Analele Ştiinţifice ale Universităţii „Al. I. Cuza” Iaşi
s. II a. Biologie vegetală, 2021, **67**: 17-22
http://www.bio.uaic.ro/publicatii/anale_vegetala/anale_veg_index.html
ISSN: 1223-6578, E-ISSN: 2247-2711

HISTO-ANATOMICAL STUDIES IN *TUSSILAGO FARFARA* L. A SPECIES WITH MEDICINAL POTENTIAL AGAINST RESPIRATORY DISEASES

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Abstract: This study aimed to describe the histo-anatomy of *Tussilago farfara* L. species from the Asteraceae family, with medicinal importance in Romania for the alternative treatment of respiratory diseases (asthma, laryngitis, cough, emphysema) and other disorders. The chemical composition of Coltsfoot includes more than 150 chemical substances (triterpenoids, sesquiterpenoids, alkaloids) with different medicinal proprieties (expectorant, antimicrobial, antitussive) and contraindications (pregnancy, lactation, hepatic disorders). The vegetal material used in this study was collected from the waterside of river Sirețel in the village Sirețel from Sirețel commune in Iași County. The cross-sections were performed manually through vegetative organs (rhizome, stem, and leaf) with the help of a hand microtome and a botanic razor. The structures of the sections were highlighted by double coloration (iodine green and ruthenium red), the observation was performed on a Novex microscope. The characteristics structures observed by us (epidermis, vascular bundles, trichomes, angular collenchyma, assimilating parenchyma, stomata, mesophyll) correspond with Toma and Rugină (1998) observations and descriptions.
Keywords: *Tussilago farfara*, histo-anatomy, respiratory diseases.

Introduction

Tussilago farfara L. is a plant from the Asteraceae family (Chirilă et al., 1987), *Tussilago* is from the Latin *tussis* for cough, *farfara* refers to the whitish, cottony down on the underside of the leaves (Seal and Seal, 2008). These plants are known with the common names: Coltsfoot, British Tobacco, Coughwort, Horsefoot (Lim, 2014). In Romania, Coltsfoot is recommended for respiratory diseases: bronchitis (Chirilă et al., 1987), laryngitis, breath whooping, asthma, cough, silicosis, emphysema (Constatinescu et al., 1986), tuberculosis, and pneumonia. For medicinal purposes, infusion and decoction are used (Mohan, 2009), made from leaves and flower buds. Due to its phytochemistry which consists of approximately 150 compounds (phenolic acids, sesquiterpenoids, pyrrolizidine alkaloids, triterpenoids) (Chen et al., 2021), this species presents different medicinal proprieties (antioxidant (Wei et al., 2020), anti-inflammatory, antitussive, expectorant, antimicrobial, anticancer, against SARS-CoV-2) (Yang et al., 2020; Chen et al., 2021), and contraindications (pregnancy, children under 2 years, lactation, people who suffer from alcoholism, heart disease, hepatitis) (Idrisova et al., 2014; Anton, 2015).

Tussilago can be found on the brooks shores and other wet places, especially where the soil is clay-like (Constantinescu et al., 1986). This species can be confounded with *Petasities* sp. (Chirilă et al., 1987), for example, *Petasites frigidus* (L.) Fr. and *Petasites hybridus* (L.) G. Gaertn. & al., the difference between these species can be observed

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microscopically (Upton et al., 2011). *Petasites* sp. do not present biseriate glandular trichomes, upper epidermis present sinuous walls, palisade layer consist of one or two rows of cells, while the aerenchyma of mesophyll it is not so large. Also, the upper leaf surface is covered by trichomes (Upton et al., 2011).

In Romania, we found one full description of *Tussilo farfara* made by Toma and Rugină (1998). In their study authors made schematic drawings of structures found in the rhizome, stem, and leaf, and described the presence of large medullary rays between the vascular bundles from the rhizome The authors also have described the stomata, and long pluricellular, uniseriate trichomes, and well-differentiated sclerenchyma in the structure of some vascular bundles on the stem. In the structures of the petiole, they observed the presence of a few anomocytic stomata (leaf is amphistomatic), tector trichomes, and median nervure which was prominent on both sides of the blade. In the phloem authors observed laticiferous elements.

