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SOME ASPECTS OF THE MORPHO-ANATOMICAL FEATURES OF THE MEDICINAL PLANT *ARNICA MONTANA* L.

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Abstract

The objective of this work was the morphological and anatomical study of vegetative organs in the species Arnica montana L. it belongs to the Asteraceae family. The genus includes approximately 30 species, herbaceous, perennial, has yellow or orange flowers, reaches a height of approximately 60 cm. It originates from the mountain regions of Europe, North America. It is commonly found in alpine meadows, on wetlands and in forests. Arnica montana L. is an endangered medicinal plant species endemic to Europe. Traditionally used in Romania for its anti-inflammatory and analgesic properties, it stimulates blood circulation, reduces edema, has a healing and antimicrobial effect. After analyzing the morphology and anatomy of arnica leaves, it was found that in both epidermises there are peri tectors and glands, short and long, the mesophyll is bifacial type, differentiated into palisade tissue with 1-2 rows of cells, under upper epidermis and lacunate tissue, with 4-5 rows of cells, with intercellular spaces, below the lower epidermis. The leaf blade is amphistomatic, with stomata in both epidermises.

Key words*: root anatomy, leaf anatomy, endemic, morphology.*

INTRODUCTION

The genus *Arnica* includes 32 species, which are distributed in the boreal zone and mountainous regions of Europe and North America. *Arnica montana* L. is native to Europe and can be found in its natural habitats from the mountainous regions of southern Iberia to southern Scandinavia and in the Carpathian Mountains of Romania (Pljevljakušić et al., 2014). Some species of the *Arnica* genus are medicinal plants widely used in both the pharmaceutical and cosmetic industries. Since 1977, *A. montana* L. has been declared a natural monument. Due to the fact that *Arnica montana* L., a species that was once quite widespread in Romania, has seen a significant decline in its populations in recent years, it has been included in Annex 5 of the Government Emergency Ordinance OUG 57/2007 (Antofie, 2012). Although *Arnica montana* is a protected species under the EU Habitats Directive and the EU regulation on trade in flora and fauna, it is currently listed only on the IUCN Red List as a species of "least concern" at the European level (https://eunis.eea.europa.eu/species/Arnica%20montana).

The inflorescences of *Arnica montana* L. are widely used in various types of phytopharmaceuticals, such as ointments, creams, and gels, due to their anti-inflammatory action, which is used in the local treatment of contusions and sprains (Pljevljakušić et al., 2014). The inflorescences of *Arnica montana* are characterized by antiseptic, anti-inflammatory, antifungal, antioxidant, and antisclerotic properties (Gaspar et al., 2014). They exhibit cardiotonic and cardiotoxic effects (Malarz et al., 1993) anxiety-reducing and analgesic effects (Ahmad et al., 2013), insecticidal effects (Ahmad et al., 2013), and hepatoprotective activity (Marchishin, 1983).

Plant extracts demonstrate antioxidant, antibacterial, anti-inflammatory, antifungal, and immunomodulatory activities, as well as antitumor, choleretic, cholagogue, diuretic, antirheumatic, anticancer, and musculoskeletal pain-relieving effects (Macêdo et al. 2004;

Kriplani et al., 2017; Rotar, 2005; Žitek et al., 2022; Nieto-Trujillo et al., 2021; Schmidt, 2023; Milani et al., 2023; Röhrl et al., 2023; Attiq et al., 2018; Sugier et al., 2020; Parafiniuk et al., 2023; Röhrl et al., 2023; Verre et al., 2024). Gautam et al., 2018, studied *Arnica* for Carpal Tunnel Syndrome, which generally affects women over the age of 30. Epistaxis can often be treated with *Arnica* (May Loo, 2009). Animal studies with microcurrent and *Arnica* gel have proven effective in treating skin lesions through anti-inflammatory effects and by reducing the presence of macrophages in the skin (Zacarias et al., 2023).

As a result of numerous chemical studies on *Arnica* species, approximately seventy sesquiterpene lactones, more than eighty flavonoids, phenolic acids, thymol derivatives, diterpenes, and pyrrolizidines have been identified (Kowalski et al., 2015). The use of *Arnica* before and after surgery to reduce bruising, swelling, pain, and soreness has been documented, with some variations in potency (strength) and dosing frequency (Burke, 2017).

