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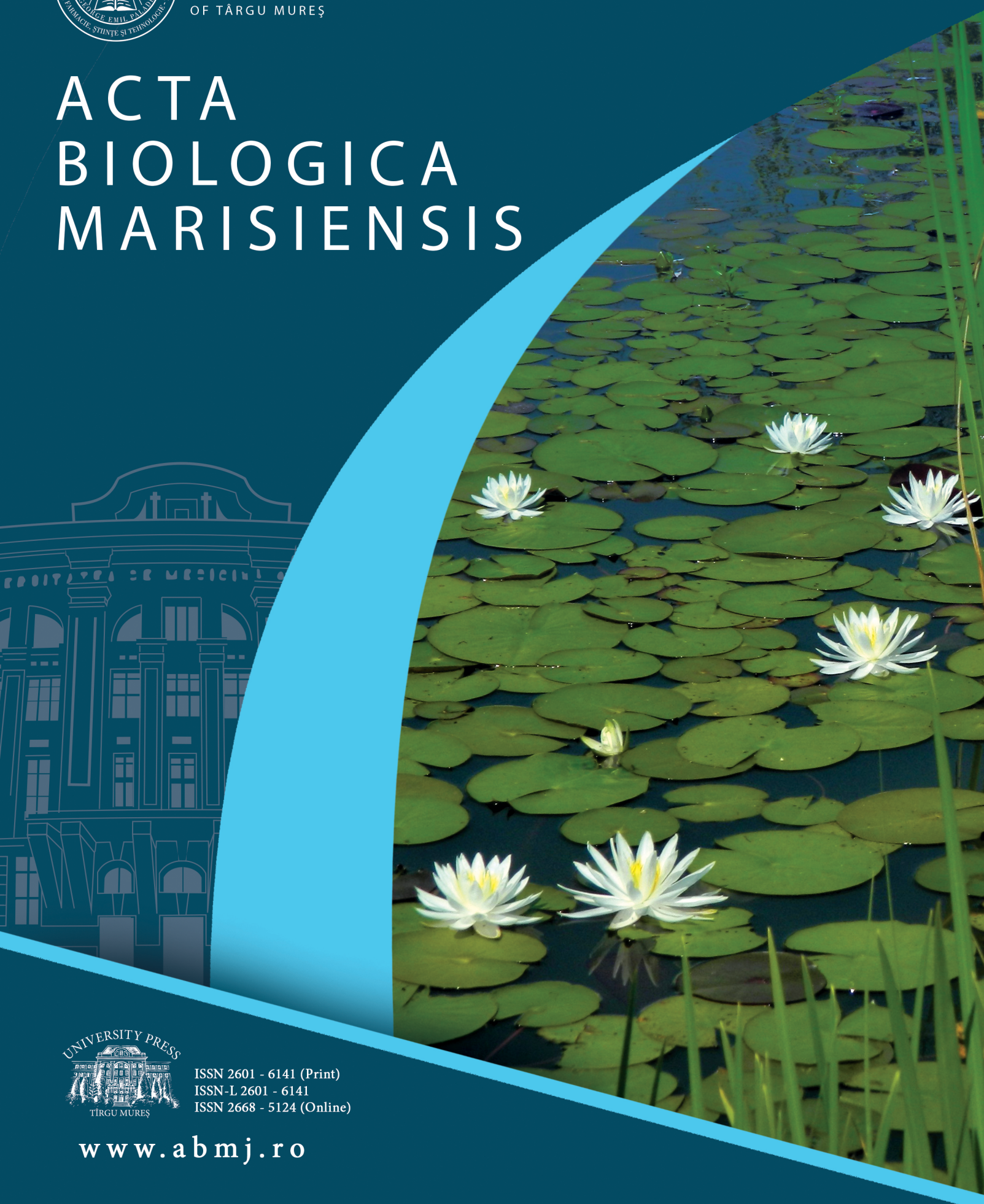

