 Arnica Flower CO$_2$-Extract – Approved Efficacy in Topical Treatment

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Abstract
Supercritical CO$_2$-extraction is the state-of-the-art technology for the production of standardised Arnica-extracts with high concentration of bioactive sesquiterpene lactones (SQLs). This provides the base for the development of valuable dermatological products with good efficacy, whilst simultaneously minimising the allergenic potential. A further advantage is that the lipophilic CO$_2$-extract does not contain polar components e.g. flavonoids which are suspected of allergenic effects.

Traditional use of Arnica preparations is supported by new pharmacological and clinical studies. Besides the application in the case of sports and accident related injuries, Arnica is increasingly used for treatment of age-related inflammatory diseases of the bones and joint apparatus. Topical treatment with Arnica preparations can be ranked today among rational phytotherapy. Arnica should not be administered orally or applied to open wounds where absorption can occur. The predominant opinion in literature that Arnica SQLs have strong allergenic potential could not be confirmed in recent investigations. Highly concentrated plant extracts standardised to bioactive ingredients have an enormous potential in skin care and dermatology when used properly.

Introduction
Knowledge about the healing power of Arnica can be traced back to the 13th century$^{[1]}$. Arnica preparations have traditionally been used for the treatment of backache, bruising, cramps, fibrositis, relief of muscular and rheumatic pain and stiffness, sports injuries and sprains.

Recent clinical studies for topical treatment of inflammatory diseases with Arnica preparations support the traditional use of Arnica for a variety of injuries and muscle or joint pains. The genus Arnica belongs to the Asteraceae family and comprises about 32 species which are divided into five subspecies: andropurpurea, arctica, austromontana, chamonissonis and montana. The European Pharmacopoeia monograph specifies only Arnica montana. The height of Arnica montana ranges from 30-60cm. It is a perennial plant with a round, hairy stem and one to three flower stalks. Flowers are yellow-orange ray flowers around a cluster of tubular center flowers. The distribution area of Arnica montana is mainly the alpine Mediterranean region from the Pyrenees in the North of Spain to the Balkans, from the valleys up to 2500m altitude.
The main ingredients of *Arnica montana* flowers are SQLs of the pseudoguaianolide type 0.3-0.9%\(^\text{(2)}\), flavonoids 0.4-0.6%\(^\text{(3)}\), essential oil 0.23-0.35%\(^\text{(2)}\), polyacetylene\(^\text{(4)}\), polysaccharides\(^\text{(5)}\) and triterpenes\(^\text{(6)}\).

The SQL composition of different origins of *Arnica* varies considerably even if the total concentration is relatively constant\(^\text{(7,8)}\). Two chemotypes of *Arnica montana* are identified. The helenalin type is native to Central Europe and the dihydrohelenalin type, which contains only traces of helenalin, is native to Spain. SQLs consist mainly of esters of helenalin and 11\(_\alpha\),13-dihydrohelenalin with short-chain fatty acids such as acetic, isobutyric, isovaleric, methacrylic and tiglic acids\(^\text{(9)}\). These compounds are known as helenanolides, i.e. pseudoguaian-7\(\beta\),8\(\beta\)-olides.

**CO\textsubscript{2}**-extraction is the best technology for the extraction of *Arnica* flowers, since it works under gentle conditions without temperature stress and the influence of oxygen. Supercritical **CO\textsubscript{2}**-extracts completely preserve the lipophilic plant ingredients, they are highly concentrated and do not contain any solvent residues. Esterified SQLs cannot be obtained by steam distillation. Furthermore, the SQLs are concentrated many times higher in the **CO\textsubscript{2}**-extract than in alcoholic extracts. The genuine *Arnica* flower **CO\textsubscript{2}**-extract is standardised by the addition of sunflower oil to 4% SQLs so that it can be easily dosed. The supercritical **CO\textsubscript{2}**-extract is unique in terms of concentration and composition of bioactive ingredients (see Table 1). It is also available in EU-organic quality and USDA-NOP quality and meets the strict criteria of natural cosmetic standards e.g. BDIH, COSMOS, ICADA, NATRUE, NPA, NSF ANSI 305.

