

URALENSIS LICORICE ROOTS EXTRACT GLABRIDIN FOR NOVEL SKIN-WHITENING



**novel functional ingredients
for multi-purpose formulations**



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CAMPO® Multi-Purpose Cosmetic Base Chemicals & Active Ingredients

CAMPO® Novel Functional Active Cosmetic Ingredient & Raw Materials

Campo Licorice Extract



Description

The generic name derives from the Greek *glykys* and *rhiza*, a sweet root. It is a perennial, herbaceous plant with a thick rhizome of a dark, reddish-brown outside, and yellowish inside, from which its stolons and very long rootlets spring. The leaves are imparipinnatisect, that is, formed by a series of almost opposite leaflets, in pairs, and a central, apical leaflet; they are all oval-elliptic, the base being slightly more rounded than the tip. They contain numerous oil glands, which make them sticky. The almost sessile flowers are arranged in

raceme, emerging corolla is 5-petalled, of a delicate blue tending towards violet. The fruit is a pod, which contains a few dark-colored, slightly oval seeds.

There are two main varieties of this plant: the *Typica* which is known commercially as Spanish licorice and the *Glandulifera* which is generally called Russian licorice. The former comes from Spain and Italy, the latter from Turkey, U.S.S.R. and the countries around Asia Minor towards India.

Parts used:

Rhizomes & roots

Chemical compounds:

Glycyrrhizin, saponin, glucose, gum, sucrose, flavanoids, phytosterol, glabridin (the active component of licorice extract, which is responsible for its whitening effect.)

Ethnobotany

The root boiled in water with some Maidenhead and figs makes a good drink for those who have a dry cough or hoarseness, wheezing or shortness of breath, and for pains in the chest and lungs. It is also good for pains of the reins, strangury and heat of the urine. The juice is also effectual in all diseases of the lungs and chest. A strong decoction of the root given to children loosens the bowels and takes off feverish heats, which attend costiveness.



The juice or extract is made by boiling the fresh roots in water, straining the decoction and, when the impurities have settled, evaporating it over a gentle heat till it no longer sticks to the fingers. It is better to cut the roots into small pieces before boiling to obtain maximum extraction. A pound (454g) of Licorice in 3 parts of water boiled down to 2 parts is best for all purposes. The juice can be obtained by squeezing the roots between two rollers. When made carefully, it is sweeter than the root itself.

Modern uses: A widely used remedy for coughs and lung complaints. It is soothing, expectorant and anti-Spasmodic. For coughs with sore throat it is often combined with Linseed and made into an infusion. One ounce (28g) of powdered Licorice and one teaspoonful of powdered Linseed are simmered in 3 pt of water for 20 minutes. The same can be taken for stomach ulcers.

People with high blood pressure should not use Licorice, as it may exacerbate this condition.

Whitening properties of Licorice Roots

Consumer demand for plant derived ingredients is gaining popularity. This keen interest from natural active materials has affected scientists in the cosmetic industry to come up with whitening agents from these natural sources. Toward these developments, cosmetic companies combine a whitening main active and other plant extracts to enhance the efficacy of their products. One remarkable observation that has been proven effective and has a perceivable result is the inclusion of the licorice extract.

The main active component of licorice roots extract is glabridin. This substance inhibits tyrosinase activity, DOPA chrome tautomerase and spontaneous conversion, while also preventing melanin formation.

Tyrosinase is an enzyme relating to the formation of melanin. In Melanocytes, tyrosinase is synthesized in the lysosome on the surface of the rough-surfaced endoplasmic reticulum. Then, it is modified by saccharide and activated in Golgi-associated endoplasmic reticulum of lysosome (GERL). Activated tyrosinase is secreted as a coated vesicle from GERL and is fused with premelanosome. Promelanosome is considered to be formed in Golgi body or smooth-surfaced endoplasmic reticulum. Melanin formation progresses in this way and melanosome is filled with polymerized melanin polymer.

It has been said that tyrosinase is the only enzyme, which is related to the melanin formation. Synthesis after Dopaquinone is considered to be spontaneously occurred. However, recent research indicates that there are three kind of enzyme including tyrosinase, which is related to the melanin formation.

