
HerbEx Licorice Extract

Skin-Whitening, Anti-Inflammatory



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1. GENERAL DESCRIPTION

1.1. Introduction



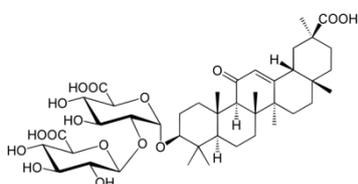
Licorice CG is an lipophilic extract obtained from the roots of *Glycyrrhiza glabra* L. (syn. *Liquiritae officinalis* Moench., Fam. Fabaceae) that is useful as a cosmetic ingredient. *G. glabra* is a perennial herb native to southern Europe, Asia and the Mediterranean, and it is cultivated extensively in Russia, Spain, Iran and India. The plant grows to a height of 1 to 1.5 meters and has dark green leaflets, yellow, blue, or violet flowers, and sweet-flavored rhizomes.

Licorice is one of the most popular and widely consumed herbs in the world. In Indian scripture, Ayurveda, it is used to relieve 'vata' and 'kapha' inflammations, eye diseases, throat infections, peptic ulcers, arthritic conditions, and liver diseases. Its effectiveness in treating these conditions is attributed to the expectorant, emollient, anti-inflammatory, antiviral, anti-hepatotoxic and antibacterial properties of its potent constituents. Licorice has been extensively researched for its medicinal and food uses because of its useful properties. The roots and stolons of the plant contain approximately 5 ~ 9% by weight glycyrrhizin, also known as glycyrrhizic or glycyrrhizic acid. Glycyrrhizin is more than 50 times sweeter than cane sugar. Thus, it can be used as a natural sweetener. Licorice may also be used in cosmetic applications. Its skin whitening, anti-inflammatory, antimicrobial and antioxidative properties make it a useful ingredient in topical applications.

2. COMPOSITION OF HerbEx Licorice Extract

Licorice contains glycyrrhizin, saponins, asparagine, sugars, resin, bitter principles, a volatile oil, and other compounds. The main constituents of licorice are the triterpenoid saponin glycyrrhizin and a mixture of calcium and potassium salts of glycyrrhizic acid. Other constituents include triterpenoid saponins (glabridin, glycyrrhetol, glabrolide, and isoglabrolide), isoflavones (formononetin, neoliquiritin, glabrone and hispaglabridin), triterpene sterols (onocerin, β -amyrin and stigmasterol), and coumarins (herniarin and umbelliferone). The hydrophobic fraction of licorice contains glabridin, the main ingredient, and several flavonoids, while the main constituents of the hydrophilic fraction are glycyrrhizin and glycyrrhetic acid.

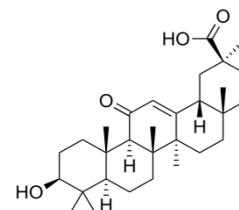
2.1. Glycyrrhizic Acid



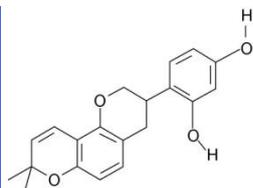
Glycyrrhizic acid (Glycyrrhizin), a saponin glycoside, is one of the compounds obtained from the root extract of licorice. This molecule has been well known for centuries, in traditional medicine, for its anti-inflammatory efficacy. Upon hydrolysis, the glycoside is converted to the aglycone glycyrrhetic acid. Glycyrrhizic acid possesses antiviral properties. It has been reported to promote the activation of interferon and to inhibit the growth of several DNA and RNA viruses. It inactivates herpes simplex virus particles irreversibly. Glycyrrhizic acid's antiviral activity is attributed to its ability to interact with the protein structure of the virus and interfere with its cycle. It inhibits the cytopathic growth and activity of the virus, thus preventing it from attacking healthy cells. Glycyrrhizic augments host resistance against *Candida albicans*, in subjects with thermal injuries. This is probably by inducing CD4 T cells, which suppress type 2 cytokines produced in burn-associated injuries.

2.2. Glycyrrhetic Acid

Glycyrrhetic acid is a pentacyclic triterpenoid derivative of the β -amyrin type. It has expectorant and antitussive properties. It is widely used as a flavoring agent and is frequently employed to mask the taste of bitter drugs such as aloe and quinine. Glycyrrhetic acid has also been reported to possess anti-inflammatory properties. It is believed to inhibit the enzymes that metabolize the prostaglandins, PGE₂ and PGF₂, to their inactive metabolites. This increases the level of prostaglandins in the digestive system. These prostaglandins inhibit gastric secretion but stimulate pancreatic secretion and mucous secretion in the intestines. This may be the reason that glycyrrhetic acid is said to relieve peptic ulcers.



