# Regional added value with medicinal plants in the Rhenish mining area

by Dr. Lena Grundmann

**Herbs and medicinal plants offer an almost infinite source of pharmaceutically useful substances for phytopharmaceuticals and have a high value-added potential in the agricultural, cosmetics and food industries. We intend to use this potential as one element to establish a model region for the bioeconomy in the Rhenish mining area and thus help to manage structural change.**

Of the approximately 50,000 plant species used for medicinal purposes worldwide, only 900 species are cultivated and currently about 90 percent of the medicinal plants needed in Germany are imported. In addition, most of the raw material required for phytopharmaceuticals derives from wild collections. This approach is neither sustainable nor ecologically sound and therefore wild collections have been severely restricted as a result of the Nagoya Protocol coming into force. Furthermore, the active ingredient content in wild plants varies considerably, often leading to unacceptable quality losses.

In the department "Functional and Applied Genomics", to-gether with other partners Fraunhofer UMSICHT and Forschungszentrum Jülich, we therefore dedicated ourselves to the sustainable production of sufficient quantities of high-quality medicinal plants in the BMBF-funded project "Circular PhytoREVIER". We focus our activities on the establishment and consolidation of a highly efficient and economically viable process chain: from the breeding of yield-optimized plants and the development of new and effective cultivation and harvesting technologies to the efficient extraction and supply of the active ingredients from the raw material. The focus of our R&D activities is on transforming valuable medicinal plants into crops adapted to agricultural conditions by means of selection and modern plant breeding (including proof-of-concept studies using genome editing). Together with our partners, we want to develop innovative processes for the targeted control and increase of the active ingredient content through biological, chemical and physical stress.

## Focus plant arnica

One of the focus plants is the medicinal plant *Arnica montana* L. Its bright yellow and aromatic flower heads are used in a variety of phytopharmaceutical preparations. For skin application, they are processed into tinctures as well as ointments, creams or gels. The main field of application is in all non-bleeding, i.e. blunt injuries such as bruises, swellings, sprains and bruises. The pharmacological properties of the flower heads are primarily attributed to the sesquiterpene lactones helenalin and dihydrohelenalin and their derivatives. In nature, these serve the arnica plants as antifeedants against herbivores and defense against microorganisms.

It is known that both the composition and content of sesquiterpene lactones (SLs) depend on a variety of different abiotic and biotic factors that control their biosynthesis and accumulation. In flowers, SL content varies from 0.3 to 1 g per 100 g dry weight. The European Pharmacopoeia (Ph. Eur. 11.0 Arnica flowers No. 1386) requires a minimum content of sesquiterpene lactones of 0.4 g per 100 g in the dried drug Arnicae flos (arnica flowers). Depending on the origin, two different chemotypes are distinguished, which are clearly differentiated in their SL composition: *Arnica montana* subsp. *montana* of Central European origin has mainly helenalin/esters and *A. montana* subsp. *atlantica* of Spanish origin has dihydrohelenanin/esters as main sesquiterpene lactones (Fig.1A).

## Sesquiterpene lactone biosynthesis in arnica: first steps elucidated

Knowledge of the biosynthesis of desired products is an essential prerequisite for innovative breeding of yield-optimized plants. For *Arnica montana*, the biosynthesis of sesquiterpene lactones (SLs) has not yet been elucidated. Based on studies in closely related species, scientists assume that the initial steps of biosynthesis are conserved in the Asteraceae family, they proceed identically up to the intermediate product germacrene A acid (GAA). For arnica, the genes responsible for this process have not yet been described and characterized, and no genomic sequences of Arnica are available in public databases. Therefore, we used sequence data from closely related species such as sunflower (*Helianthus annus*) to derive the corresponding genes from arnica. In this way, we started by generating and sequencing fragments of the corresponding genes. With the help of various PCR techniques, we succeeded in completing the gene sequences in the next step. Thus, in both chemotypes we identified seven genes coding for key enzymes of terpene biosynthesis and ten candidate genes possibly coding for enzymes of helenalin biosynthesis.

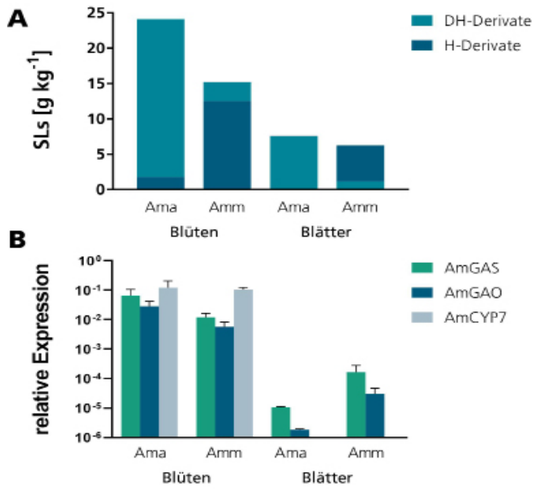


*Fig. 1: SL content in flowers and leaves of two Arnica chemotypes A. montana subsp. montana (Amm) mainly helenalin derivatives, A. montanta subsp atlantica (Ama) mainly dihydrohelenaliv derivatives (A). Comparative expression analysis of potential SL biosynthetic genes* AmGAS, AmGAO, AmCYP7 *in flowers and leaves (B).*

We can detect SLs in both flowers and leaves of arnica plants, with higher content in flowers (Fig.1A). These results can be explained by biosynthesis in both organs and/or interorgan transport. To verify this, we performed expression analyses: *AmGAS*, *AmGAO*, and *AmCYP7* genes are expressed more than 100-fold higher in flowers than in leaves (Fig.1B). The conclusion: SLs biosynthesis is enhanced in the flower; however, it also occurs in the leaf; in addition, SLs could also be transported from the leaf to the flower.

For the candidate genes, we also used expression studies to clarify the exact nature of the involvement of the gene or gene product in the biosynthetic pathway. Scientists usually use heterologous expression systems such as the yeast *Saccharomyces cerevisiae* for these analyses. Fraunhofer IME in Münster has a yeast production strain specifically designed for the biosynthesis of selected secondary metabolites. We stably integrated the coding sequences of the candidate genes, either individually or in combination, into the yeast genome. A few days after induction, we harvested the cultures, extracted potential biosynthetic products and performed GC/MS analysis. Thus, AmGAS1 was identified as a functional germacrene synthase and, based on this, AmGAO1 was also characterized as a functional germacrene A oxidase (Fig. 2).

The first steps of SL biosynthesis towards GAA in arnica could be successfully elucidated and serve as a basis for the characterization of additional identified candidate genes (*AmCYPs*) for the subsequent steps towards the synthesis of active helenalin and dihydrohelenalin derivatives and for the establishment of a cell culture-based drug production platform.

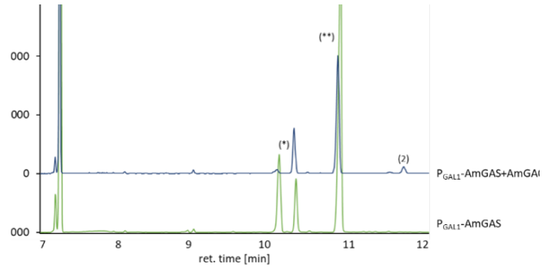


*Fig. 2: By GC/MS analysis, a first precursor (germacrene A, peak (1)) of SL biosynthesis was identified in AmGAS and AmGAS-AmGAO expressing yeast cultures. According to the additional AmGAO gene present in AmGAS-AmGAO expressing yeast cultures, another peak (2) was detected here, which is characteristic of Germacrene A acid (GAA).*

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