It is reported that in melanin formation pathway by way of 5,6-dihydroxyindole-2-carboxylic acid (DHICA), DOPA-chrome to DHICA. Likewise the existence of DHICA oxidase or TRP1 is reported. It catalyzes the conversion of DHICA into Indole-5, 6-quinone-2-carboxylic acid. In addition, these two enzymes have the function to stabilize tyrosinase.

In addition to the inhibition of tyrosinase activity, the inhibition of the activity of these two enzymes would be another important key issue for the development of whitening products.

Inhibitory effect of Licorice Roots Extract on:

A. Tyrosinase

Conc. Ext. (mg/ml)	Melanin Formation*	% Inhibition
0	26 ± 3	
10 ⁻³	-3 ± 1	110 ± 4
10 ⁻⁴	10 ± 1	59 ± 4
10 ⁻⁵	24 ± 2	5 ± 8
10 ⁻⁶	26 ± 2	0 ± 8
10 ⁻⁷	25 ± 2	3 ± 8
10 ⁻⁹	30 ± 2	-15 ± 8

*** pmol/24h/1.5x10⁶ cells**

Licorice Roots Extract was added to B16F10 derived Tyrosinase, and melanin formation was measured using ¹⁴C-tyrosine.

B. Culture Cell

Conc. Ext. (mg/ml)	Melanin Formation*	% Inhibition
0	398 ± 20	
10 ⁻²	165 ± 10	60 ± 3
10 ⁻³	237 ± 18	41 ± 5
10 ⁻⁴	319 ± 24	20 ± 6
10 ⁻⁵	364 ± 30	8 ± 8
10 ⁻⁶	389 ± 17	2 ± 4
10 ⁻⁷	394 ± 12	1 ± 3
10 ⁻⁸	410 ± 24	-3 ± 6

*** pmol/24h/1.5x10⁶ cells**

HM-3-KO human melanoma cells were cultured with DMEM, which contains Licorice Roots Extract. After 3 days the cells were harvested, solubilized and the melanogenic activities were measured using ¹⁴C-tyrosine.

C. DOPAchrome Tautomerase

Conc. Ext. (mg/ml)	DOPAchrome Tautomerase Activity*	% Inhibition
0	323 ± 24	
10 ⁻²	95 ± 10	71 ± 3
10 ⁻³	82 ± 8	75 ± 2
10 ⁻¹⁰	82 ± 6	75 ± 2

*** nmol/24h/1.5x10⁶ cells**

HM-3-KO human melanoma cells were cultured with or without Licorice Roots Extract for 3 days. Then the cells were harvested, solubilized and DOPAchrome tautomerase activity was measured by the concentration of DHICA using HPLC.

D. DHI Production

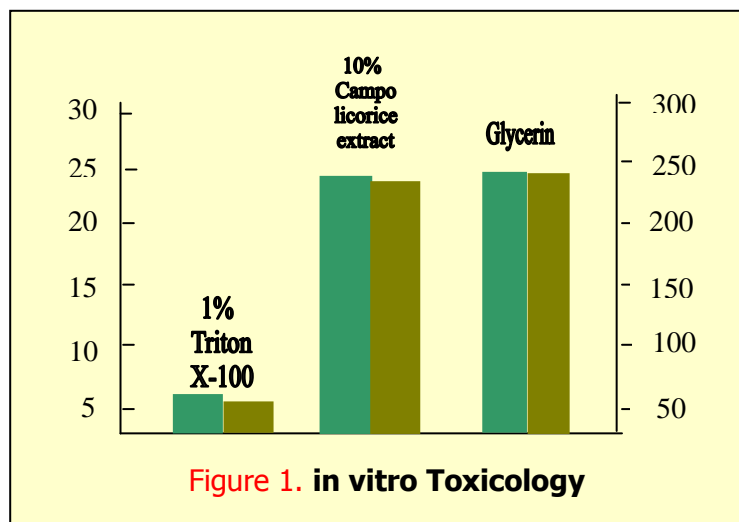
Conc. Ext. (mg/ml)	Spontaneous DHI Production*	% Inhibition
0	14.0 ± 2.6	
10 ⁻²	4.2 ± 0.8	70 ± 6
10 ⁻³	3.3 ± 0.7	76 ± 5
10 ⁻¹⁰	3.1 ± 0.8	78 ± 6

• µg/h/1.5x10⁶ cells

HM-3-KO human melanoma cells were cultured with or without Licorice Roots Extract for 3 days. Then the cells were harvested, solubilized and spontaneous DHI production was measured by the concentration of DHI using HPLC.

