



Glechoma Bio Extractive® 'C' G

P-00025648

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Botanica®
An Evonik company.

My plant extracts, naturally



Glechoma hederacea L.

Glechoma Bio Extractive®

First Aid for Irritated Skin





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1 Summary

Glechoma hederacea, which is also known as ground-ivy belongs to the **family of Lamiaceae** and is widespread in moderate climates of Eurasia. It is a herbaceous, perennial and evergreen plant, which blooms during April to June with funnel shaped bluish-violet colored flowers. *Glechoma hederacea* grows on **meadows and in forests**. It prefers **moist shaded areas**.

Ancient European folks used ground ivy as **medicinal herb**. It can be used as tea to treat bronchitis and cough or to treat poorly healing wounds and skin diseases like psoriasis. *Glechoma hederacea* has **antibacterial, antioxidative** and **anti-inflammatory** activities. In cuisine, it can be cooked as vegetable or used as **spicy addition to salads or dips**. The ground-ivy extract is also used in cosmetics. The **INCI Glechoma Hederacea Extract** is known in the EU, US, China and Japan.

Botanica uses **organic certified** flowering *Glechoma hederacea* herb. The Glechoma Bio Extractive® is manufactured using an in-house developed **gentle extraction process** which provides a **concentrate of the extract**.

The constituents of Glechoma Bio Extractive® were analyzed with high performance thin layer chromatography and identified the phenolic constituents **rutin**, a flavonoid, **and rosmarinic acid**, an ester of caffeic acid. It has a minimum **antioxidant capacity** of 2 g/l expressed as rosmarinic acid.

In vitro screening for biological activities of Glechoma Bio Extractive® revealed that it can be used for cosmetic products which are dedicated to inflammatory skin disorders or irritated skin. It can support **healing and calming** and help to **reduce itching and pain sensation**.

2 General Information

2.1 Occurrence and Classification

Glechoma hederacea, which is known as gill-over-the-ground, ground-ivy or gill herb belongs to the **family of Lamiaceae** and is widespread in moderate climates of Eurasia. It has been introduced to Northern America.

Family	<i>Lamiaceae</i>
Genus	<i>Glechoma</i>
Species	<i>G. hederacea</i>





2.2 Characteristics

Glechoma hederacea is a **herbaceous, perennial and evergreen plant**. The square stem creeps on the ground, forms side shoots every 8 to 15 cm and roots at the nodes. *Glechoma*



hederacea forms flat roots. The main shoot can get 50 cm to 2 m long. The leaves are arranged crosswise and are roundish heart-shaped and coarsely grooved. The hairiness of the plant parts is variable but the leaves usually have a hairy upper surface.

Glechoma hederacea grows on **meadows and in forests**. It prefers **moist shaded areas**, but can also deal with sunny locations.

In the flowering season (April-June), *Glechoma hederacea* forms upright flowering shoots which are 10 to 30 cm high. Two to five flowers emerge in the axils of the stem leaves. The flowers are funnel shaped and blue, bluish-violet to lavender.

2.3 Use

Ancient European folks used ground ivy as **medicinal herb** in order to treat pus and other toxic body secretions. Nowadays, its healing properties has got slightly forgotten, although ground ivy can be used to treat gastrointestinal catarrhs, diarrhea, bronchitis and cough [1]. Tea from fresh ground ivy helps against chronic colds or chronic problems with the sinuses. Traditional Chinese medicine uses it against pneumonia and nephritis [2]. In Italy, it is traditionally used to treat arthritis and rheumatism by placing the rubbed leaves on the affected body parts [1].

Glechoma hederacea juice or its infusions are used to treat poorly healing wounds and skin diseases (even psoriasis) [1]. *Glechoma hederacea* was shown to have **antibacterial** [3], **antioxidative** [4] and **anti-inflammatory** [5] activities.

The leaves are collected in spring and can be cooked as vegetable, in former times e.g. as Holy Thursday soup. Due to its resin-aromatic, mint-like taste, it can be used as **spicy addition to salads or dips**. Before the cultivation of hops, the Saxons and the English used ground ivy as preservative and source of bitter for beer [6]. Therefore, ground-ivy is also known as alehoof or tunhoof. Its antioxidative activity was approved in a study and proposed its application as vegetable antioxidative food additive, e.g. for animal fat or vegetable oil [7].

The ground-ivy extract is used in cosmetics for skin care and as tonic for hair and skin [8]. The **INCI Glechoma Hederacea Extract** is known in the EU, US, China and Japan.