SQLs are generally analysed by GC or HPLC\(^\text{(10)}\). Comparative quantitative analysis with HPLC, GC and spectrophotometry showed similar test results\(^\text{(11)}\). Wagner developed and validated a GC-MS method for separation and quantitative analysis of the SQLs from *Arnica* flowers\(^\text{(12)}\). Helenalin and 11\(_\alpha\),13-dihydrohelenalin derivatives are detected in the SIM mode by their specific molecular masses. TLC is the preferred method for identity control and visualisation of constituents. The standardised *Arnica* flower **CO\textsubscript{2}**-extract is specified as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of Helenalin and Dihydrohelenalin Esters</td>
<td>4%</td>
</tr>
<tr>
<td>Triterpenediol Esters</td>
<td>2.0-4.5%</td>
</tr>
<tr>
<td>Helenalin and Dihydrohelenalin</td>
<td>~0.1%</td>
</tr>
<tr>
<td>Content of Essential Oil</td>
<td>0.2-0.4%</td>
</tr>
</tbody>
</table>

**Table 1. The Standardised *Arnica* Flower **CO\textsubscript{2}**-extract**
Besides SQLs, Arnica CO₂-extract also contains esters of the triterpenediols arnidiol, faradiol, maniladiol and calenduladiol which contribute to the anti-inflammatory efficacy⁶,¹³.

**Pharmacology**

In general Arnica is applied topically with good efficacy for symptomatic treatment of complaints connected with inflammation. In order to achieve anti-inflammatory efficacy, the active ingredients have to penetrate and subsequently permeate the skin. It has been demonstrated that the SQLs of different Arnica preparations show a comparable penetration in and permeation through the stratum corneum. The permeated concentration is sufficient for anti-inflammatory efficacy. It is interesting that isolated SQLs have a significantly poorer penetration performance than the SQLs of an Arnica extract and dihydrohelenalin and its derivatives have a better penetration than helenalin derivatives. A gel preparation showed a decrease of the penetration rate over time, whereas the penetration rate of ointments remained constant over time¹⁴,¹⁵.

A literature review reveals that the SQLs of Arnica montana have a broad spectrum of effects when applied topically:

- analgetic
- anti-arthritic
- antibacterial
- anticancer
- anti-inflammatory
- antimycotic
- antiplatelet.

These indications are demonstrated in vitro¹⁶,¹⁷,¹⁸,¹⁹,²⁰ and in vivo²¹,²²,²³,²⁴ and they correlate closely with the therapeutic areas of Arnica flower preparations²⁵.

Arnica preparations have been used topically for centuries for the treatment of injuries, sprains, bruises and haematomas²⁶,²⁷,²⁸,²⁹. In recent decades, pharmacological investigations with SQLs of Arnica have confirmed their effectiveness. The latest research in the field of chronic and age-related diseases proves the good efficacy e.g. in the case of rheumatic and inflammatory conditions such as osteoarthritis³⁰,³¹ and chronic venous insufficiency³²,³³,³⁴.

Osteoarthritis comprises a group of musculo-skeletal disorders. It is a chronic degenerative disease of the joints, especially of the hips, knees and fingers which can lead to degradation of joint cartilage and subchondral bone. This disease is prevalent in an ageing society. Symptoms of osteoarthritis are pain, stiffness, joint deformities and reduced mobility. Osteoarthritis is typically treated with analgesics as well as with non-steroidal antiphlogistics.

The efficacy of an Arnica montana gel was investigated in an open multicentre study with 53 women and 26 men with mild to moderate osteoarthritis of the knee. The gel was applied twice daily and after two weeks the symptom relief was comparable to topical treatment with a Diclofenac-gel³⁰. After three and six weeks significant improvement was demonstrated.