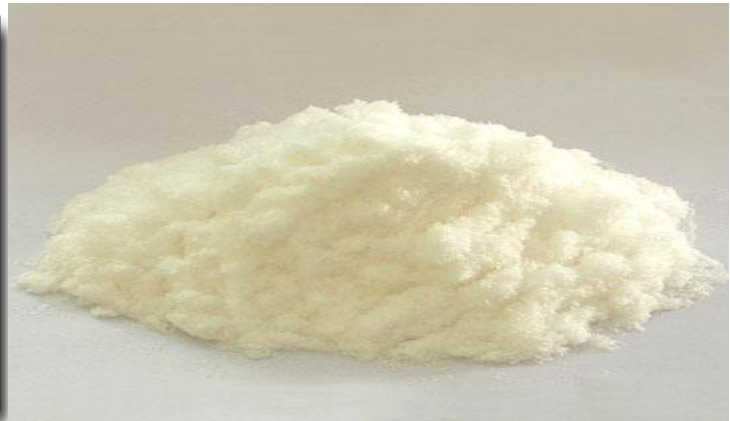
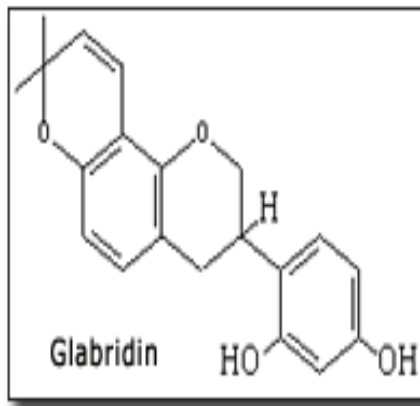
Campo Licorice extract was also safety tested using a variety of in vivo and vitro protocols. The CAMVA was used to determine irritancy. This in vitro assay determines the irritancy of a test compound based on its ability to induce hemorrhage on the chorioallantoic membrane of a chicken egg. Two other in vitro tests were run on Campo Licorice Roots Extract-EpiDerm and Epi-Ocular. EpiDerm is a three - dimensional system composed of human epithelial cells to which the test compound is applied. After incubation, the number of viable cells is measured using the MTT conversion assay.

An ET_{50} is determined, which gives an idea of potential skin toxicity. EpiOcular is a three-dimensional system composed of stratified human keratinocytes to which the test material is applied. After incubation, the number of viable cells is measured using the MTT conversion assay. An ET_{50} is determined, which gives idea of possible ocular irritation . Results are shown in Figure I.



A fifty -person RIPT was run on Campo Licorice Roots Extract to assess its ability to induce skin irritation and sensitization. The method is modified from the 200 person methodology cited in the reference Appraisal of the Safety of Chemicals in Food, Drugs, and Cosmetics. The material was tested at 100% concentration and underwent nine inductive patchings.

GLABRIDIN



GLABRIDIN PURE

CAMPO GLABRIDIN PURE with hydroxyl group at 4' position is the pure isolated compound of isoflavan, a sub-class of the polyphenolic flavonoids fraction of licorice roots extract of *Glycyrrhiza uralensis*. It is known for its beneficial effects on the skin due to its anti-inflammatory and antioxidant properties. In addition, **Campo Glabridin Pure** inhibits melanogenesis.

Campo Glabridin Pure is being the main isoflavan compound of the polyphenolic flavonoid fraction of *Uralensis licorice* roots extract; of which pure isolate is hydro-alcoholic solubilized and freeze-dried into crystalline powder.

It is known for its beneficial effects on the skin due to its anti-inflammatory and antioxidant properties. In addition, **Glabridin** inhibits melanogenesis. Some researchers have established that this effect may be due to the inhibition of tyrosinase activity (1,3).

COSMETIC USAGE

CAMPO GLABRIDIN PURE with hydroxyl group at 4' position has several unique properties that are useful for cosmetic applications.