In another study, Upton and collaborators (2011) observed some differences between young and adult leaves of *Tussilago farfara*. Young leaves are covered by tufts of uniseriate trichomes and biseriate glandular trichomes, while the adult leaves are glabrous. The indumentum can obscure the epidermal cells, but it can be removed by processing, and then can be observed large anomocytic stomata (35 µm) and cells that present wavy anticlinal walls. In the tissue of the leaf blade, sometimes can be observed sphaerocrystals of inulin. Akçin (2007) observed that anomocytic stomata are more common on the lower epidermis.

Świeboda and Brunarska (1975) have described the histo-anatomy differences between plant samples of *Tussilago farfara* collected from sites exposed to industrial contamination and sites which was not exposed to industrial contamination. The authors did not observe the differences between the histo-anatomy structures of the root, stolon, and petiole, but observed the damage of leaf blade in the cells from the upper epidermis and the first palisade parenchyma layer in the *Tussilago farfara* collected from sites that were exposed to contamination.

In another study, Bota and collaborators (2018), studied the anatomy of *Tussilago farfara*, with the purpose to identify a correlation between the anatomical region of plants and the number of active compounds. They concluded that there is a large variation of the polyphenolic profile between the vegetative organs. In another study, Muravnik and collaborators (2016) researched the morphology, anatomy, ultrastructure, and histochemistry of trichomes found in *Tussilago farfara*. Trichomes can be found on the leaves, peduncles, phyllaries, and bracts. The authors observed that capitate glandular trichomes do not present a subcuticular cavity, but present biseriate long stalk and multicellular head. In the head cells of glandular trichome were identified terpenoids and phenols, polysaccharides. Also, the authors had shown that the quantity of organic acids and other compounds is higher in the organs that present glandular trichomes. And they concluded that the medicinal proprieties of coltsfoot are provided by the substances (organic acids, polyols, sugars, terpenoids) produced in capitate glandular trichomes.

Materials and methods

The vegetal material used in this study represented by one specimen of *Tussilago farfara* L., which is from the Asteraceae family, genus *Tussilago* (Sell and Murrell, 2005) was collected on 08.03.2019 from the waterside of river Sirețel (which is a watercourse of

the Siret River) in the village Sirețel from Sirețel commune in Iași County, Western Moldavia, Romania. The collected vegetal material was fixed in 70% ethanol. The cross-sections through the vegetative organs of *Tussilago farfara* L. were cut manually using hand microtome, botanic razor, and the elder pith as support. The sections were colored with iodine green and ruthenium red (Andrei et al., 1981), after that they were analysed in a Novex microscope and photographed with Sony Cyber-shot DSC-W730.

Results and discussions

Rhizome (Plate I, Figs. 1-5)

The contour of the cross-section is round. The epidermis cells have an isodiametric shape and a slightly thicker outer wall. The epidermis is covered by a cuticle that forms a characteristic relief. The cortical parenchyma is thick, homogeneous, the cells have different sizes, they are smaller near the epidermis, their size grows until the central cylinder. Between the cells in some places can be observed aerial spaces, which are more and bigger in the apical region of the rhizome. In the basal region of the rhizome can be observed 5 vascular bundles which are collateral open, because between phloem and xylem they present procambium. Endodermis presents Casparian strips, which are implicated in the transportation of water and inorganic substances between cortical parenchyma and the vascular bundle. The central cylinder is composed of 26 vascular bundles (collateral open). Some of them are connected in the region of phloem and xylem. Vascular bundles are separated by medullary rays. The cells of the pith are disorganized, resulting in a big aeriferous lacune. In the apical region of the rhizome can be seen 4 vascular bundles. The central cylinder is composed of 16 vascular bundles (collateral open), some of them are connected and have different sizes. The pith is homogenous, does not present aeriferous lacune, but between the cells can be observed air spaces.

The stem (Plate I, Figs. 6-9)

The contour of the cross-section is round. The epidermis has cells with a thicker outer wall and a thin cuticle. In some places, can be observed long non – glandular trichomes and few glandular trichomes. The bark is differentiated into angular collenchyma and assimilating parenchyma. The cortical parenchyma is thinner than that of the rhizome. Between the cells can be observed aeriferous spaces. In the peripheral position, some cells of the assimilating parenchyma are disorganizing, resulting in big air cavities that are strongly elongated tangentially. The central cylinder consists of the vascular bundles (collateral open) arranged in a circle. The bundles alternate with each other and have different sizes. The pith is larger than the one observed in the rhizome, but it is disorganized, resulting in a big aeriferous lacune, bigger in the middle and upper level of the stem.