Arnica reduces pain and has an anti-inflammatory effect after surgeries (Iannitti et al., 2016) and reduces bruising following rhinoplasty surgeries (Chaiet & Marcus, 2016). Some studies indicate that the essential oil from the roots (2-4%) and rhizomes (3-6%), which mainly contains thymol and derivatives with bactericidal and fungicidal activity, contributes to these effects (Kohlmünzer, 2003).

MATERIALS AND METHODS

In this study, the morphological and anatomical characteristics of the vegetative organs of *Arnica montana* L. were investigated.

The plants used in the experiment were collected from the Blana Bucegi experimental center, under the coordination of the Research and Development Institute for Pastures in Brașov (ICDP Brașov), during the period of July 5-10, 2024.

After collection, they were transferred and acclimatized in the greenhouse of the Medicinal and Aromatic Plants Laboratory within the same institute.

These steps ensured optimal conditions for plant development prior to conducting the morphological and anatomical research (Figure 1).

The research material consisted of young plants at the 6-8 leaf stage. At this stage, the leaf of *A. montana* is fully developed and represents a stable biological unit that can be effectively analyzed to obtain precise data. The use of fresh material is important, as it allows for the best results in structural analysis, with the cells and tissues being in an active state, unaltered by dehydration processes that may occur during preservation.

The study of leaf anatomy is of great importance in taxonomy, as both the internal and external structures of leaves are often used for plant classification and for understanding their evolution and adaptability. A detailed knowledge of these features significantly contributes to deciphering the phylogenetic relationships between species and deepening our understanding of plant diversity in nature.

The microscopic examination was carried out on cross-sections of leaves treated with chloral hydrate for clarification, facilitating the observations. The study was conducted in the Botany Laboratory - Plant Morphology and Anatomy Department of the Faculty of Horticulture at USAMV of Bucharest, using Leica and Optika binocular microscopes, instruments that allow detailed observation of the internal structures of the leaf. Photographs were taken with a Motorola digital camera, using objectives of different magnifications to capture details at

various levels, which are essential for precise analysis of leaf anatomy.

Figure 1 a, b. - a - Arnica obtained from seeds;
b - Arnica obtained from the mother plant

Research on the morphology of *Arnica montana* L. has been conducted by Sugier et al., 2022, Parafiniuk et al., 2023. Knowledge of anatomical elements is crucial in the field of taxonomy.

Studies related to the anatomy of the species under study, as well as other species from the Asteraceae family, have been conducted by Pljevljakušić, 2012; Kostina & Muravnik, 2019; Milan et al., 2006; and Kromer, et al., 2016.

RESULTS AND DISCUSSIONS

Morphology and Anatomy of the Root and Leaf of *Arnica montana* L.

Morphology and Anatomy of the Root

Transverse sections were made through the fibrous root of *Arnica montana* (Figure 2).

Figure 2. Fragment from the fibrous root of Arnica

In transverse section, the root has a circular contour (Figure 3 a, b).

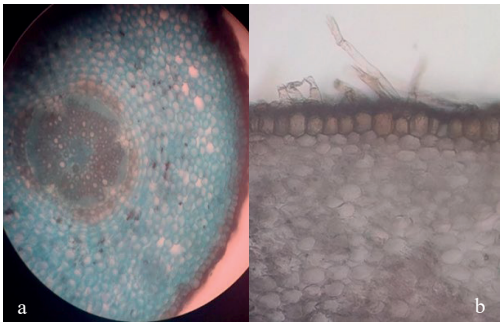

Figure 3 a, b – a - Root - Transverse section - general appearance; b - Rhizodermis with absorbent hairs

The outer surface of the root is covered by rhizodermis, with a single row of cells and absorbent hairs, cortex with parenchymal cells, and intercellular spaces (Figure 3), along with secretory canals, which are rounded or oval in shape. The central cylinder contains the pericycle, xylem, and phloem, while the pith consists of parenchymal cells with thin walls at the center of the section (Figures 4 and 5).

