

Documentation: Houseleek Bio Extract 'C' G (CH) P-00025259

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Sempervivum Tectorum

Houseleek

The unusual alpine "Aloe Vera"





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1. Summary

The Latin botanical name Sempervivum stands for “always” or “forever” (semper) and “living” (vivus). This plant is very resistant to difficult growth conditions and keeps its leaves in summer and winter. Sempervivum tectorum is commonly known as “houseleek”.

The thick leaves can store water, making the plant capable to live on stony, sunny surfaces, mostly in the mountain, subalpine and alpine belts. Sempervivum tectorum is usually planted in the average mountain area, the Alps and foothills of the Alps on roofs and walls. People believe that the plant protects buildings against lightning, thunder and fire [1].

Sempervivum tectorum is used as medical herb, spice and salad. Recent medical research data present houseleek leaf extract as an antimicrobial, antinociceptive, cooling, astringent and antioxidative substance.

The Innovative glycerin houseleek leaf extract (concentration 0.1% to 1%) shows novel activities for cosmetic use. The tested houseleek leaf extract activates the Nrf2 pathway, shows an antagonistic activity against TrPV1, enhances glucose uptake in HaCaT keratinocytes, is a PPAR α and PPAR γ agonist, a powerful agonist of cannabinoid receptor type 1, a potent tyrosinase inhibitor and shows skin lightening effects.

Houseleek leaf extract activates cellular mechanisms which are involved in skin healing and regeneration, helps skin repair, moisturizes and refirms the skin. Additionally, it seems to be beneficial in the treatment of ageing effects, wrinkles or fine lines, sensitive and sun damaged skin.

Houseleek extracts with the INCI Sempervivum Tectorum Extract have been used in Cosmetics (see e.g. EU 1996/335) for decades. The INCI is also known in China and Japan.

2. Classification

Family	Crassulaceae
Genus	Sempervivum
Species	tectorum





3. General Information on Sempervivum Tectorum

3.1. Botanical Description



Sempervivum tectorum was first described in 1753 by Linnaeus and is a rosette-forming succulent, evergreen and perennial plant, which spreads by offsets. It is an extremely diverse plant, in particular regarding the diameter of the rosette, width, color and type of escalation of the rosette leaves.

Sempervivum tectorum in Central Europe exists also in other synonyms: alpinum and schottii. It can grow up to 15 cm tall by 50 cm wide and develops grey-green, tufted, sessile leaves, with a diameter of about 4-10 cm, which are often suffused with rose-red. In summer Sempervivum bears clusters of reddish-purple flowers, in multiples of 8-16, on hairy erect flat-topped stems [2, 3].

3.2. Use

Historically, houseleek (*Sempervivum tectorum* L.) leaves were used as a medicinal herb for external treatment of injuries, burns, rashes, open wounds, bee or insect stings, burns, sore nipples, corns, freckles, throat infections, uterine neuralgia, dysplasia and amenorrhea, against catarrhs of the eyelids, canker sores, fungal infections in the mouth, headaches, chapped or cracked skin, deafness, ear pain, and as a vermifuge and febrifuge. Houseleek leaves are used in tooth holes against toothache. Houseleek juice was used to treat eye infections. The plant is also sometimes used internally in the treatment of dysentery. The effectiveness of the drug has not been proven in mentioned application areas [1]. The Commission D of German BGA/BfArM mention *Sempervivum tectorum* as a treatment against nodular hardening in the skin, tongue and nipples, and additionally against warts [18].

Modern research confirmed *Sempervivum tectorum* juice for treating ear pain. The juice possessed antimicrobial activity towards clinical isolates of bacteria linked to otitis [4]. Furthermore study data mention antinociceptive, cooling, astringent effects, antioxidant activities of *Sempervivum tectorum* extracts [5, 6, 7].

Additionally, houseleek is used as a spice or salad. Leaves and young shoots can be eaten in a salad or as additive to drinking water, giving a refreshing taste, common in the Swiss canton of Grisons [1].





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3.3. Content

The drug *Sempervivum minoris folium* is also known under the following synonyms: *Folia Sedi magni*, *Folia Sempervivum majoris* and *Herba sempervivi tectori*. The main component of the houseleek leaf are L-malic acid and their calcium salts. Dried leaves contain about 5.3 – 9.7% isocitric acid [1]. Polyphenol, tannin, proanthocyanidine and flavonoid contents of lyophilized houseleek leaves were determined by spectrophotometry. Numerous catechin and proanthocyanidine compounds and main flavonoids aglycone and kaempferol were identified [8]. In another study, ten flavonol glycosides and sixteen phenolic acid compounds were detected. Three kaempferol glycosides were quantified which may reduce sensitivity to painful stimuli [5].

