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HISTO-ANATOMICAL CONSIDERATIONS ON SOME ROMANIAN *INULA* L. SPECIES, WITH PHARMACOLOGICAL ACTION

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Abstract: In the present study, the anatomy of vegetative organs *of Inula* species was investigated. Some of these species have a proven pharmacological action, while the medicinal value of other is still presumable. The presence of secretory canals in the structure of underground organs in some species is assumed to be related to helenin, a compound with multiple medicinal properties.
Key words: secretory canals, helenin, pharmacological, hairs.

Introduction

Inula genus *(Asteraceae* family) includes about 120 species, widespread in temperate regions of Europe and Asia [25]. In Romanian flora 10 species can be found, but one of these has not been anymore confirmed in the older [25] or recent botanical works [26; 11]. Moreover, there were included 6 [25] or 11 hybrids [26], which were not mentioned any longer by Ciocârlan [11].

All *Inula* species of Romanian flora are perennial, having rhizome [25]; only *I. conyza* D.C. has well branched fusiform root, but here is no mention about rhizome.

Among *Inula* species growing in our country, *Inula helenium* L. is considered a medicinal herb [4; 37; 34; 28; 29; 38; 13; 1; 2]. *Inula conyza* [28] and *I. britannica* L. [29; 13] are also recognized as medicinal plants. But many species *of Inula,* included in this study, were known and used in Romanian popular medicine [5; 27; 9; 10; 6; 3; 7]. Some recent works of pharmacognosy, phytochemistry and pharmaceutical technology deal with some composite plants [8; 19; 32]. Only Bruneton [8] refers specifically to *I. helenium,* stating that its underground organs contain eudesmanolides (alantolactone, isoalantolactone), germacronolides, triterpenes, sterols and inulin. Perrot and Paris [28], in their paper about medicinal plants of France, point out that *I helenium* L. contains helenin in the root (a complex of terpene lactones). For the same species, Barnes et al. [2] stated that inulin could reach up to 44 % of total constituents. Here is no place to extend the comments regarding the pharmacological actions of helenin. Attention should be paid only on the possible implications of these constituents in plant systematics. It has been suggested *that Asteraceae* differ from the clade *Calyceraceae* and *Goodeniaceae* mainly in producing various types of sesquiterpene [33]. In addition, the chemical nature of constituents produced by *Inula* species must be carefully checked, in order to avoid possible confusions and misunderstandings, as in the case of Nyárady [25], who refers to helenin as inulin and *vice versa.*

The anatomical structure of *Inula* species has been less studied. In handbooks of dicotyledons anatomy [30; 23] or in angiosperms anatomical monographs [24] there is mentioned only *Inula* genus. The same is also true in the case of extensive works referring on secretory hairs from *Lamiaceae* and *Asteraceae* taxa [20]. In some atlases of microscopy

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of medicinal plants [35; 36] here is taken under investigation only *I. helenium*. Lemaire [21] refers to hairs of *I. helenium* only accidentally. Mansfield [22] seems rather confusing when deals with inulin in relation to *I. helenium*, without saying anything about helenin. We must emphasize that in the atlas published by Terpilo [35], the author asserted that in the underground organs of this species secretory canals are localized in the primary and periderm, as well in the xylem. Unfortunately, the author was not able to clearly distinguish neither between rhizome and root (histological approach), neither between periderm and secondary phloem. Finally, but not in the last, Svoboda and Svoboda [31] in their atlas related to secretory structures of aromatic and medicinal plants, do not included any mention of any species of *Inula.*

Therefore, as derived from the above presented data, we can assume that the information about anatomy of vegetative organs of *Inula* species is very scarce. As far we know, only *Inula helenium* was considered for investigations by some authors.

In this context, the aim of our work is to develop an exhaustive study relative to the comparative anatomy on *Inula* species from Romanian flora. We will focus mainly on histological features with taxonomic value, as well as on some ecological anatomy considerations, drawing attention on the structure and position of secretory canals and non-glandular (protecting) and secretory hairs.

Material and methods

The plant material for this study was collected from Natural Reserve Valea lui David (Iasi) and belongs to the following species of *Inula*: *I. britannica* L. (from a salinized area), *I. germanica* L., *I. oculus-christi* L., *I. salicina* L. subsp. s*alicina* and *I. salicina* L. subsp. *aspera* (Poir.) Hayek*. Plants collected in anthesis stage in the summer of 2010 were fixed and preserved in 70% ethanol; then all vegetative organs were transversally sectioned. The sections were stained with fast green and ruthenium red and finally included in glycerol gelatin. Permanent slides were examined with a light microscope and micrographs have been taken using a NOVEX (Holland) microscope, with an A 95 Canon photo digital camera.

