

ARNICA MONTANA L. AS A MEDICINAL CROP SPECIES

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Abstract

The increased demands on the market for pharmaceutical plants from the wild put a high pressure for their future conservation and therefore they need protection. Thus, species such as Arnica montana L well known for their medicinal effects in a range of human diseases became for long time a target for being collected from the wild. Still, since 1977 the species is declared as nature monument and later on it is protected under the current legislative framework for nature protection. However the transfer of the plant into the crop field my further support the access to this genetic resource without any harm on the wild species. The scope of this article is to analyse if Arnica montana may change the profile of volatile oil if it is transferred from the wild into the crop field. Based on morphometry and biochemical analysis it can be considered that arnica may become a medicinal crop plant as no changes in their volatile oil composition was observed.

Key words: *Arnica montana, crops, field cultivation, pharmaceuticals, Romania*

INTRODUCTION

The drug market is fast heading toward the replacement of common drugs of chemical synthesis with those of natural origin and in particular derived from plants. But it is necessary to pay special attention to the origin of plant extracts especially when the species are to become endangered and therefore new conservation measures should be in place. Thus, *Arnica montana* L., a species quite common in Romania until recent years they have been recognized that it was a drastic reduction in their population [4, 10, 15, 18, 19, 20, 23, 24]. As a consequence this species was included in Annex 5 of the Governmental Emergency Ordinance GEO 57/2007 [1]. Therefore in order to cover the current market demands it is necessary to establish in vitro micropropagation protocols [13, 16, 25] and specialized farms for medicinal crops, especially in areas where climatic conditions are most favourable to the species, in our case the mountain region.

Arnica is a herbaceous plant that belongs to the Asteraceae family, that is particularly prevalent in sub-alpine region, through hayfields and wet pastures throughout the Carpathians and recognized as a acid-

neutrophil species [3]. The rhizome is cylindrical, thick, from which fibrous roots are forming. The stems is erect, cylindrical, simple, high up to 60 cm rarely branched, with short hairs, finished with an inflorescence. Yellow flowers are arranged in a head, ligulae are marginal, and tube shape are central [3].

The flowers are rich in active pharmaceutical compounds such as volatile oil, arnidiol, arnisterina, faradiol, astragalin and carotenoids; the roots contain, in addition to volatile oil and caffeic acid, fumaric acid and succinic also inulin and thymol, etc. The most important feature, in terms of therapeutic compounds are Sesquiterpene lactones (especially helenalin) [17].

Flowers extracts are used in human and veterinary medicine, including homeopathy [6]. The active principles have antiseptic (e.g. treatment of burn), inflammatory (e.g. rheumatic, anti-hemorrhoidal) anti-sclerotic, choleric, cholagogue, diuretic, blood pressure regulator, etc [24]. Although some studies have shown a potential for toxic side effects even at high doses of alcohol extraction, a recent study has disproved these claims, however recommending the use of Arnica preparations based on only for external

use [3] Besides flowers of pharmaceutical interest are roots and rhizomes [8, 14, 15].

The scope of this research is to study the behaviour of arnica as a crop plant during the first year after transplanting from the wild in the field and to identify valuable genotypes. Peculiarities such as the optimum momentum for transplanting or factors controlling the growth and multiplication were studied as important for developing the agricultural technology in order to obtain plants as well as seeds. Also, chemical analysis of volatile oil extracts originating from flowers were realized in order to reveal if the cultivation of the species may affect its pharmaceutical qualities.

MATERIALS AND METHODS

Plant material consisted of *Arnica montana* L. seeds harvested from native plants from the wild namely Valea Frumoasei (Alba) genotypes G1 - G3 and Cindrel Mountains (Sibiu) genotypes G4 - G6 (Figs. 1 and 2). The seedlings were obtained in heated seedbeds, and in the phase of 1-2 true leaves, they have been transferred in pots under controlled conditions [3, 7].

Planting method The experimental plots consists in row at a distance of 1m between rows and 0.5 m between seedling into the row. The working scheme consist in 3 variants (accounting for propagation method) located in three repetitions. Each variant included 10 plants.

Plant extracts have been obtained from dried flowers after harvesting [22]. It was prepared a mixture of ligulate and tubular flowers, dolls predominantly bulky. This extract was submitted to volatile oil analysis.