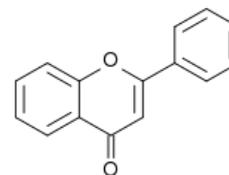
2.3. Glabridin



Glabridin is the main compound in the hydrophobic fraction of licorice extract. It is known for its beneficial effects on the skin due to its anti-inflammatory and antioxidative properties. In addition, glabridin inhibits melanogenesis. Some researchers have established that this effect may be due to the inhibition of tyrosinase activity.

2.4. Flavonoids

The flavonoids in licorice are mainly responsible for its yellow coloring; however, they possess potent properties. The flavonoid components of licorice root exhibit antispasmodic action. Liquirtin, the main flavonoid, exhibits anti-inflammatory activity. Isoliquirtin, glabridin, liquirtigenin, rhamnoliquirtin, neoliquirtin, licoflavonol, licoisoflavones A & B, licoisoflavone, formononetin, glabrol, glabrone, glabrene, hispglabridin A, hispglabridin B are some of the other flavonoids. These flavonoids are potent antioxidants and work to protect the cells of the liver. Preclinical tests (in-vitro) have shown that the flavonoids are capable of killing *Helicobacter pylori*, the bacteria that causes most ulcers and stomach inflammation.



3. ANALYTICAL STUDY

3.1. GC/SIM Quantitation of Fragrance Allergens

In the European Union, the allergenicity of some consumer products has recently come to the forefront. The adoption of the 7th amendment of the European Cosmetic Directive 76/768/EEC requires any cosmetic product containing any of 26 raw materials (30, including isomers) identified by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers as likely to cause a contact allergy when present above certain trigger levels to be declared on the package label. Of these 26, 24 are volatile and can be analyzed by GC/MS.

No.	Substance	CAS No.	GC-Rt	Prominent Ions	Amount Detected
1	(R)-(+)-Limonene	5989-27-5	6.14	136, 93, 68*	Not detected
2	Benzyl alcohol	100-51-6	6.21	108, 91, 79*	Not detected
3	Phenylacetaldehyde	122-78-1	6.38	120, 91*, 65	Not detected
4	Linalool	78-70-6	7.26	136, 93, 71*	Not detected
5	1,4-Dibromobenzene	106-37-6	8.69	236*, 155, 75	Not detected
6	Allylanisole (Estragole)	140-67-0	8.80	148*, 133, 121	Not detected
7	Methyl 2-octynoate	111-12-6	8.82	123, 95*, 75	Not detected
8	Citronellol	106-22-9	9.20	156, 95, 69*	Not detected
9	<i>cis</i> -Citral (neral)	5392-40-5	9.42	109, 94, 69*	Not detected
10	Geraniol	106-24-1	9.59	154, 123, 69*	Not detected
11	<i>trans</i> -Citral (geranial)	5392-40-5	9.84	109, 94, 69*	Not detected
12	Cinnamaldehyde	104-55-2	9.87	131*, 103, 77	Not detected
13	4-Methoxybenzyl alcohol	105-13-5	10.02	138*, 212, 109	Not detected
14	Hydroxy-citronellal	107-75-5	10.06	157, 139, 59*	Not detected
15	Methyl 2-nonynoate	111-80-8	10.24	153, 137, 79*	Not detected
16	Cinnamyl alcohol	104-54-1	10.34	134, 105, 92*	Not detected
17	Eugenol	97-53-0	11.06	164*, 149, 103	Not detected
18	Methyleugenol (4-Allyl-1,2-dimethoxybenzene)	93-15-2	11.65	178*, 163, 147	Not detected
19	<i>cis</i> -Isoeugenol	97-54-1	11.73	164*, 149, 131	Not detected
20	Coumarin	91-64-5	12.18	146*, 118, 90	Not detected
21	Isoeugenol	97-54-1	12.27	164*, 149, 131	Not detected
22	α -Isomethylionone	127-51-5	12.68	206, 150, 135*	Not detected
23	2-(4- <i>tert</i> -Butylbenzyl)propionaldehyde (BMHCA)	80-54-6	13.26	204, 189, 147	Not detected
24	α -Amylcinnamaldehyde	122-40-7	14.67	202, 145, 129*	Not detected
25	Hydroxy-methylpentyl-cyclohexenecarboxaldehyde (HMPCC (Lyrall))	31906-04-4	14.77	192, 136, 59*	Not detected
26	Hydroxy-methylpentyl-cyclohexenecarboxaldehyde (HMPCC)	31906-04-4	14.85	192, 136*, 59	Not detected
27	α -Amylcinnamyl alcohol	101-85-9	15.09	204, 133, 91*	Not detected
28	α -Amylcinnamyl alcohol	101-85-9	15.32	204, 133, 91*	Not detected
29	Farnesol	106-28-5	15.47	136, 81, 69*	Not detected
30	Hexylcinnamaldehyde	101-86-0	15.78	216, 145, 129*	Not detected
31	Benzyl benzoate	120-51-4	16.00	212, 194, 105*	Not detected
32	Hexylcinnamaldehyde	101-86-0	16.06	216, 145, 91*	Not detected
33	Benzyl salicylate	118-58-1	17.11	228, 121, 91*	Not detected
34	4,4'-Dibromobiphenyl	92-86-4	18.46	312*, 310, 152	Not detected
35	Benzyl cinnamate	103-41-3	19.25	238, 131, 91*	Not detected