Results and discussions

Root

In all investigated species the roots are adventitious, originating from rhizomes and having an endogenous origin.

Rhizodermis has numerous root hairs in *I. oculus-christi* and is almost completely exfoliated in *I. britannica*. The cortical parenchyma, moderately collenchymatized or of parenchymatic type (*I. oculus-christi, I. salicina*), shows many air spaces in *I. britannica* and has very few sclerenchymatic elements.

Exodermis and endodermis are unilayered; the last presents Casparian strips and its cells are filled with a brown colored content, similar with the tannin (Fig. 1). Perrot and

* We thank Dr. Adrian Oprea for identifying the species which are subjected to investigation in this anatomical study

Paris [28] indicate that in *I. helenium* this content could be helenin (a mixture of terpene lactones), being also found in secretory canals located in underground organs of *Inula* species (Fig. 2). The nomenclature of these secretory structures is relatively disputed and here some confusing could arise, since even the classification of the secretory tissues is heterogeneous [12; 18; 15; 14; 16]. For instance, de Bary [12] refers to these secretory canals as *protogenetic secretory passages* (p. 525-526, op. cit.).

The stele could be triarch (*I. britannica*) (Fig. 3) tetrarch (*I. oculus-christi*), or polyarch (in other taxa). The two subspecies of *I. salicina* differ in the number of vascular bundles (7-8 of xylem and phloem in *I. salicina* subsp *aspera* and only 6 in *I. salicina* subsp. *salicina*). In all investigated species, the xylem vessels could be hardly distinguished from xylem parenchyma lignified cells. In the *I. britannica*, the phloem and xylem structure is mostly the result of secondary growth.

Rhizome

The epidermis is partially exfoliated in *I. oculus-christi*; epidermis has long, protective multicellular and uniseriate hairs in *I. britannica* (Fig. 4). In all five analyzed taxa, the cork (1-2 layers) is present, especially in hypodermical position.

The primary cortex contains sclerenchymatous elements (sclereids) in *I. germanica* which are more abundant in *I. britannica* (Fig. 5). In the internal layer of cortex, between periphloemic mechanical strands, helenin secretory canals can be observed (corresponding with the location of medullary rays) (Fig. 6). The size and the number of these are greater in *I. britannica* and *I. germanica* (Fig. 7). The secretory cavity is surrounded by one or more (*I. britannica*) epithelial layers. The secretory canals are more tangentially elongated in *I. salicina* subsp. *aspera* (Fig. 8) and very tight in *I. salicina* subsp. *salicina.* The cortex is bordered by a Casparian endodermis, well visible in *I. britannica.*

The stele has typical secondary growth, the vascular tissues forming two concentric rings; the xylemic ring is much thicker, with numerous phloem fibers near to the cambium, with or without (*I. salicina* subsp *aspera*) visible medullary rays, which are of parenchymatic – cellulosic nature at the level of phloem and sclerified and lignified (especially in *I. germanica*) at xylemic level. Outside of phloemic ring are located many sclerenchymatic elements strands; between them we observed above described secretory canals.

The pith is parenchymatic-cellulosic or lignified (Fig. 9) (*I. oculus-christi*), having many sclerenchymatic elements in *I. britannica.*

Stem

The contour of cross section is circular - with large undulations – in the upper part of the organ and typically circular in the other parts of the stem.

Epidermis presents long multicellular, uniseriate protective hairs, occurring in great number and being very long in *I. germanica* (Fig. 10) and *I. oculus-christi*; in *I. salicina* subsp. *aspera* they are shorter and thicker while in *I. salicina* subsp. *salicina* are completely absent. Moreover, in *I. oculus-christi*, we observed two different categories of hairs: some of these have the terminal cell very long, whip-shaped, and other are very short, with terminal cell pallet-shaped. Among all 5 investigated taxa we observed stomata only in *I. salicina*, where they are confined above the epidermis level.