The extraction method of volatile oil Only Arnica flowers have been used following the method of Bergonzi et al. 2005 [2, 21, 22]. Upon harvesting flowers were washed under tap water for 3 min and dry up to constant weight, chopped and submitted to volatile oil extraction. 50 g of dry plant material of Arnica were introduced to the distilling flask of 2 l with 700 ml of distilled water. Subsequently the flask was adapted to a distillation device. The oil collecting tube,

with an graduated increment of 0.01 ml, and the bottom of the water separator was filled with a deviation above the separator, which is then closed with a cork traversed by a channel. It was further allowed the free water flow into the refrigerator and the balloon was heated to boiling distilled at moderate speed. After completion of the distillation the vapours were allowed to run for a few minutes in order to wash traces of oil. After about 30 minutes the oil layer tube slowly descended gradually by opening the drain valve. The extraction duration was 4-5 hours /sample. At the end obtained volatile oil was measured in ml/100 g plant tissue. The resulting oil was supplemented with 1 ml benzene. The fraction oil with benzene was trapped in glass vials carefully for each rhizomes sample individually. The glass bottle included a small amount of anhydrous sodium sulphate to remove water traces. The volatile oil was extracted from the glass vial, benzene has evaporated and the oil was kept in a vial to be kept in optimal conditions until analysis without changing its properties.

The identification of compounds of pharmaceutical interest was performed using standard thin-layer chromatography (TLC) and gas-chromatography [9, 12]. During the determination of the temperature of the chromatographic column must remain constant, or, if necessary, to increase by a given program.

RESULTS AND DISCUSSIONS

Arnica montana as a crop plant

Arnica montana L. proved to maintain in the crop filed the same characteristics like in the wild. Thus the species developed well rhizomes and during the spring time emerge a rosette of oval leaves. In their midst emerge a cylindrical hairy stem, which ends in the inflorescence of 4-8 cm in diameter [11]. On the stem, 1-2 pairs of opposite leaves appears, smaller than the basilar. The stem is slightly branched, with opposite branches and ending each with one flower. The flowers are yellow-orange, sitting surrounded by bracts capital, often reddish-brown, glandular hair cells. At the edge the capitulated female flowers are in

the shape of ligule, ending with three teeth and are found only in the centre of blossom tubular flowers hermaphrodite. Both the flowers and the tubular ligule are surrounded by a papus of 8 mm long, made of stiff hairs, slightly toothed. It blooms in June up to August.



Photo 1 *Arnica montana* L.– The Cindrel Massive



Photo 2. *Arnica montana* L. – The Valea Frumoasei - 2007

Regarding the morphometry of the plant it was found that the rhizome is thick, horizontal, with numerous fibrous roots, less branched.

The stem is erect, cylindrical, 15-55 cm high, solitary, occasionally single or 1-2 branches, one anthodium, often up to 5 anthodia with a basal rosette leaves and one, often two pairs leaf stems, opposite, small bottom (rarely up under anthodium) glandulous often reddish brown.

Basal leaves are elliptical, rarely wider or lanceolate, sessile, slowly attenuated obtuse, whole or very small and spaced denticulate, hairless or rough because short hairs, with glandular cells (Table 1).

Chemical analyses The flowers (*Arnicae flos*) have to be harvested in June July at the beginning of flowering by cutting or tearing

inflorescences. Drying is done in the shadows in a thin layer. The artificial drying is realized at 40-50 °C [5, 6, 9].

Table. 1. Morphometry results on *Arnica montana* L. in the experimental field

Genotype	Plant no.	Rhizome length (cm)	Stem height (cm)	Leaves no
G1	6	5.5	45.2	6
G2	4	6.7	46.3	6
G3	9	5.3	44.8	6
G4	7	7.2	55.8	8
G5	7	7.6	58.2	10
G6	6	6.9	55.9	8

For these analyses were used dried flowers after harvesting. It was prepared a mixture of ligule and tubular flowers, dolls predominantly bulky.

The extract was performed at the end of the growing season, the volatile oil content in the organs generative (flower). The volatile oil extracted from all samples of yellow-orange inflorescences, semi-solid consistency, smells as fragrance.