In another randomised, double-blind study with 204 patients who suffered from osteoarthritis of the hands, an Arnica gel was tested in comparison to a 5% ibuprofen gel. The gel was rubbed into the affected wrists three times a day. Pain relief and hand function were investigated after 21 days of treatment. Both preparations led to equally good results regarding pain relief and functional improvement of the hand³¹.

Arnica has a strong anti-inflammatory and blood circulation-enhancing effect and thus it is very well suited for treatment of chronic venous insufficiency (CVI). Several clinical studies with Arnica gels demonstrate the significant improvement of symptoms and venous backflow as well as the decrease in venous capacity³²,³³,³⁴.

In a prospective open multi-centre observational study with 148 patients, the efficacy of a topical Arnica preparation for treatment of sub-acute ankle joint injury resulting from contusion and distortion could be confirmed. During a two-week period of observation efficacy was approved. Swelling of the ankle joint was reduced to normal compared with the ‘healthy side’³⁵.

In comparison with a placebo, an Arnica gel also showed good efficacy in the treatment of 12 patients with muscle soreness. After a treatment period of six days, symptoms reduced and no side effects or intolerability were observed³⁶.

The European Commission has approved the external use of Arnica flower for injuries and for the consequences of accidents,
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e.g. sprains, haematoma, dislocations, contusions, oedema due to fracture, rheumatic muscle and joint problems. It is also approved for the treatment of superficial phlebitis and inflammation caused by insect bites. In cases of inflammation, Arnica preparations also show analgesic and antiseptic activity. In animal studies, helenalin and dihydrohelenalin and their esters were found to have analgesic, antibiotic, antimicrobial and anti-inflammatory activity[46,23].

Mechanism of Activity
The anti-inflammatory activity of Arnica extracts applied topically has been proven in a range of in vitro studies on isolated enzymes and cellular systems. The concentration of SQLs is responsible for the pharmacological activity. Both helenalin and dihydrohelenalin and their esters already act in very low concentrations in the biochemical pathways of the cells by inhibiting the transcription factor NF-κB, a central mediator of the human immune response system. Helenalin not only inhibits the NF-κB activation but also decreases the production of many inflammatory cytokines. This prevents the recruitment of immune cells, T- and B-cells, as well as macrophages and neutrophils and thus helenalin reduces inflammation. It has been shown that helenalin can inactivate previously activated NF-κB, a decisive factor for treatment of inflammation[37].

Helenalin binds directly to thiol (sulfhydryl) groups on the amino acid cysteine in NF-κB, thus blocking its transcription activities[38]. Both the α-methylene-γ-lactone and α,β-unsaturated cyclopentenone structures in helenalin are involved in this mechanism, which explains why 11α,13-dihydrohelenalin lacking the α-methylene group, is less active and inhibits NF-κB only at higher concentrations than helenalin[38,39].

New Evidence on Allergic Potential
The allergenic potential is a difficult subject since many factors are involved in an allergic response. The Arnica species used in a formulation, the type of extract, the concentration of active ingredients, other ingredients in the formulation and, of course, the applied test model for sensitisation all have influence on the test results.

In literature the allergenic potential of Arnica has been discussed controversially in the past. Some time ago the allergenic potential of Arnica was investigated using the guinea pig skin erythema test with the result that it was classified as a strong contact allergen[40]. However the disadvantages of this test model are obvious e.g. choice of test concentration, choice of vehicle, practice of reading, use of control animals and interpretation of test results vary widely and are not standardised. This could lead to overestimation of the sensitisation hazard for certain substances[41,42].