The cortex, more or less collenchymatized (*I. britannica* and *I. germanica*) has a prominent aerenchyma (Fig. 11), as in well in the root, in *I. britannica.* In internal part of the cortex there are located sclereids (*I. germanica*) and several very small vascular bundles in *I. salicina.*

The configuration of vascular tissues is fascicular one; in the greater vascular bundles, the composing elements have a cambial origin, being visible many phloem fibers in the external zone of xylem. Close to the lower part of the stem, all vascular bundles have a secondary structure, the xylem fibers and those of periphloemic strands have the walls thicker and intensely lignified and the number of protective hairs decreases, while the number of vascular bundles increases.

Leaf

Among all investigated taxa, only *I. oculus-christi* has short- petiolate leaves, but with decurrent lamina; for this reason, the contour of cross section through the petiole is cordiform. The petiole appears to be modified by two flatted lateral-adaxial "wings", which represents the lamina. In the fundamental parenchyma of petiole, we can distinguish 7-9 vascular bundles, arranged in a particular arch. The size of these bundles decreases starting from the abaxial to adaxial side; all of them have a sclerenchymatic strand, thicker at the periphery of the phloem (Fig. 12) and thinner on the internal side of xylem.

The lamina epidermis, in surface view, has cells rather with inordinate contour, with lateral walls undulated; the amplitude of these undulations is greater at abaxial side.

The anomocytic stomata are confined only at the level of abaxial side; therefore, the limb is hypostomatic. In *I. britannica* and *I. salicina*, the lamina is amphistomatic, but the number of stomata per unit surface is higher in upper epidermis. The protective hairs – having the same shape, size and configuration as those found in the stem – lack in *I. salicina* and are more conspicuous in *I. britannica* (Fig. 13) and *I. germanica*, especially on the lower epidermis level.

The mesophyll is homogeneous, lacunae-type in *I. oculus-christi* and heterogeneous with uni-or multilayered palisade tissue (*I. germanica*) and multilayered spongy tissue in the remaining species, where the lamina has a bifacial dorsiventral structure) (Fig. 14).

The midvein is widely prominent on abaxial side of lamina, having 1 - 2 (*I. salicina*) (Fig. 15) or 3 (*I. germanica*) vascular bundles, with sclerenchymatic fibers on both poles. The lateral bundles are smaller, with sclerenchymatic fibers on both poles or only on phloemic pole. The smallest (thinnest) vascular bundles have only phloem and are surrounded by a parenchyma – sheath.

Conclusions

All *Inula* species investigated in this study differ by cytohistological features, both qualitatively and especially quantitatively. Here are differences regarding the number of vascular bundles, the thickness of periphloemic sclerenchymatic strands, presence or absence of root hairs, the size and number of secretory canals and layers of epithelial cells surrounding the glandular cavity. In addition, we found a variation in the presence of protective hairs, the frequency of sclereids in the cortex, and in the absence or presence of aerenchyma in cortical parenchyma. Some contrasts were also observed regarding the position of stomata and the number of palisade layers in the structure of lamina.

The two subspecies *of I. salicina* differ by the frequency of secretory canals from rhizome; they are many and more tangentially prolonged in *I. salicina* subsp. *aspera.* The stomata are located in the lower epidermis in *I. salicina* subsp. *aspera* and in the both epidermis, in *I. salicina* subsp. *salicina.* In this last mentioned subspecies, the protective hairs lack, while in *I. salicina* subsp. *aspera* the hairs are thick (Fig. 16).

As an ecological anatomy observation, we underline the presence of aerenchyma in the cortex of root and stem of *I. britannica,* a plant collected from a salinized area, with water table localized near to the soil surface (for extensive comments, see the paper of Grigore and Toma [17] and references therein).

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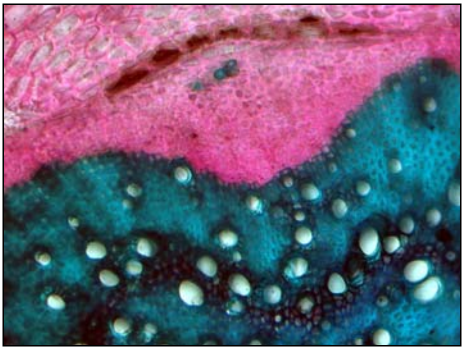
Explanation of plates

Micrographs of cross section through:

Plate I: **Fig. 1.** Root *of Inula britannica* (X200); **Fig. 2**. Root of *I. salicina* subsp. *aspera* (X200); **Fig. 3**. Root of *I. britannica* (X100); **Fig. 4, 5.** Rhizome of *I. britannica* (X400); **Fig. 6**. Rhizome of *I. oculus-christi.*
Plate II: Fig. 7. Rhizome of *I. germanica* (X100); **Fig. 8**. Rhizome of *I. salicina* subsp. *aspera* (X200); **Fig. 9**. Rhizome of *I. oculus-christi* (X200); **Fig. 10**. Stem of *I. germanica* (X200); **Fig. 11.** Stem of *I. britannica* (X400); **Fig. 12**. Petiole of *I. oculus-christi* (X400).
Plate III: Fig. 13. Lamina of *I. britannica* (X400); **Fig. 14**. Lamina of *I. salicina* subsp. *aspera* (X200); **Fig. 15.** Lamina of *I. salicina* subsp. *aspera* (X200); **Fig. 16**. Lamina of *I. salicina* subsp. *aspera* (X400);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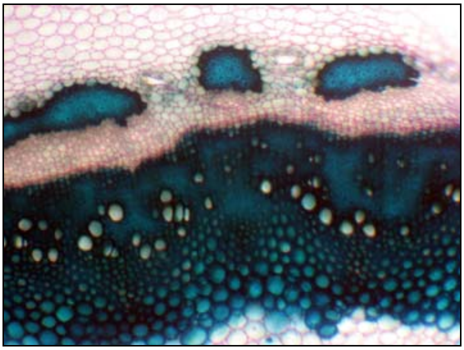
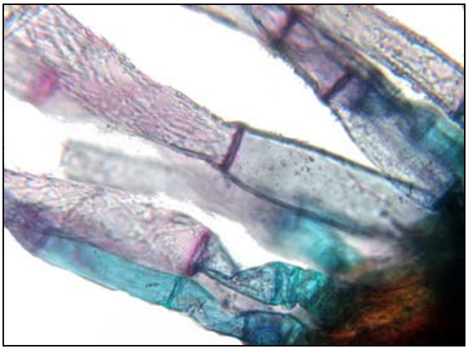


Fig. 2


Fig. 3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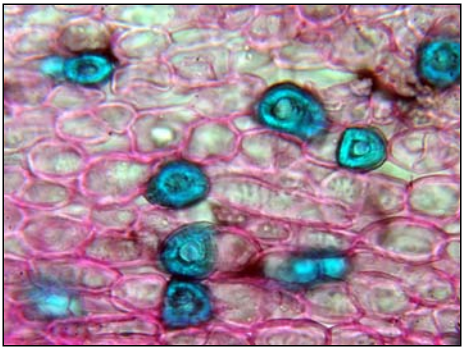
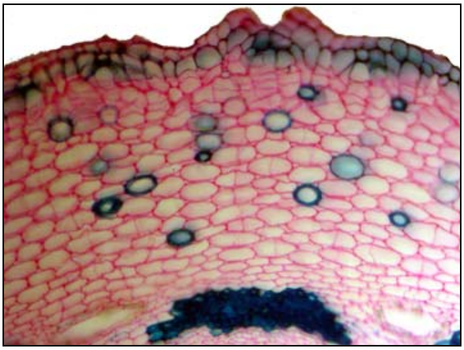


Fig. 5


Fig. 6



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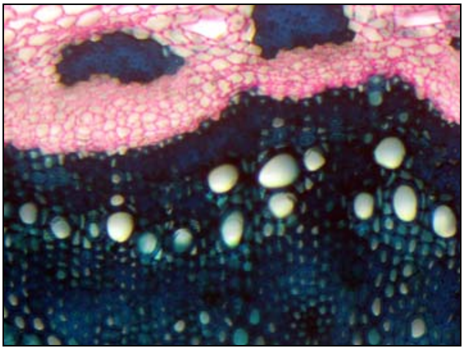

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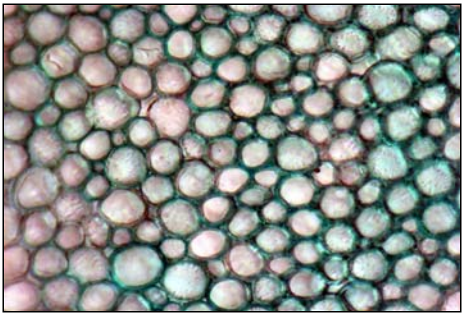
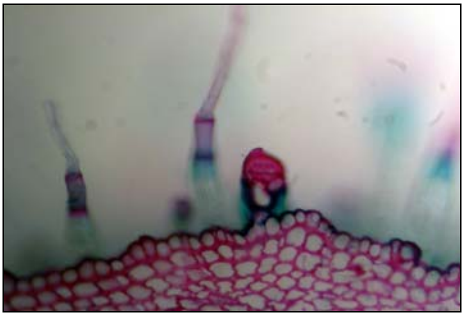