4. Production of Concentrated Houseleek Extract 'C' G

The concentrated houseleek extract is produced using a specific extraction process developed by Botanica. It is a gentle extraction process used to obtain a concentrated extract.

To obtain a concentrated extract known as 'C' G, we use a Drug-Extract ratio (DER) of 1:1. The process consists of two steps: firstly, the plant is extracted into a circulating ethanol/water mixture. The alcohol is then evaporated and the concentrated extract that remains is transferred into a glycerin/water mixture (and is entirely free of ethanol by the end of the process).

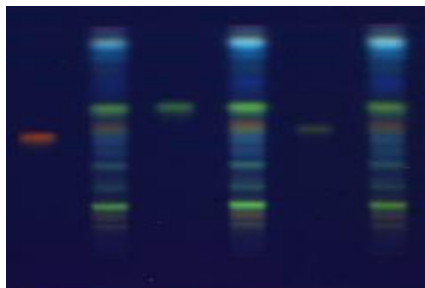
Our quality control laboratory then validates various parameters: organoleptic criteria, density, refractive index, etc. In order to check for microbial contamination, the samples are sent to an external microbiological laboratory. Retention samples are also stored for each production batch.





5. Analytical Tests

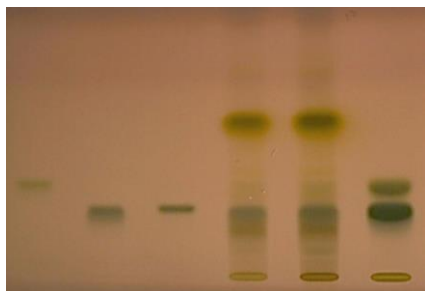
Flavonoid- „Screening“:



Track	Description
1	Hyperosid
2	Houseleek Bio Extract (CH)
3	Kaempferol-3-Glucosid
4	Houseleek Bio Extract (CH)
5	Luteolin-4-Glucosid
6	Houseleek Bio Extract (CH)

The HPTLC fingerprint above demonstrates a flavonoid pattern of high diversity. One of the flavonoids could be assigned to Kaempferol-3-glucoside which is associated with the reduction of sensation of pain. Other flavonoids like hyperoside or luteolin-4-glucoside could not be identified.

Carbohydrate- „Screening“:



Track	Description
1	Glucose
2	Fructose
3	Saccharose
4	Houseleek Bio Tincture (CH)
5	Houseleek Bio Tincture (CH) (20)
6	Aloe Vera Bio Leaf Juice Tincture

It can be demonstrated that houseleek is rich in various carbohydrates, comparable to Aloe vera leaf juice. The concentration and distribution varies, of course, but both plants offer a healthy portion of nutrients for the skin.





6. Properties and Activities of Houseleek Extract 'C' G

6.1. Soothing and Restorative Properties of the Houseleek Extract 'C' G

6.1.1. Cannabinoid Receptor Type 1 Agonist

Cannabinoid signaling is mentioned for regulating the permeability barrier and epidermal differentiation. Cannabinoid receptor type 1 in keratinocytes help to maintain epidermal barrier homeostasis and attenuate Th2-type allergic inflammatory responses [20]. The houseleek test results demonstrate its agonistic activity. A study by Ständer et al. about cannabinoid agonists mention a mast cell stabilizing function which helps against itching and inhibits histamine secretion [20]. This research indicates that houseleek leaf extract may be a powerful active ingredient to treat sensitive skin.

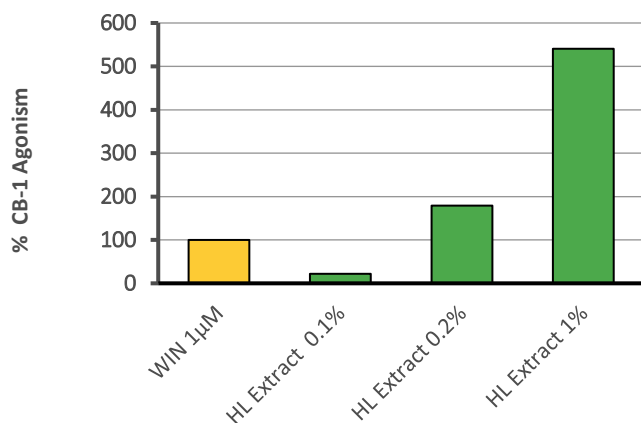


Fig.1: Houseleek leaf extract (HL Extract) showed cannabinoid receptor type 1 (CB-1) agonistic activity. WIN-55,212-2 (WIN), a cannabinoid receptor type 1 agonist, is used as positive control.