2.4 Constituents

Glechoma hederacea is reported to contain 0.03 – 0.06% volatile oils, various terpenoid components, e.g. the sesquiterpene glechomafuran, flavonoids like rutin, rosmarinic acid (1.48% in leaf, 0.10% in stem), the bitter principle glechomin and other constituents [1,9].



3 Manufacturing the Glechoma Bio Extractive[®]

Botanica uses **organic certified** flowering *Glechoma hederacea* herb. The dried herb is used to manufacture the Glechoma Bio Extractive[®]. The Glechoma Bio Extractive[®] contains about 80% BIO glycerin and the rest are plant ingredients and water (for more details about composition, see specification).

It is manufactured according to a specific extraction process which was developed at Botanica and provides a **gentle extraction process** and a **concentration of the extract**.

For the production of that so-called 'C'G concentrate, a drug:extract ratio of 1:1 is used. The manufacturing process consists of two stages. First, the drug is extracted with a circulating ethanol-water mixture. Secondly, the alcohol is evaporated and the plant ingredients are



dissolved in a mixture of glycerin and water. The concentrated extract is therefore largely free of alcohol. From each production lot Botanica tests various quality parameters, e.g., appearance, density or the antioxidant capacity of the Glechoma Bio Extractive[®]. Further, each lot is microbiologically controlled.





4 Analytical Tests

4.1 HPTLC Analysis

Botanica performed high performance thin layer chromatography (HPTLC) analyses to determine the constituents of the Glechoma Bio Extractive[®].

4.2 Flavonoids

Flavonoids are widely distributed in plants. Flavonoids are polyphenols on the basis flavan and vary significantly in their chemical structure. Flavonoids are often yellowish - the Latin word *flavus* means yellow. Flavonoids have the capacity to absorb the most energetic solar wavelengths, i.e., UV-A and UV-B. Furthermore, flavonoids are known as **antioxidants** and **radical scavengers** and have **anti-inflammatory** and **antimicrobial activities** [10,11]. Figure 1 shows the flavonoid profile of the Glechoma Bio Extractive[®], which was analyzed by HPTLC and identified **rutin** as one of the main flavonoids of the Glechoma Bio Extractive[®]. Rutin was found a candidate for the treatment of atopic dermatitis and allergic contact dermatitis [12]. Results of another study demonstrated its efficacy as sun protection agent [13].

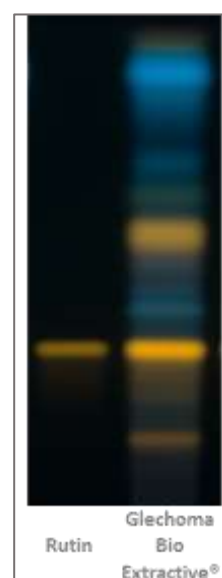


Figure 1. HPTLC Flavonoids of Glechoma Bio Extractive[®]

4.3 Rosmarinic Acid

In addition to rutin, **rosmarinic acid** was identified as another main component of the Glechoma Bio Extractive[®] by using an HPTLC method to detect tannins (Figure 2). Rosmarinic acid is a water-soluble phenolic compound and an ester of caffeic acid (Figure 3). Rosmarinic acid is known for its **antioxidant**, **anti-inflammatory** and **antimicrobial activities** [14].

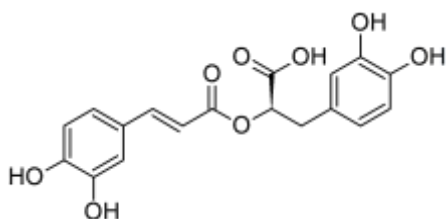


Figure 3 . Chemical structure of rosmarinic acid

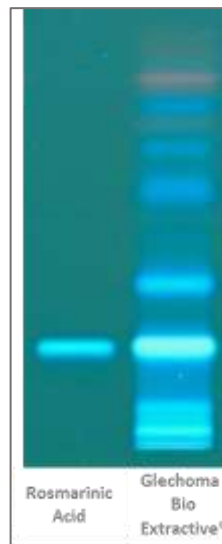


Figure 2. HPTLC Tannins of Glechoma Bio Extractive[®]

4.4 Antioxidant Capacity

The antioxidant capacity (AOC) was measured based on Ph.Eur. 2.8.14. Gallic acid was used as reference standard and the AOC results expressed as mass of a polyphenol per sample volume. The Glechoma Bio Extractive[®] 'C' G in Bio Glycerin-water P-00025648 has an antioxidant capacity of minimum 2 g/l expressed as rosmarinic acid (see specification).