The leaf (Plate I, Figs. 10-11)

The cross-section through the petiole has a semi-circular contour. The outer wall of epidermal cells of the petiole is thicker. Between the cells can be observed aeriferous spaces. After the lower epidermis can be seen aeriferous lacune. The hypodermic parenchyma is poorly cholenchymatized. In the fundamental parenchyma can be observed 9 vascular bundles (collateral open) of different sizes, arranged on an arch. After the upper epidermis, in the central zone of the petiole can be observed a big aeriferous lacune. The leaf blade

epidermis presents cells with a polygonal contour. The side walls are straight or slightly wavy. The median nervures have a collenchyma in a hypodermic position and a central vascular bundle (collateral open). The mesophyll is differentiated into palisade mesophyll with large cells, after the upper epidermis, and spongy mesophyll, after the lower epidermis. In the spongy mesophyll can be observed numerous aeriferous lacunes.

In their study Toma and Rugină (1998), also describe the presence of conductive vascular bundles which are collateral open, and the presence of disorganized pith cells which form a big aeriferous lacune.

Conclusions

In Romania, *Tussilago farfara* is used for the alternative treatment of respiratory diseases (emphysema, asthma, cough) and other disorders. Our observations relating to the histo-anatomy of *Tussilago farfara*, correspond with the observations of other authors (Świeboda and Brunarska, 1975; Toma and Rugină, 1998; Akçin, 2007; Upton et al., 2011; Bota et al., 2018). The characteristic structures of *Tussilago farfara* are vascular bundles which are collateral open, big aeriferous lacune in the pith of stem and rhizome, air spaces between cells found in cortical parenchyma and central cylinder, Casparian type endodermis (in rhizome), amphistomatic leaf with a normocytic stoma. According to Muravnik and collaborators (2016), the glandular trichomes by their secretions are implicated in the medicinal proprieties of Coltsfoot.

Acknowledgements

We would thank the Integrated Centre for Environmental Science Studies in the North-East Development Region – CERNESIM, Alexandru Ioan Cuza University, and the Department of Experimental and Applied Biology, NIRDBS - Institute of Biological Research.

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EXPLANATION OF THE PLATES

PLATE I

Fig. 1. Cross-section through rhizome (basal region), ensemble image (al - aeriferous lacune, cp - cortical parenchyma, ep - epidermis).
Fig. 2. Cross-section through rhizome (apical region), ensemble image (ep - epidermis, al - aeriferous lacunae, cp - cortical parenchyma, vb - vascular bundle (collateral open), pi - pith, cb - cortical bundle).
Fig. 3. Cross-section through rhizome (apical region) (ep - epidermis, cp - cortical parenchyma, vb - vascular bundle, pi - pith).
Fig. 4. Cross-section through rhizome (basal region): detail (cp - cortical parenchyma, cb - cortical bundle, en - endodermis, pe - perycicle, al - aeriferous lacunae).
Fig. 5. Cross-section through rhizome (apical region): detail (vb - vascular bundle)
Fig. 6. Cross-section through stem (lower level): ensemble image (ep - epidermis, cl - collenchyma, cp - cortical parenchyma, vb - vascular bundle, pi - pith, al - aeriferous lacunae).
Fig. 7. Cross-section through stem (middle level): ensemble image (gt - glandular trichome, ep - epidermis, cl - collenchyma, cp - cortical parenchyma, vb - vascular bundle (collateral open), pi - pith, al - aeriferous lacunae).
Fig. 8. Cross-section through stem (middle level): detail (gt - glandular trichome, ep - epidermis, cl - collenchyma, cp - cortical parenchyma, vb - vascular bundle, al - aeriferous lacunae).
Fig. 9. Cross-section through stem (upper level): detail (ep - epidermis, cl - collenchyma, cp - cortical parenchyma, vb - vascular bundle, al - aeriferous lacunae).
Fig. 10. Cross-section through the petiole: ensemble image (ue - upper epidermis, le - lower epidermis, al - aeriferous lacunae, vb - vascular bundle).
Fig. 11. Cross-section through foliar lamina (middle region): detail (ue - upper epidermis, pp - palisade parenchyma, sp - spongy parenchyma, vb - vascular bundle (collateral open), al - aeriferous lacunae, cl - collenchyma, le - lower epidermis).