These properties are among others:

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

CAMPO GLABRIDIN PURE, with its unique structure is the main compound as a pure isolate of Isoflavan of the polyphenolic flavanoids fraction of licorice extract is known for its beneficial effects on the skin due to its anti-inflammatory and skin whitening properties (1.)

Other Licorice flavonoids such as Glycyrrhizin and glycyrrhetic acid are also known to have anti-inflammatory properties (4).

The pure isolated fraction containing the Isoflavan Glabridin Pure is known to have an inhibitory effect on melanogenesis (3).

Some researchers have established that this effect may be due to the isoflavan Glabridin's constituent structure with a hydroxyl group at the 4' is well-related to the unique functional ability to inhibit tyrosinase activity (1, 2, 3).

Both in-vitro and in-vivo studies were carried out to study the inhibitory effects of Glabridin on melanogenesis and inflammation (2).

SKIN WHITENING EFFECT / INHIBITION OF MELANOGENESIS

In a comprehensive study carried out by Yokota, T. et al. (2),

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** alcoholic 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 with hydroxyl group at the 4'.

MECHANISM OF ACTION

Glabridin may inhibit melanogenesis by one of two mechanisms:

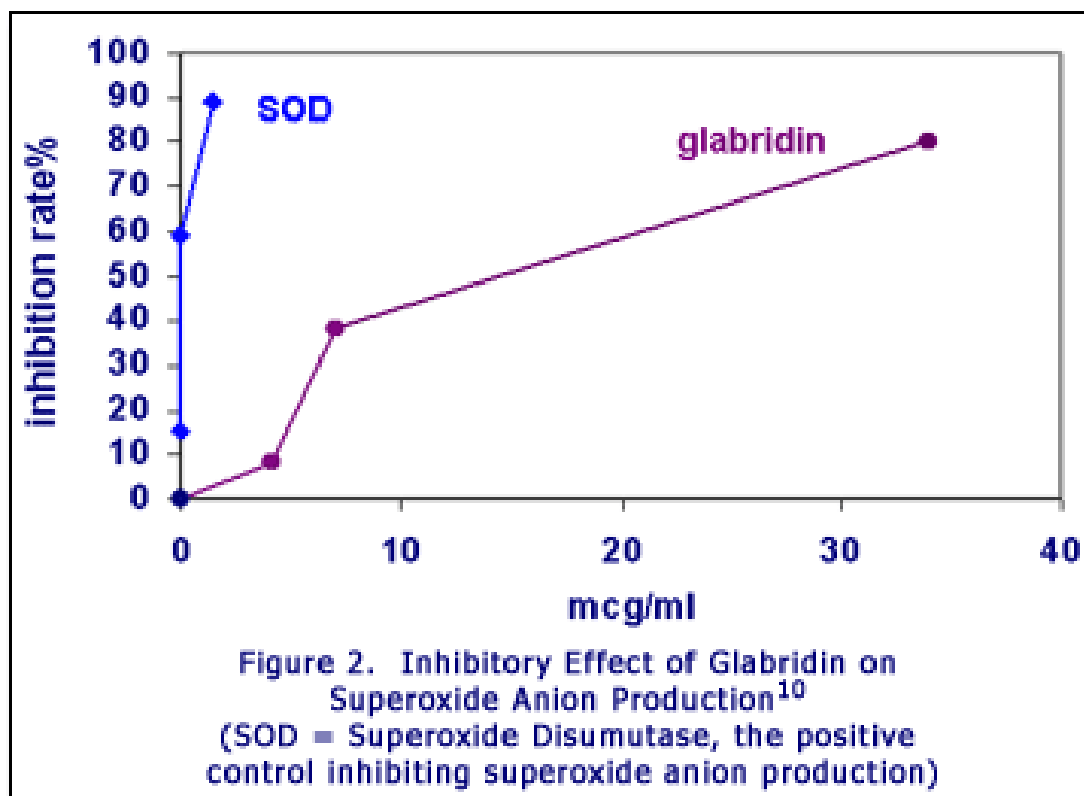
1. Inhibition of the production of active oxygen species: ($O_2^{\cdot-}$)
2. Inhibition of tyrosine: Human tyrosinase is an essential enzyme, which regulates the production of melanin, a group of brown to black pigments in the skin of humans.