5 *In vitro* Screening of the Glechoma Bio Extractive[®]

In order to identify the biological activities of Glechoma Bio Extractive[®], an *in vitro* screening was performed.

It was shown that the Glechoma Bio Extractive[®] activates different biological pathways *in vitro*:

- Inhibition of the STAT-3 transcription factor, which could lead to a **reduction of inflammation and lesions** in skin disorders such as psoriasis or **very irritated skin** (see 5.1).
- Agonist effect on the cannabinoid receptor type 2 (CB2), which could contribute to the **maintenance of the epidermal homeostasis** and a **reduction of inflammation and pain sensation**. In cosmetics, CB2 activation is associated with a **skin soothing and calming** effect (see 5.1).
- Antagonist effect on transient receptor potential vanilloid 1 (TRPV1), which could support **skin barrier recovery and reduction of pruritus (itching sensation) and skin irritation** (see 5.2).
- **Antioxidant effect by induction of the Nrf2 pathway**, which may be complementary to the actions described above since oxidative stress may contribute to the pathogenesis of skin disorders (see 5.3).

The following chapters describe the biological activities, which were identified by *in vitro* screening in detail.





5.1 Anti-inflammatory Effect and Epidermal Homeostasis Linked to STAT-3 Inhibition and CB2 Agonism

STAT-3 is a transcription factor and central regulator of inflammatory skin conditions like psoriasis [15]. It has been reported as being involved in the initiation but also in the maintenance of the skin disorder [15]. Glechoma Bio Extractive® shows an inhibition effect of STAT-3 activation (Figure 4). Inhibition of STAT-3 activation could lead to a **reduction of inflammation and lesions in skin disorders** such as psoriasis or very irritated skin [15]. *In vitro* testing demonstrates that Glechoma Bio Extractive® inhibits STAT-3 activation (Figure 4).

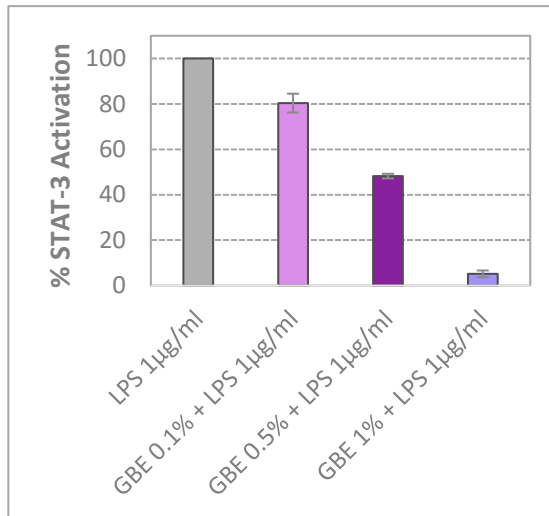


Figure 4. STAT-3 is activated by 1 µg/ml Lipopolysaccharide (LPS). Increasing concentrations of Glechoma Bio Extractive® (GBE) inhibit STAT-3 activation. Results are represented as the percentage of inhibition considering 100% the value of LPS-induced STAT-3 activation.

Cannabinoid receptors are involved in the regulation of epidermal homeostasis, skin inflammation and pain sensation [16]. For example, stimulation of the cannabinoid receptor type 2 (CB2) in keratinocytes evokes the release of analgesic opioid peptides [17]. The agonist effect of the Glechoma Bio Extractive® on CB2 (Figure 5) could contribute to the **maintenance of the epidermal homeostasis** and a **reduction of inflammation and pain sensation**. In cosmetics, CB2 activation is associated with a **skin soothing and calming** effect.