Fig. 9



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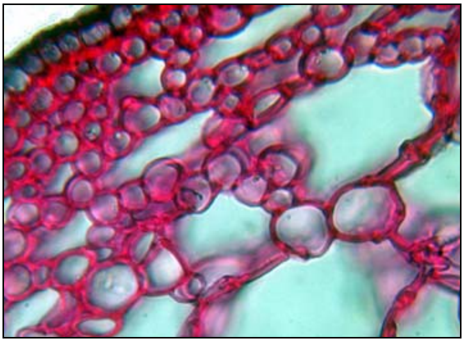

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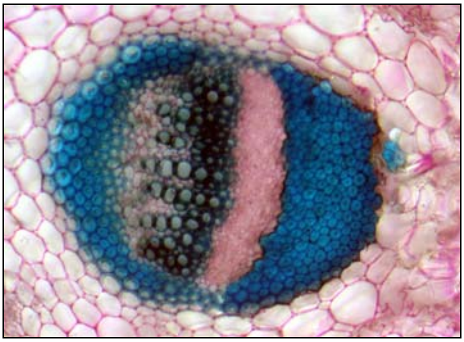

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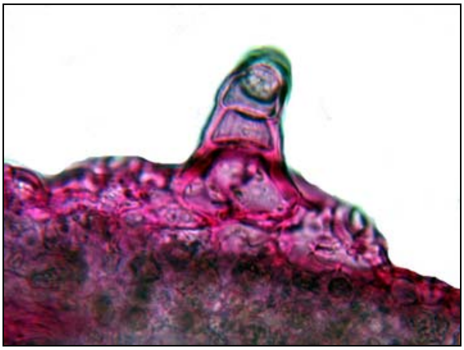
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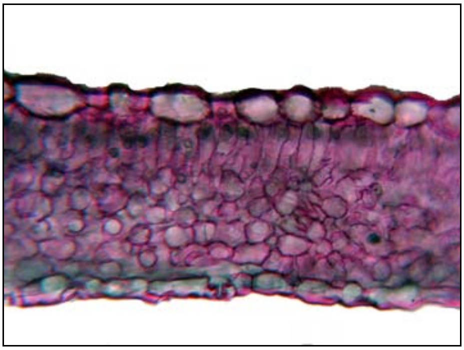
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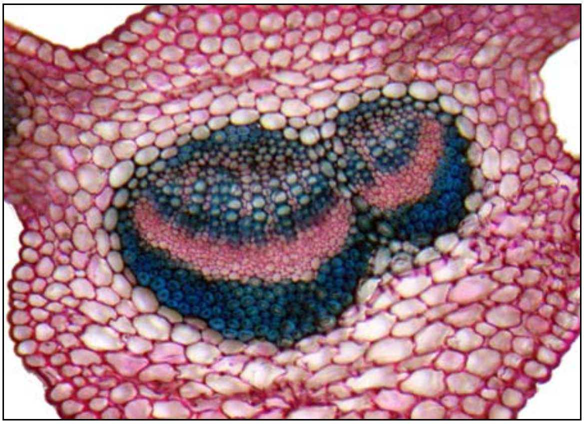
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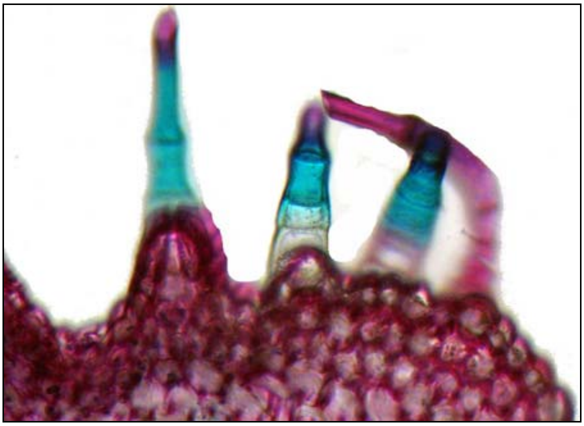
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