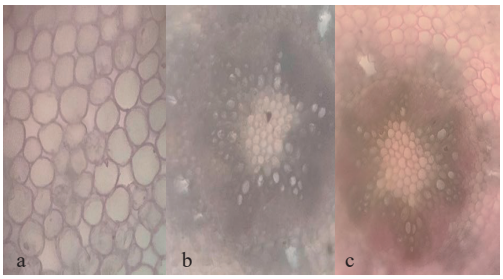

Figure 4 a, b, c – a - Root - Transverse section - cortical parenchyma with intercellular spaces;
b, c - Secretory canals and central cylinder

The structure of the fibrous root from plants obtained from seeds and from cuttings was the same, and thus the sections will not be presented separately.

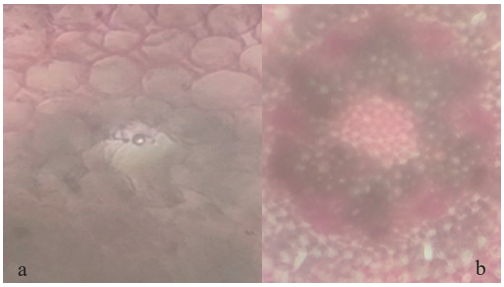

Figure 5 a, b – a - Root - Transverse section - secretory canal with oil droplet; b - Central cylinder with pith at the center

Morphology and Anatomy of the Leaf

Anatomy of the Petiole

The petiole is very short, with the leaf being nearly sessile.

It contains bicolateral vascular bundles embedded in parenchymal tissue, which are surrounded by a unistratified epidermis. Characteristic trichomes are present on the leaf surface (Figure 6 a, b, c).

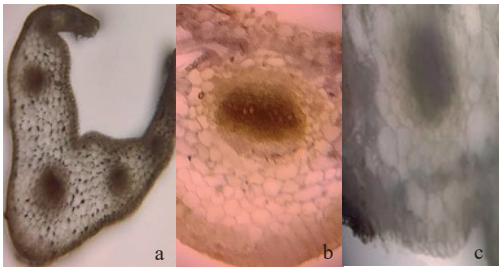

Figure 6 a, b, c – a - petiole overview; b, c - Petiole with the main vascular bundle - detail

Micromorphology and Anatomy of the Leaf

Leaves from both seed-grown and cutting-propagated plants were analyzed. Since no significant structural differences were observed, only the elements that stood out are mentioned. The leaves exhibited long and short multicellular protective trichomes on the margins, as well as glandular trichomes, both sessile and pedicellate, uniseriate and multiseriate, moniliform, of the same types (Figure 7 a,b,c, Figure 8 a,b,c, Figure 9 a,b,c,d, Figure 10 a, b, c, d).

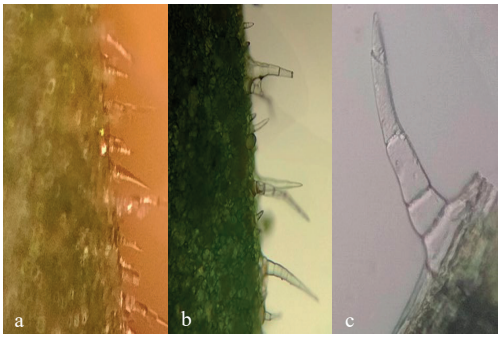

Figure 7 a, b, c – a - Short and long multicellular glandular trichomes on the leaf margin; b - Protective and secretory hairs on the leaf margin; c - Detail of multicellular protective hair (plant obtained from seeds)

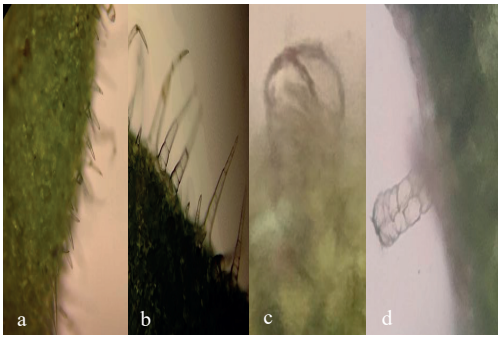

Figure 8 a, b, c, d – a, b – Short and long multicellular protective trichomes on the leaf margin; c – Bicapitate glandular trichome; d – Multiseriate glandular trichome (on the mother plant).