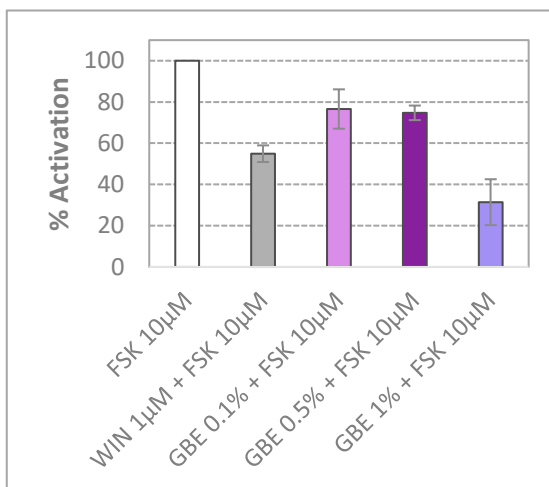


Figure 5. GBE shows an agonist effect on Cannabinoid receptor type 2 (CB2). CB2 agonists are associated with the inhibition of cAMP (cyclic adenosine monophosphate) synthesis. For testing CB2 agonists *in vitro*, the cells were treated either with increasing concentrations of Glechoma Bio Extractive® (GBE) or with WIN 55,212-2 (WIN). WIN is a potent CB2 agonist and is used as positive control. Then, cAMP is induced by 10 µM forskolin (FSK) and the inhibitory effect of the CB2 agonists explored. GBE shows an agonist effect on CB2 which is shown by lower %activation.



5.2 Antipruritic (Anti-itching) and Soothing Effect Linked to TRPV1 Antagonist Effect

The Transient Receptor Potential (TRP) channels are intimately involved in cutaneous processes that include the initiation of pain, temperature, and itch perception, the maintenance of epidermal homeostasis, the regulation of hair follicles and sebaceous glands, and the modulation of dermatitis [16].

Transient Receptor Potential Vanilloid 1 (TRPV1), a member of the TRP group, is present in epidermal nerve fibres, hair follicle outer root sheath epithelial cells and keratinocytes [16]. It is activated by pain-inducing stimuli, such as capsaicin (CAP), a pungent vanilloid compound. CAP is an agonist of TRPV1. TRPV1 activation by high doses of CAP has been shown to lead to cell death [18]. TRPV1 is involved in **pain and itch sensation, skin inflammation** and dermatitis [16]. Inhibiting TRPV1 by means of an antagonist could limit these various phenomena.

Glechoma Bio Extractive® shows an antagonist effect on TRPV1 (Figure 6). Due to this antagonist effect on TRPV1, the Glechoma Bio Extractive® could **support skin barrier recovery to reduce** dermatitis-like symptoms, **pruritus and skin irritation** [19,20,21].

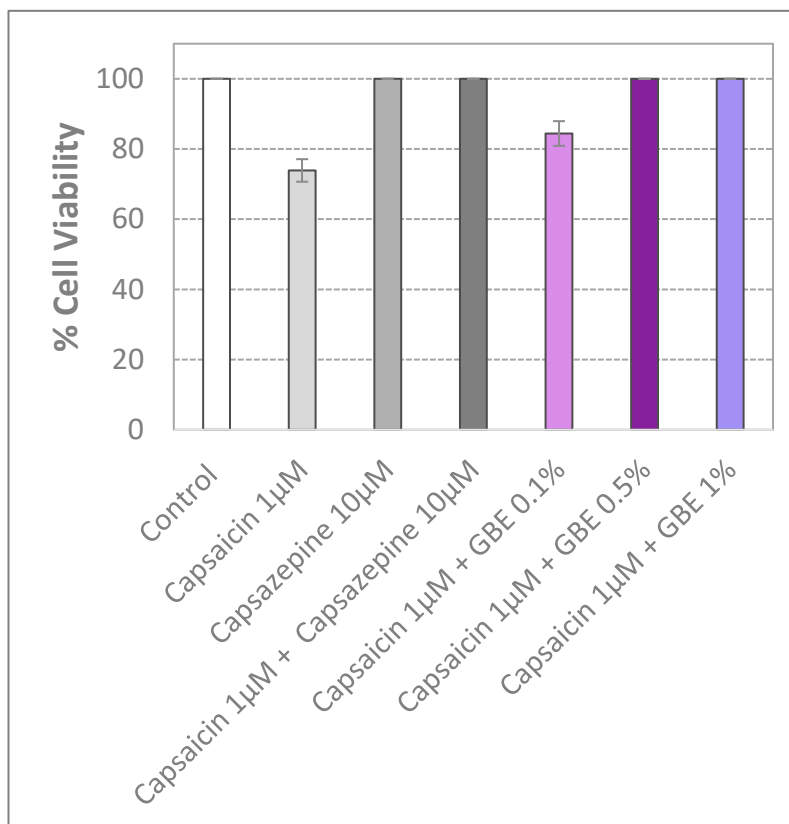


Figure 6. Glechoma Bio Extractive® (GBE) shows an antagonist effect on TRPV1. The cells are incubated with increasing concentrations of GBE. Then they are treated with the agonist capsaicin. Capsaicin activates TRPV1 which leads to cell death. Capsazepine is used as the positive control of TRPV1 antagonism. Capsazepine alone has no effect on cell viability but it overrides the effect of capsaicin by inhibiting TRPV1. Results are represented as % cell viability considering the control (untreated cells) as 100% cell viability .