This extract was reported in ml per 100 g vegetable oil. Based on these results the flowers of species *Arnica montana* L. may contain volatile oil between 0.2 and 3.8 g /100 dry plant.

Qualitative analysis performed with gas - chromatography aimed to elucidate the composition and among these compounds we are mentioning the following: terpenes, fatty acids and alcohols triterpenes (Fig.1.).

It can be concluded that there was thus identified the following structure of the essential oil arnica composition with no change between cultivated and wild plants:

-monoterpenes oxygenated compounds: thymol, moldo-hydroquinone dimethyl ether, thymol and methyl ether thymil iso-butyrate.

- compounds other m-ethyl phenol, fatty acids representing almost 50% of the composition, including palmitic acid, linoleic, myristic acid, caffeic acid and angelic; triterpene alcohols of which have been identified arnidol, faradiol, carnaubilic alcohol;

- lactone sesqui-terpenes belonging to the class of pseudo-guaianolidic: arnisterine, astragaline, izo-quvereitine;

- carotenoids: xanthophyll, xanthophyll-epoxy, zeaxanthine.

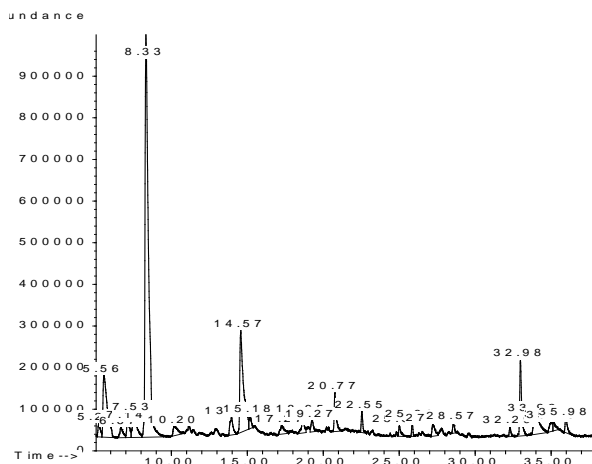


Fig. 1. Gas-chromatography diagram representing the volatile oil components in dry flowers of *Arnica montana* L. which is the same in control and cultivate plants

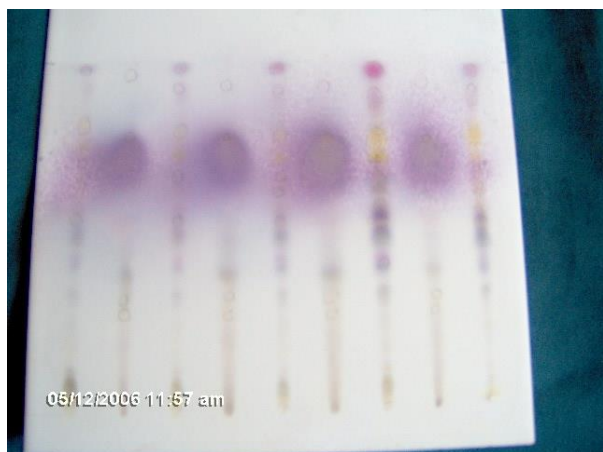


Fig.2. Thin -layer Chromatography for volatile oil extracts in *Arnica montana* L. The control is the first in the left of the series of extracts on the figure.

Thin-layer chromatography and gas - chromatography allowed the identification and quantification of all volatile oil compounds in arnica for comparing them with the control plants from the wild (fig 4). Thus it can be mentioned: mono-terpenes oxygenated compounds thymol 12%, dimethyl ether of hydroquinone 15.7%, the ether of methyl-thymol and thymol -isobutyrate 11.3% in similar concentrations and proportions both in the wild and as a crop plant [17, 21, 22, 26, 27].

CONCLUSIONS

The transfer from the wild into the crop field of *Arnica montana* proves to not alters the composition in volatile oil extracts in the dried flowers. The Arnica plants cultivated in the crop filed proved to maintain their botanical characteristics as well no change was identified between the control from the mountain and the cultivated plants. As a consequence the final conclusion of this study is that *Arnica montana* may be transferred into the crop field, treated as a crop plant without any negative effect on the composition of volatile oils.

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