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6.1.2. TrPV1 Receptor Antagonist

The houseleek extract is a TrPV1 receptor antagonist

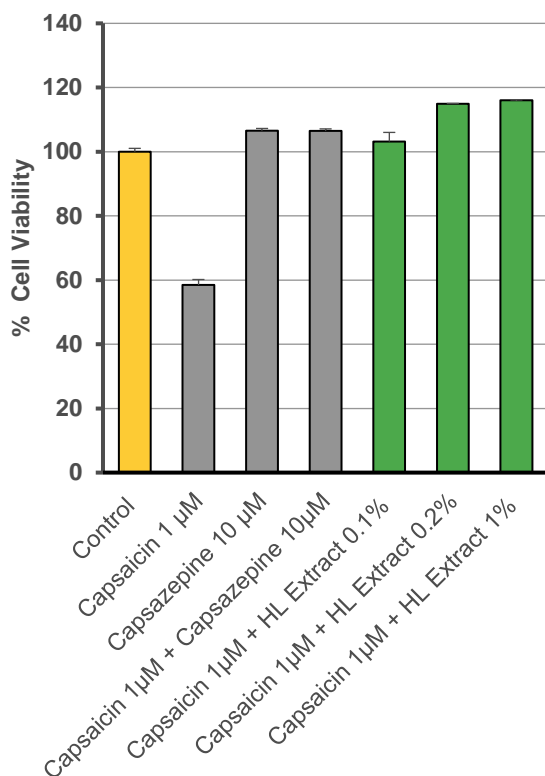


Fig.2: Capsaicin, a TrPV1 receptor agonist, is used as a negative control. Capsazepine, an antagonist, is used as a positive control. The untreated cells (control) are calibrated for 100% cell viability.

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TRP channels are involved in cell homeostasis, survival and proliferation, endocrine and exocrine secretory processes and immune as well as inflammatory mechanisms. TRP channels are stimulated by external environmental factors like UV light, cold, heat, air pollution and chemical substances. They play a role in the recognition and physiology of sensitive skin.

TrPV1, a member of the TRP group, are present in the keratinocytes and the free nerve fibres; they are activated by capsaicin and thermal stimuli [11]. They are involved in inflammation, irritation and pain phenomena [12]. Inhibiting them by means of an antagonist makes it possible to limit these various phenomena. Furthermore, TrPV1 inhibitors can also be used to prevent skin ageing, improve wrinkles and improve skin tone.

Tests show that treatment using houseleek extract 'C' G P-00025259 has an antagonistic effect against TrPV1 receptors. Houseleek extract can also be used to soothe sensitive skin or skin that has been sensitised by the sun and in anti-ageing formulations.





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6.1.3. PPAR alpha Receptor Agonist

The houseleek extract is a PPAR α . receptor agonist

PPAR (Peroxisome Proliferator-Activated Receptors) nuclear receptors belong to the superfamily of nuclear receptors which are ligand activated transcription factors. There are three PPAR isoforms: α , β/δ and γ .

PPAR α is involved in skin barrier function, activation of fatty acid oxidation, regulation of inflammation and plays a role in cell differentiation, proliferation and healing of skin wounds [13, 14].

Topical treatment with a PPAR α agonist had a positive effect on atopic dermatitis and stimulated sebaceous lipid production. PPAR α activators or compounds that positively regulate PPAR α gene expression may help to counteract ageing processes and can reduce the adverse effects of UVB [19].

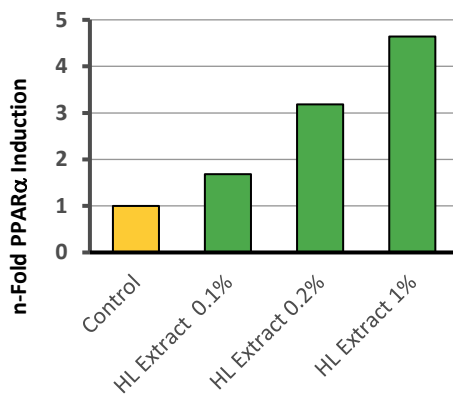


Fig.3: PPAR α activation. Untreated skin cells (Control) are set as 1. Houseleek leaf extract (HL Extract) shows agonistic effects for PPAR α in a concentration dependent manner.