5.3 Antioxidant Effect by Induction of the Nrf2 Pathway

As cellular response to oxidative stress, the **transcription factor Nrf2 is activated**, which induces the expression of antioxidant enzymes, so-called antioxidant response elements (ARE) [22,23]. Low or moderate oxidative stress, e.g., caused by low or moderate concentrations of reactive oxygen species (ROS), **protect the cell against damage** by inducing different antioxidant responses and re-establishing or maintaining redox homeostasis [24].

In vitro testing demonstrates an induction of the Nrf2 pathway by Glechoma Bio Extractive® with a clear dose effect (Figure 7).

This antioxidant effect may be complementary to the actions described in the sections above since oxidative stress may contribute to the pathogenesis of skin disorders. A high level of oxidative stress leads to an increase of the release of inflammatory mediators. Boosting antioxidant capacity of the skin by induction of the Nrf2 pathway and, therefore, the protection machinery of the cell, might avoid this additional trigger caused by oxidative stress.

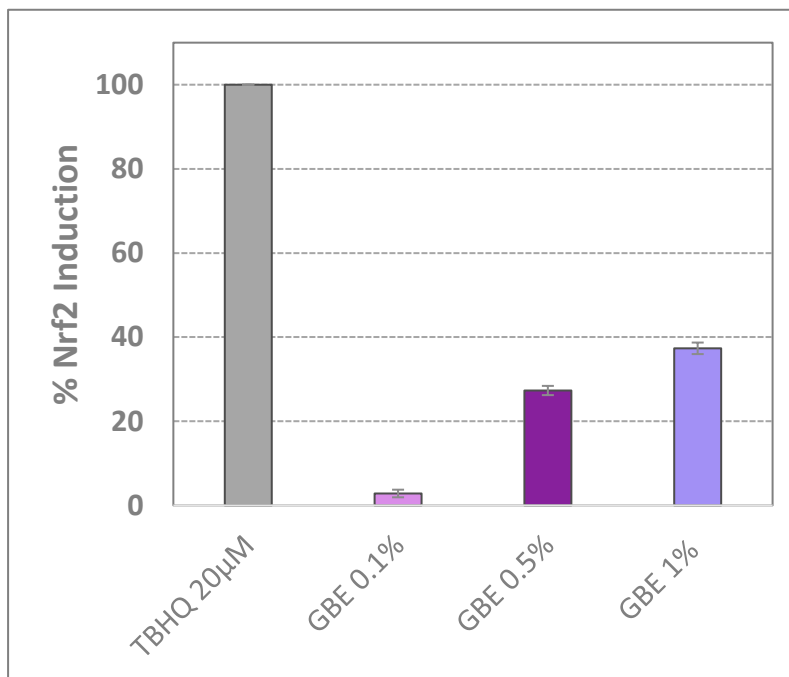


Figure 7. Glechoma Bio Extractive® (GBE) induces Nrf2 with a clear dose effect. The cells were either treated with increasing concentrations of GBE or with Tert-butylhydroquinone (TBHQ, 20 µM). TBHQ is used positive control for Nrf2 induction. Results are represented as % Nrf2 induction considering the value induced by TBHQ as 100%.





5.4 Summary of Dermatological Activities

Table 1 below provides an overview of the dermatological activities of the Glechoma Bio Extractive® based on the results described in the chapters above.

Table 1. Dermatological activities of the Glechoma Bio Extractive®

	Anti-oxidative	Anti-inflammatory	Skin Repair	Anti-irritant	Soothing	Calming	Anti-itching
Flavonoids	X	X					
Rosmarinic Acid	X	X					
Antioxidant Capacity	X						
Inhibition of STAT-3		X	X	X			
Agonist Effect on CB2		X				X	X
Antagonist Effect on TRPV1		X			X	X	X
Activation of Nrf2	X	X					

Glechoma Bio Extractive® can be used for cosmetic products which are dedicated to inflammatory skin disorders or irritated skin. It can support **healing and calming** and help to **reduce itching and pain sensation**.

6 Acknowledgement

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