitis*. 1995;33(6):435-436.
21. Gomes, J., Pereira, T., Vilarinho, C., Duarte, Mda L., and Brito, C. Contact dermatitis due to *Centella asiatica*. *Contact Dermatitis*. 2010;62(1):54-55.
22. Jorge, O. A. and Jorge, A. D. Hepatotoxicity associated with the ingestion of *Centella asiatica*. *Rev Esp.Enferm.Dig*. 2005;97(2):115-124.
23. Yunianto, I., Das, S., and Mat, Noor M. Antispermato-genic and antifertility effect of Pegaga (*Centella asiatica* L) on the testis of male Sprague-Dawley rats. *Clin Ter*. 2010;161(3):235-239.
24. Siddique, Y. H., Ara, G., Beg, T., Faisal, M., Ahmad, M., and Afzal, M. Antigenotoxic role of *Centella asiatica* L. extract against cyproterone acetate induced genotoxic damage in cultured human lymphocytes. *Toxicol In Vitro*. 2008;22(1):10-17.
25. Yen, G. C., Chen, H. Y., and Peng, H. H. Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. *Food Chem Toxicol*. 2001;39(11):1045-1053.
26. Pittella, F., Dutra, R. C., Junior, D. D., Lopes, M. T., and Barbosa, N. R. Antioxidant and cytotoxic activities of *Centella asiatica* (L) Urb. *Int J Mol Sci*. 2009;10(9):3713-3721.
27. Babykutty, S. Padikkala J. Sathiadevan P. P. Vijayakurup V. Azis T. K. A. Srinivas P. and Gopala S. Apoptosis induction of *Centella asiatica* on human breast cancer cells. *Afr.J.Trad.CAM*. 2009;6(1):9-16.
28. Bian, D. Zhang J. Wu X. Dou Y. Yang Y. Tan Q. Xia Y. and Gong Z. Asiatic acid isolated from *Centella asiatica* inhibits TGFb1-induced collagen expression in human keloid fibroblasts via PPAR-g activation. *Int.J.Biol.Sci*. 2013;9:1032-1042.
29. Hashim, P. The effect of *Centella asiatica*, vitamins, glycolic acid and their mixtures preparations in stimulating collagen and fibronectin synthesis in cultured human skin fibroblast. *Pak.J.Pharm.Sci*. 2014;27(2):233-237.
30. Suguna, L. K. Sivakumar P. and Chandrakasan G. Effects of *Centella asiatica* extract on dermal wound healing in rats. *Indian J.Exp.Biol*. 1996;34:1208-1211.
31. Shetty, B. S. Udupa S. L. Udupa A. L. and Somayaji S. N. Effect of *Centella asiatica* L (Umbelliferae) on normal and dexamethasone-suppressed wound healing in Wistar albino rats. *Int.J.Low Extrem. Wounds*. 2006;5:137-143.
32. Arpaia, M. R. Ferrone R. Amitrano M. et al. Effects of *Centella asiatica* extract on mucopolysaccharide metabolism in subjects with varicose veins. *Int.J.Clin.Pharmacol.Res*. 1990;10:229-233.
33. Soumyanath, A. Zhong Y. P. Gold S. A. et al. *Centella asiatica* accelerates nerve regeneration upon oral administration and contains multiple active fractions increasing neurite elongation *in vitro*. *J.Pharm.Pharmacol*. 2005;57:1221-1229.
34. Defillipo, P. P., Raposo, A. H., Fedoce, A. G., Ferreira, A. S., Polonini, H. C., Gattaz, W. F., and Raposo, N. R. Inhibition of cPLA2 and sPLA2 activities in primary cultures of rat cortical neurons by *Centella asiatica* water extract. *Nat Prod.Commun*. 2012;7(7):841-843.
35. Gray, N. E., Morr, J., Kelley, J., Maier, C. S., Stevens, J. F., Quinn, J. F., and Soumyanath, A. Caffeoylquinic Acids in *Centella asiatica* Protect against Amyloid- β ; Toxicity. *J Alzheimers Dis*. 2014;40(2):359-373.
36. Punturee, K. Wild C. P. Kasinrerck W. and Vinitketkumnuen U. Immunomodulatory activities of *Centella asiatica* and *Rhinacanthus nasutus* extracts. *Asian Pacific Journal of Cancer Prevention*. 2005;6:396-400.