Anatomy of the Lamina

The leaf of *Arnica montana* in transverse section shows the upper epidermis, the lower epidermis, and the mesophyll with vascular bundles (Figure 9 a, b, c, d).

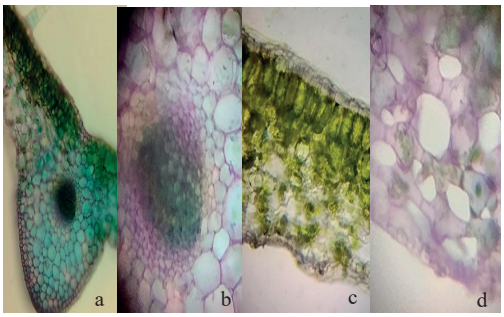

Figure 9 a, b, c, d – a, b - Transverse section of the lamina and main vein - overview and detailed view of the vascular bundle; c - Transverse section of the lamina

The mesophyll is bifacial, differentiated into palisade tissue consisting of 1-2 rows of chloroplast-rich cells located beneath the upper epidermis, and lacunose tissue formed by 4-5 rows of isodiametric cells with intercellular spaces and spherical secretory canals, located beneath the lower epidermis, containing bicolateral vascular bundles. The leaf is amphistomatic, with more stomata in the lower epidermis compared to the upper epidermis. Both epidermises contain protective trichomes, including uniseriate, short and long, slightly curved multicellular trichomes, as well as glandular trichomes - short and globular, as well as long, uniseriate, multiseriate, and bicapitate.

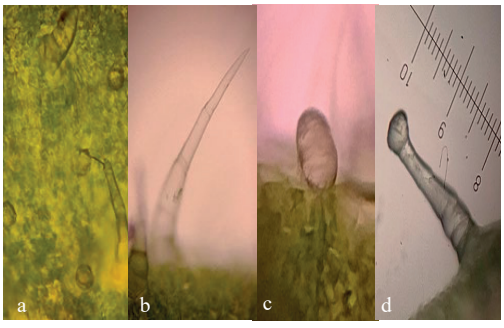

Figure 10 a, b, c, d – a - Upper epidermis with protective and glandular trichomes; b - Protective trichome; c - Sessile glandular trichome; d - Multicellular uniseriate glandular trichome (seed-grown plant)

In leaves of cutting-propagated plants, multi-seriate bicapitate glandular trichomes predominate.

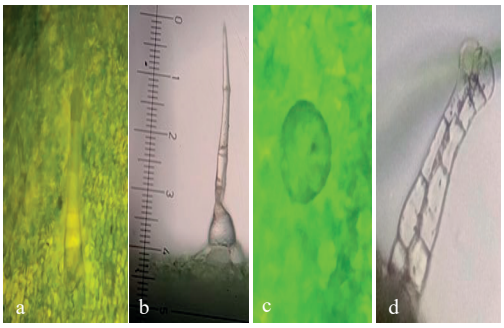

Figure 11 a, b, c, d – a - Upper epidermis with protective and secretory trichomes; b - Protective trichome; c - Glandular trichomes; d - Multiseriate glandular trichome (mother plant)

In the epidermises, there are both long and short multicellular protective trichomes and glandular trichomes, which are sessile, spherical, and pedicellate, uniseriate and multiseriate (Figure 10 a, b, c, d; Figure 11 a, b, c, d, ; Figure 12 a, b, c; Figure 13 a, b, c).

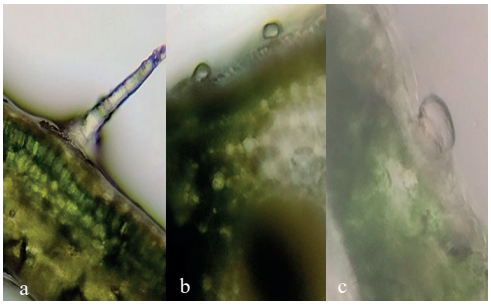

Figure 12 a, b, c – a - Upper epidermis with protective trichomes; b, c - Glandular trichomes, overview and detail (transverse section - mother plant)