4. PROPERTIES OF LICORICE

4.1. Applications of Licorice

Licorice has been used medicinally for its demulcent, diuretic, emollient, expectorant, laxative and estrogenic properties. Traditionally, licorice has also been used to relieve asthma, bronchitis, fevers ulcers, and cancers. Today, it is widely used to treat coughs and colds and as a digestive aid. In Japan, physicians have used licorice to treat chronic hepatitis B. Glycyrrhizinic acid (Glycyrrhizin) from licorice interferes with hepatitis B surface antigen and works synergistically with the interferon against hepatitis A virus. Licorice has also been used for treating hepatitis C.

Licorice has several properties that are useful for cosmetic applications. They include:

- Skin whitening property or the ability to inhibit melanogenesis
- Anti-inflammatory property
- Antioxidative property

Glabridin is known for its beneficial effects on the skin due to its anti-inflammatory and skin whitening properties. Glycyrrhizin and glycyrrhizinic acid are also known to have anti-inflammatory properties. The hydrophobic fraction containing glabridin and other flavonoids is known to have an inhibitory effect on melanogenesis. Some researchers have established that this effect may be due to the constituents' ability to inhibit tyrosinase activity. Both in-vitro and in-vivo studies were carried out to study the inhibitory effects of glabridin on melanogenesis and inflammation.

4.2. Skin Whitening Effect / Inhibition of Melanogenesis

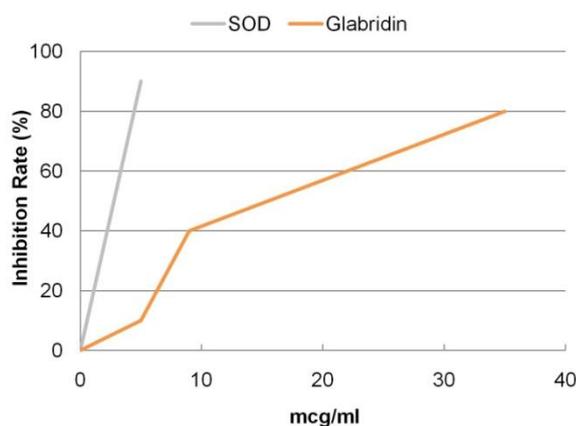
In a comprehensive study carried out by Yokota, T. *et al.*, the inhibitory effects of glabridin on melanogenesis as well as inflammation were examined. The structure-function relationship of glabridin was also studied. Topical skin-depigmentation activities of the active component, glabridin, were examined using UVB-induced pigmented skins of brownish guinea pigs. A 0.5% glabridin solution was applied topically to the skin. Topical application of glabridin significantly reduced pigmentation induced by UVB radiation on the backs of the brownish guinea pigs. Skin samples were also taken from each of the glabridin treated areas for histological studies. The treated tissue was stained with 0.1% DOPA and the inhibition of melanogenesis was evaluated by counting the number of DOPA-positive melanocytes/mm² under an optical microscope. Epidermal histological studies performed showed that DOPA-positive melanocytes reduced in number on the skin treated with glabridin. Treatment with glabridin also lightened the skin color due to inhibition of melanogenesis. The authors concluded that the glabridin present in Licorice roots inhibits both melanin synthesis and inflammation. They also observed that these properties of glabridin were related to its structure.