It is a known fact that a number of reactions (e.g. inflammatory, etc.) are induced when human skin is exposed to UV radiation⁽⁵⁾.

The membrane phospholipids of the skin tissue are damaged by UV-induced active oxygen.

Histological changes occur in the skin that manifest as erythemas and skin pigmentation **(6,7)**.

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 2**, **Glabridin** inhibited superoxide (active oxygen) formation at concentrations from 0.33 $\mu\text{g/ml}$ to 33.3 $\mu\text{g/ml}$.

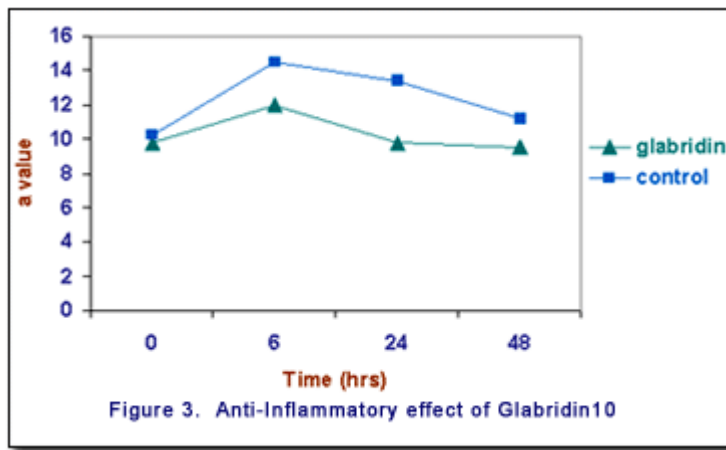


Thus, **Glabridin** may be useful for treating conditions like melasma or pigmentation of skin due to sun-exposure.

ANTI-INFLAMMATORY EFFECTS

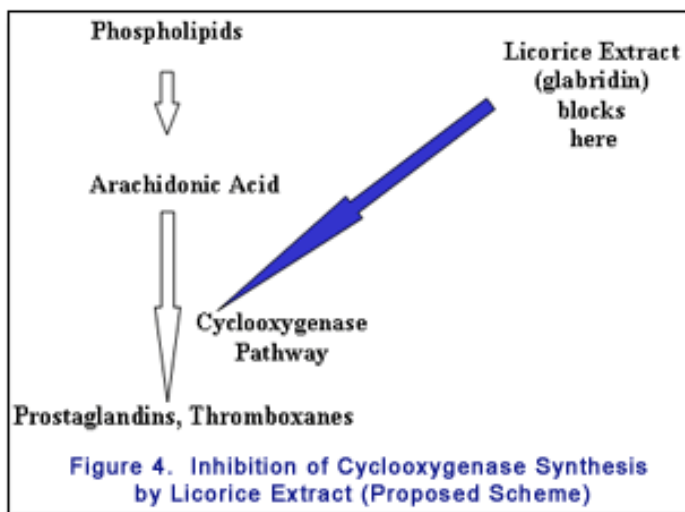
An assay was performed to test the anti-inflammatory activity of **Glabridin** when used for topical application (2).

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 3**, 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 (2).

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 µg/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 **(5, 6, 8)**

ANTI-OXIDANT EFFECT

As discussed in the assay performed to test the inhibition of superoxide production by **Glabridin**, it can be said that **Glabridin** has an antioxidant effect in addition to its skin-whitening (anti-melanogenetic) and anti-inflammatory properties.

CAMPO GLABRIDIN PURE is used topically is documented to reduce the amount of corticosteroids in dermatological infections. This is probably by inhibiting 11 - beta hydroxysteroid dehydrogenase which is responsible for the conversion of cortisol to corticosterone and thus, potentiating the effects of steroids **(9, 10)**.

COSMETIC APPLICATIONS

CAMPO GLABRIDIN PURE possesses potent and effective anti-inflammatory, antioxidant 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.).

CAMPO GLABRIDIN PURE 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% **GLABRIDIN PURE**, galacturonic acid, lactic acid, kojic acid, ascorbyl palmitate and tocopheryl linoleate. **(11)**

It is said to lighten the skin color by inhibiting melanin formation, mainly by the inhibition of tyrosinase activity.