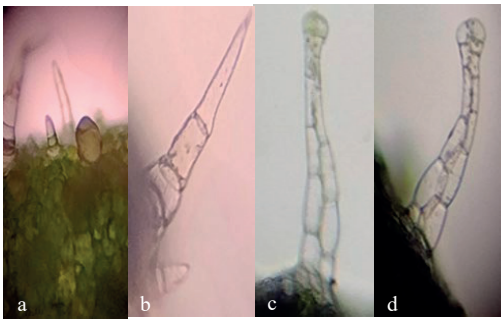

Figure 13 a, b, c, d – a - Lower epidermis with long and short multicellular protective trichomes and glandular trichomes; b - Long multicellular protective trichomes and short trichomes; c, d - Multiseriate glandular trichomes (mother plant)

On the lower epidermis of the leaf, protective trichomes, multicellular and glandular, spherical, and multiseriate, are observed (Figure 13 a, b, c, d).

In species of Asteraceae, trichomes are predominantly biseriate (Appezzato-da-Gloria et al., 2012 and others).

Glandular trichomes accumulate various products of primary and secondary metabolism, which, in addition to being essential elements for species identification, also contribute to the medicinal properties of the plant (Gopfert et al., 2005; Muravnik et al., 2016, 2019).

In the upper epidermis, rare stomata are observed, and the epidermal cells have undulated walls, with no intercellular spaces. The epidermis at the level of the main vein contains polygonal cells and stomata (Figure 14 a, b, c, d).

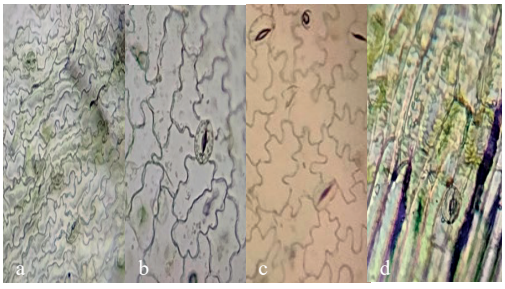

Figure 14 a, b, c, d – a, b - Upper epidermis of seed-grown Arnica; c - Upper epidermis of cutting-propagated Arnica (mother plant); d - Upper epidermis above the main vein (mother plant)

In the lower epidermis, more numerous stomata and glandular trichomes are observed, and the epidermal cells have undulated walls, with no intercellular spaces (Figure 15 a, b, c; Figure 16 a, b, c).

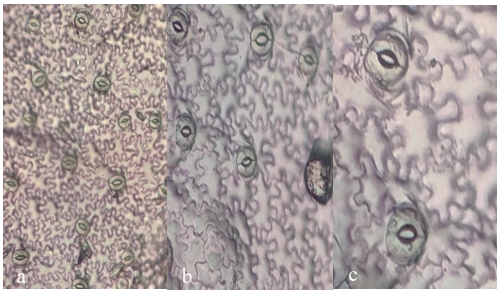

Figure 15 a, b, c – a, b - Lower epidermis of seed-grown Arnica; c - Upper epidermis of the mother plant

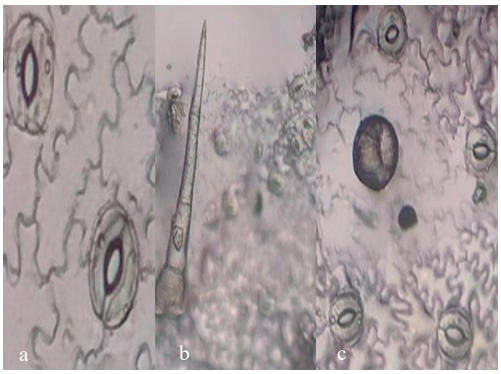

Figure 16 a, b, c – a, b, - Lower epidermis of seed-grown Arnica; c - Lower epidermis of the mother plant

CONCLUSIONS

Morphological and anatomical analyses are crucial for taxonomy, systematic botany, and for verifying the authenticity of semi-processed material obtained from this medicinal plant.

These morphological and anatomical analyses are being carried out for the first time in Romania for this species, and the detailed characteristics are presented in the Results and Discussion.

Both in the root and in the leaves, structures secreting essential oils, used for medicinal purposes, were observed.

The morphological and structural characteristics are similar in plants obtained from seeds and those obtained from cuttings, with the exception that in plants propagated through cuttings, multiseriate glandular trichomes predominated on the leaves.

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