Results shown in figure 3 show that the houseleek extract is a PPAR α agonist. It stimulates the synthesis of cellular lipids for skin barrier reconstruction; by stimulating the synthesis of sebaceous lipids and contributes to skin healing.





6.2. Antioxidant and Cellular Activity-boosting Properties of the Houseleek Extract 'C' G

6.2.1 Glucose Uptake in Keratinocytes

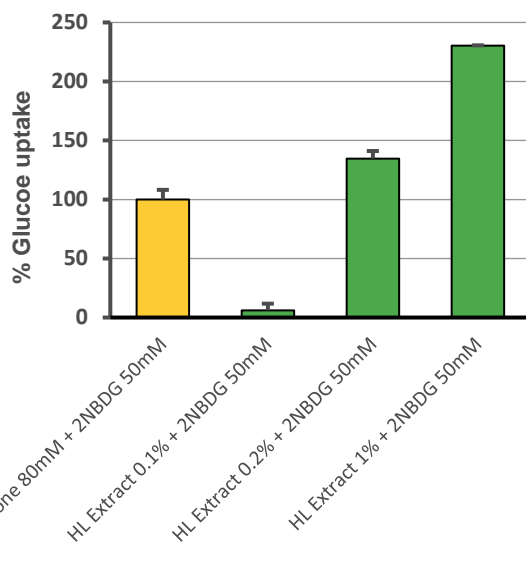


Fig.4: Houseleek leaf extract (HL Extract) showed increased glucose uptake in keratinocytes. Rosiglitazone + 2NBDG is the positive control.

Glucose plays a role in the energy metabolism of keratinocytes. It is associated with cell regeneration and renewal.

Houseleek leaf extract induces glucose uptake in keratinocytes in a dose-dependent manner. Houseleek leaf extract at a concentration of 0.2% demonstrates a significant glucose stimulation (30.4% more than positive control). This activity is important to promote recovery of cellular activity, which is an essential process for tired, dry, stressed or aged skin.





6.2.2. Nrf2 Pathway Activation

Nrf2, a nuclear transcription factor, plays an antioxidant role within the cells. Inactive when in cytoplasm, this transcription factor, which is bound to two Keap1 molecules, is released from these two proteins when subjected to stress and travels to the nucleus where it triggers synthesis of ARE antioxidant proteins (Antioxidant Response Element).

At keratinocyte and melanocyte level, Nrf2 protects the cells against mutation during the process of keratinisation and melanogenesis. Furthermore, Nrf2 involvement in healing wounds and inhibiting inflammation is also essential to maintaining healthy, intact skin [10].

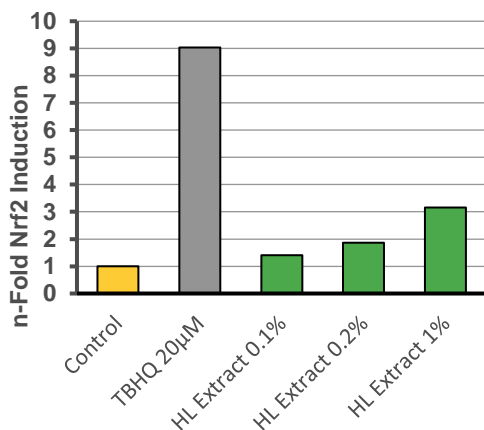


Fig.5: Houseleek leaf extract (HL Extract) slightly activated the Nrf2 pathway in HaCaT-ARE-Luc cells at the highest tested concentration. HaCaT-ARE-Luc cells were treated with HL Extract. Luciferase activity was measured in the cell lysates and results are represented as the n-fold induction over basal levels. Tert-Butylhydroquinone (TBHQ) is used as positive control. Untreated cells (Control) are set as 1.

Houseleek leaf extract stimulates the Nrf2 pathway in a concentration dependent manner. The highest effect, a 3-fold induction, is observed with 1% houseleek leaf extract.

In summary, Nrf2 allows the skin to protect itself naturally against oxidant attacks and prevents oxidative stress. Anti-oxidative activities of houseleek leaf extract should facilitate preventing skin dryness, reducing the elastin and collagen breakdown caused by excess oxidative stress. These results indicate the potential use of houseleek leaf extract containing cosmetic formulations for anti-ageing, sun care, skin repair and moisturizing products.