4.3. Mechanism of Action

Glabridin may inhibit melanogenesis by one of two mechanisms:

- Inhibition of the production of active oxygen species: O₂
- Inhibition of tyrosine: Human tyrosinase is an essential enzyme, which regulates the production of melanin, a group of brown to black pigments in the skin and eyes of humans.

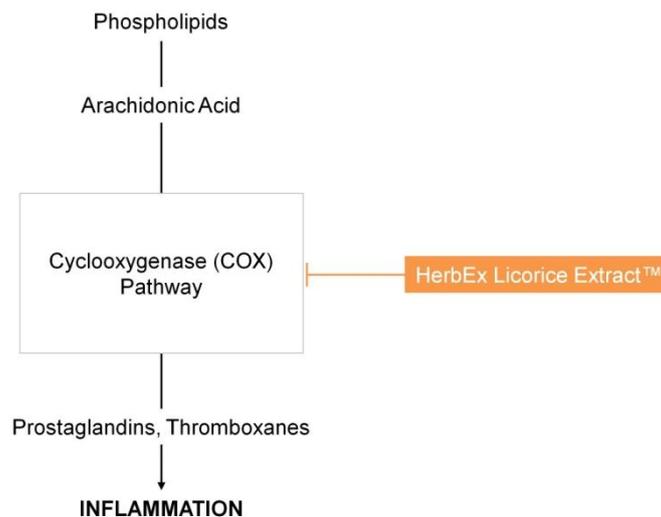
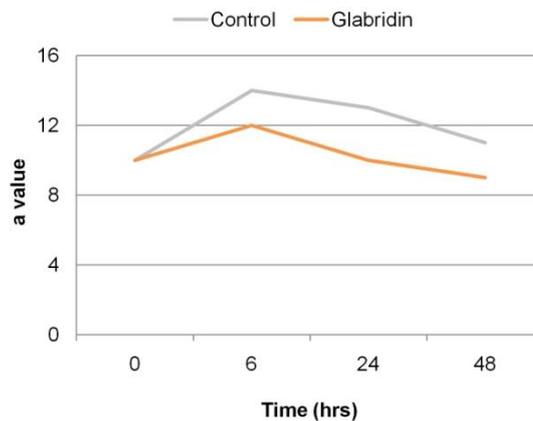
It is a known fact that a number of reactions (e.g. inflammatory, etc.) are induced when human skin is exposed to UV radiation. The membrane phospholipids of the skin tissue are damaged by UV-induced active oxygen. Histological changes occur in the skin that manifest as erythema and skin pigmentation. Active oxygen is one of the species that induces skin pigmentation. Thus, prevention of its production is linked to inhibition of melanogenesis.



To test this, an assay was performed to study the inhibitory effect of glabridin on superoxide anion production. As shown in Figure, glabridin inhibited superoxide (active oxygen) formation at concentrations from 0.33 µg/ml to 33.3 µg/ml. Thus, licorice may be useful for treating conditions like melasma or pigmentation of skin due to sun-exposure.

4.4. Anti-Inflammatory Activity

An assay was performed to test the anti-inflammatory activity of glabridin when used for topical application. UVB-induced pigmented skins of guinea pigs were treated with 0.5% glabridin solution. It was observed that glabridin decreased the inflammation induced by UVB irradiation on the skin. The erythema manifested as redness in skin color is indicated by a^* values. The extent by which the inflammation decreased was calculated by recording the a^* values (of a $L^*a^*b^*$ colorimeter) before irradiation, after irradiation and after the topical application of glabridin. The a^* value increases with the appearance of erythema. As shown in Figure (up), the a^* values of the skin treated with glabridin were lower than those of the control, indicating a decrease in the inflammation. An assay was performed to determine the inhibitory effect of glabridin on cyclooxygenase activity. Cyclooxygenase is an enzyme that metabolizes arachidonic acid into prostaglandins, which are mediators that initiate the inflammatory cascade reaction. It was observed that addition of 6.25 $\mu\text{g/ml}$ glabridin inhibited the cyclooxygenase activity with respect to the control. The positive control in this experiment was indomethacin, a known cyclooxygenase inhibitor. It is believed that glabridin has the anti-inflammatory effect through the arachidonic acid cascade by inhibition to cyclooxygenase.