PLATE I

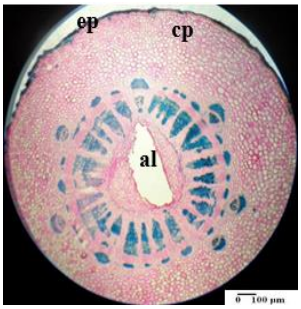


Figure 1

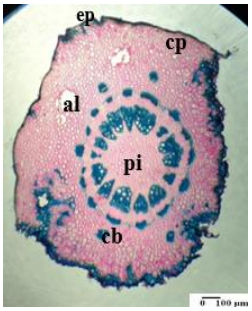
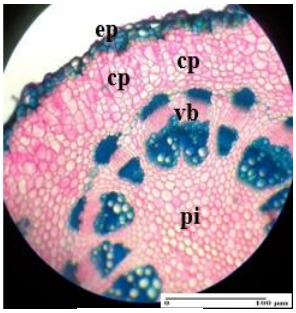


Figure 2



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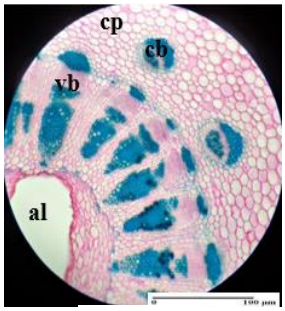

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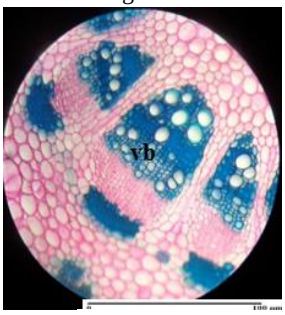

Figure 5

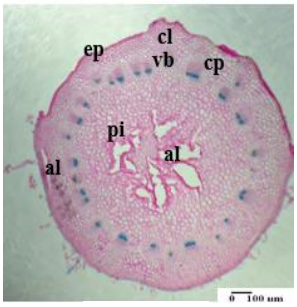

Figure 6

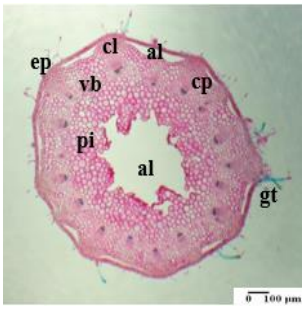

Figure 7

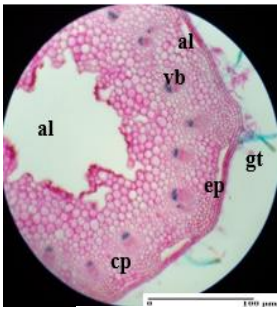

Figure 8

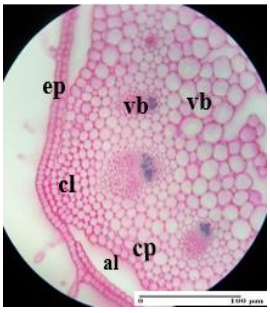

Figure 9

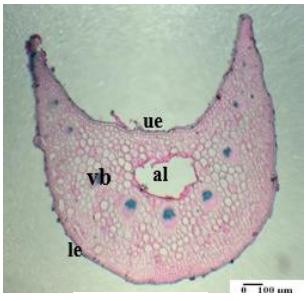

Figure 10

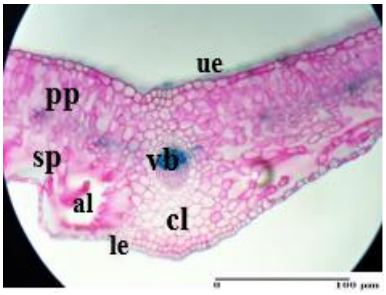

Figure 11