6.3. Properties of the Houseleek Extract 'C' G for Brighter Skin and Even Complexion

6.3.1. Tyrosinase Inhibition

Houseleek leaf extract treatment showed an inhibitory effect on tyrosinase. The inhibitory effects were dose-dependent and higher than that of kojic acid, a well-known tyrosinase inhibitor and already at 0.2% concentration. These results indicate that houseleek leaf extract is a potent tyrosinase inhibitor, which could be used in skin whitening products. Houseleek leaf extract could be beneficial to protect sensitive and sun exposed skin.

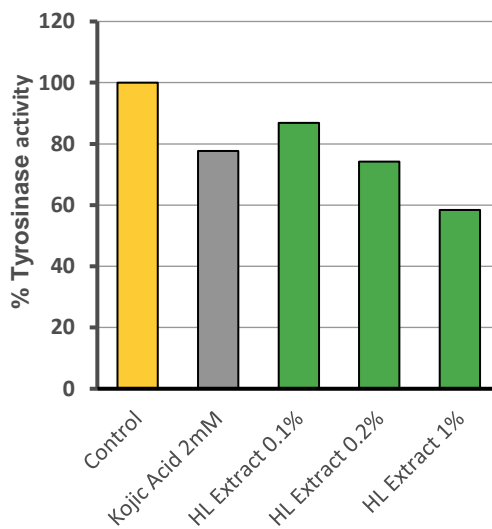


Fig.7: Effect of houseleek leaf extract (HL Extract) on tyrosinase activity in B16 melanoma cells. Cells were treated with houseleek leaf extract or kojic acid as a positive control. Results are represented as percentage. Untreated control cells (Control) are set to 100%.

6.3.2. Melanin Synthesis Inhibition

Houseleek leaf extract inhibits melanin synthesis comparable to kojic acid. This confirms the skin-lightening abilities of houseleek leaf extract.

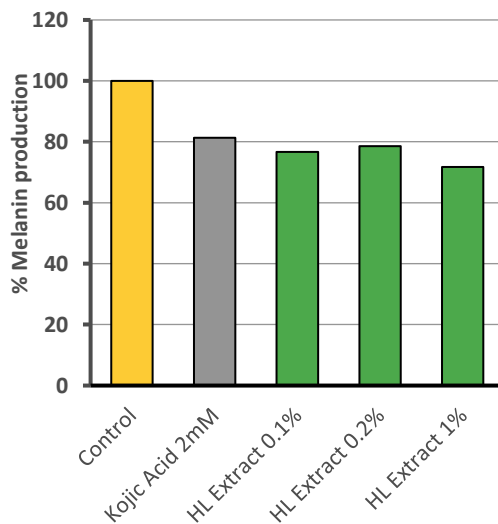


Fig.8: Effect of houseleek leaf extract on melanin synthesis in B16 melanoma cells. Melanin content assessment was performed as described in Methods. Briefly, cells were treated with the doses indicated of kojic acid as a positive control. Results are represented as percentage. Untreated control cells (control) are set to 100%.



6.4. Summary of Dermatological Activities of Houseleek Extract 'C' G

	Sensitive Skin	Calming	Skin Repair	Cellular Energy Booster	Skin Tone Evenness	Lightening	Skin Protection	Antioxidant Activity
Cannabinoid Receptor Type 1	X		X	X				
TRPV1	X		X					X
PPAR alpha Agonist				X				X
Glucose Uptake					X			
Transcription Factor Nrf2					X			X
Tyrosinase Inhibitor						X	X	
Melanin Synthesis Inhibitor						X	X	

7. Toxicological Data of the Houseleek Extract 'C' G

Following toxicity tests were conducted to determine the degree of toxicity of the houseleek Bio Extrakt 'C' G (CH) P-00025259:

- AMES Test which is a biological assay to assess the mutagenic potential of chemical compounds.
- MCA-CHEM Test which is a test to evaluate the irritating potential after application on the chorion-allantoic membrane of the Hen's egg.
- PTC-CHEM Test which is a test to evaluate the phototoxic potential.
- TCFS Test which is a test to evaluate the sensibilisation potential on volunteers.

All the tests showed negative results.

8. Acknowledgement

We would like to thank VivaCell Biotechnology GmbH and SimCosmetic Biotech for testing the houseleek leaf extract, as well as I. Le Fur.





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