A new in vitro and in vivo study at the department of allergology at the renowned dermatological clinic of Freiburg demonstrates that topically applied Arnica preparations are not strong contact allergens[43]. The investigations were performed by using the mouse contact hypersensitivity model, which is today the standard model for allergic contact dermatitis assessment[44,45]. The anti-inflammatory and allergenic potential of alcoholic Arnica tinctures from Arnica montana Spanish type and Central European type were investigated beside the effect of pure helenalin and 11α,13-dihydrohelenalin[43]. It was found that Arnica tinctures as well as helenalin or dihydrohelenalin are only weak contact sensitisers. Another study which is in accordance with these results demonstrates that the SQLs of helenalin and dihydrohelenalin type did not play a major role in positive allergic tests with Arnica. There was also no difference found between helenalin and dihydrohelenalin type of SQLs regarding allergic reaction[46,47].

Further investigations, however, reveal that the SQLs have a dual effect: at high concentration they are anti-inflammatory by inhibiting NF-κB DNA binding through alkylation of NF-κB p65[48,49] and at low concentration they have a pro-inflammatory and pro-allergic effect as a result of NF-κB activation and hapten modification of proteins to generate antigenic T cell determinants[49,50]. In vitro tests with human keratinocytes and dendritic cells of mouse and human have shown that the balance between these opposing effects is dose dependent. The standardised Arnica CO2-extracts with a high concentration of SQLs are therefore the first choice for the development of products with good efficacy and control over allergic reaction.

The anti-inflammatory activity of dihydrohelenalin is lower than that of helenalin. Therefore the concentration of dihydrohelenalin derivatives must be approximately 10-fold higher than the concentration of helenalin derivatives, in order to achieve the same anti-inflammatory effect. In the mouse model contact hypersensitivity could not be induced even if the Arnica tinctures and SQLs are applied undiluted to inflamed skin.
The findings above are supported by recent clinical studies. In spite of extensive use of Arnica preparations, the frequency of allergy occurrence is surprisingly low, if used properly. A proper use requires analytical, well documented extracts and formulations with defined and standardised composition of active substances. Furthermore, indication of dosage ranges is crucial for achieving good efficacy and controlling allergic response. This is confirmed by several recent studies. Only three volunteers out of 213 who reacted positively to the composite mix (Arnica, Camomile, Feverfew, Tansy and Yarrow) showed a positive reaction to a 0.5% ether extract of Arnica\(^{51}\). In another study with the same setting, only five of 443 volunteers showed a positive reaction\(^{52}\). In a European multi-centre study on allergic contact dermatitis, only one volunteer out of 475 developed an allergy\(^{53}\).

Contact dermatitis of Arnica preparations described so far were performed almost without exception with Arnica tinctures whereby it was not always clear if they were used in the correct dilution\(^{54}\). These statements are in accordance with the results of a Danish study demonstrating that sensitisation occurred mainly from the topical use of Arnica tinctures which had highly variable SQL concentrations\(^{55}\). In the tinctures and infusions SQLs were partly detected in a very low concentration or only in traces whereas other analysed samples, e.g. an Arnica CO\(_2\)-extract was much higher in SQLs\(^{56}\). It seems therefore reasonable to suppose that such low concentrated SQL formulations can cause allergy due to the dual effect of SQLs. It also suggests that the presence of other allergens in low SQL infusions and tinctures which elicited positive allergenic reactions\(^{56}\). Also ether extracts contain not only SQLs, but also other potential allergens\(^{51}\). Arnica should not be taken internally, except in the form of a homeopathic dilution. Arnica should not be applied to injured skin where absorption can occur.

**Conclusion**

Supercritical Arnica extracts are the first choice for the development of high quality dermatological products. They are analytically well documented and contain a high and standardised concentration of the active SQLs while simultaneously the allergenic potential is under control.

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Author's Biography

Dr. Peter May studied chemistry at the University of Saarland and received his Ph.D. in 1991 in metal-organic chemistry. For the last twelve years he has been working at Flavex Naturextrakte in Sales and Marketing.

Dr. May has been the author of articles on plant science and phytotherapy in several publications. Flavex is a producer of specialty botanical extracts for cosmetics and for the food supplement industry on the basis of supercritical CO2-extraction.