4.5. Antioxidative Activity

As discussed in the assay performed to test the inhibition of superoxide production by glabridin, it can be said that glabridin has an antioxidative effect in addition to its skin-whitening (anti-melanogenetic) and anti-inflammatory properties. Licorice used topically is documented to reduce the amount of corticosteroids in dermatological infections. This is probably by inhibiting 11- β hydroxysteroid dehydrogenase which is responsible for the conversion of cortisol to corticosterone and thus, potentiating the effects of steroids.

5. AREAS OF COMMERCIAL VALUE

Licorice possesses potent and effective anti-inflammatory, antioxidative as well as melanogenesis-inhibiting properties. Thus, it would be a good ingredient for various cosmetic and/or medicinal skin care products (e.g. creams, lotions, body wash products, etc.). Licorice is used in skin-whitening creams, and there are a number of patented formulations for this purpose. One patented formula for a skin whitening cream contains 0.05% Licorice extract, galacturonic acid, lactic acid, kojic acid, ascorbyl palmitate and tocopheryl linoleate. It is said to lighten the skin color by inhibiting melanin formation, mainly by the inhibition of tyrosinase activity.

6. CLINICAL SAFETY EVALUATION

SUMMARY (#27)

Title	Clinical safety evaluation by single patch test of HerbEx Licorice		
Purpose	To evaluate the irritation potential of cosmetics on human skin		
Test center	Dermapro/Skin Research Center	Test period	23 Nov. – 26 Nov. 2009
Authentication of Test Center	Jae-sook Koh, Ph.D. Scientific Director of Dermapro/Skin Research Center DERMAPRO was validated "QUALITY MANAGEMENT SYSTEM CERTIFICATE" (Certificate No.5855) by KOTRIC Certification Center on the contract research and consulting service on human skin safety and efficacy.		
Sponsor	BioSpectrum, Inc. 202 Jeju Industry Center, 66 Jejudaehakno, Jeju City, Jeju Special Self-Governing Province 690-756, Rep. of Korea Yong-Woo Kim		
Method	<p><u>Subjects:</u> 30 healthy women (mean age 29.3 ± 8.6)</p> <p><u>Procedure:</u> 48 hours single closed patch test on the upper back.</p> <p><u>Reading and interpretation:</u> Reactions were assessed at 30 minutes and 24 hours after patch removal by the dermatologist and principal researcher according to the modification of Frosch & Kligman (1979) and CTFA safety guidelines (1981). All assessments were performed under standard lighting conditions.</p>		
Result	This material (#27) did not show any skin reaction at 30 minutes and 24 hours after patch removal in all subjects.		
Conclusion	This material (#27) did not show a significant evidence of primary irritation on human skin and was classified in the non-irritating range.		
Date of Final Report/Code	4 Dec. 2009 / DSA-PI-929/2		
Safety Test Assessor	Seung-joo Kang, M.D., Ph.D. Scientific Director of Dermapro / Skin Research Center		

7. PRODUCT INFORMATION

7.1. INCI Name

- ♦ Butylene Glycol, Water, Glycyrrhiza Glabra (Licorice) Root Extract

7.2. % Breakdown

FDA Code	INCI Name
A (≥50%)	Butylene Glycol
B (25 ~ 50%)	Water
C (10 ~ 25%)	-
D (5 ~ 10%)	-
E (1 ~ 5%)	Glycyrrhiza Glabra (Licorice) Root Extract
F (0.1 ~ 1%)	-
G (<0.1%)	-

7.3. Recommended Use Level

- We are confident in recommending HerbEx Licorice Extract as an effective ingredient of cosmeceuticals for skin problems due to skin-whitening and inflammation. In addition, HerbEx Licorice Extract can be an ingredient for a wide range of product types, from skin care to shampoos and from creams to gels etc.
- Usage levels may vary from 1% to 5%, depending on the formulation, the typical usage level averages around 2%.

7.4. How to Get the Most of HerbEx Licorice Extract

- Do not add HerbEx Licorice Extract to the formulation at temperature above 55°C, where there is a heating stage it is recommended to add after the cooling process.
- If possible, avoid any strong oxidizers and UV or direct light.

Specialty cosmetic actives from GREEN nature
World leading GREEN manufacturing and technology
Heartfelt GREEN satisfaction to customers