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VESICULAR ARBUSCULAR MYCORRHIZA INFLUENCES THE HISTO-ANATOMIC CHARACTERISTICS OF VEGETATIVE ORGANS IN *ARTEMISIA ANNUA*

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Abstract: Recent studies have shown that vesicular-arbuscular mycorrhizae stimulate plant growth in case of *Artemisia annua* plants. According to these studies mycorrhization can enhance plant height and biomasses, shoot branching and inter-nodal length, foliar glandular hair density, and nutrient status of shoots and leafs. Contradictory data were obtained in case of leaf chlorophyll content and photosynthetic rate. The effects of vesicular-arbuscular mycorrhizae on roots, shoots and leafs anatomy of *A. annua* have not been studied yet. The aim of this paper was to compare the microscopic characteristics of the vegetative organs from the *Artemisia annua* plants treated with vesicular-arbuscular mycorrhizae, with those from the control plants. *Rhizophagus irregularis* influenced the development of vascular tissues in root and stem of *Artemisia* plants by increasing their surface in the organs. Mycorrhization also reduced the percentage of lignification in the cortex of the root, increased the percentage of palisade parenchyma in leaf and had a positive effect on foliar glandular hair density. Further investigations are necessary to find out the role of these histo-anatomic alterations in the growth and development of *Artemisia* plants.

Keywords: *Rhizophagus irregularis*, anatomy, histology, root, stem, leaf, glandular hair.

1. Introduction

Recent studies have shown that vesicular-arbuscular mycorrhizae stimulate plant growth in case of *Artemisia annua* (Chaudhary et al., 2007; Kapoor et al., 2007; Awasthi et al., 2011; Huang et al., 2011; Tan et al., 2013; Fortin and Melchert, 2015; Giri, 2017; Domokos et al., 2018). According to these studies mycorrhization can enhance plant height and biomasses, shoot branching and inter-nodal length, foliar glandular hair density, and nutrient status of shoots and leafs.

Contradictory data were obtained in case of leaf chlorophyll content and photosynthetic rate (Kapoor et al., 2007; Huang et al., 2011; Rapparini et al., 2008). The effects of vesicular-arbuscular mycorrhizae on roots, shoots and leafs anatomy of *A. annua* have not been studied. The hypothesis of this work was that vesicular arbuscular mycorrhiza stimulates plant growth by changes in vegetative organ anatomy. Therefore the objective of the study was to compare the microscopic characteristics

of the vegetative organs from the *Artemisia annua* plants treated with vesicular-arbuscular mycorrhizae, with those from the control plants.

2. Materials and Methods

The plants (*Artemisia annua* Anamed A-3, Winnenden, Germany) were cultivated in 2017 in Corunca (Mureș County, 46°31’18.18’’N and 24°35’53.78’’E) as previously described in Domokos et al. (2018). For comparison of microscopic features of vegetative organs, 20 plants treated with *Rhizophagus irregularis* and 20 control plants were used. Observations were made on plants harvested in July. Sections of vegetative organs were done by hand microtome and razor. For staining iodine green and ruthenium red was utilized (Tanase et al., 2017). Microscopic images were obtained by a Motic B3 (Hong Kong) optical microscope equipped with a Canon EOS 1100D (Taiwan) camera. Leaf surface of 40 treated plants and 40 control plants was observed with a JEOL JSM-5200 (Japan) scanning electron microscope. Analysis of obtained microscopic images was performed with ImageJ Image Processing and Analysis in Java Version 1.51j8 (National Institute of Mental Health, Bethesda, MD, United States). The data did not have a normal distribution (Shapiro-Wilk test), thus for data comparing the Wilcoxon signed rank test (Past 2.17, Hammer et al., 2001) was used.

3. Results and discussions

The cross section of the *A. annua* root had a circular outline (**Fig.1.**). The root presented a secondary structure. From the external part of the root to the internal part, the following tissues could be distinguished: periderm (cork) formed by the phellogen, cortex with a few tangentially elongated secretory ducts (**Fig. 2**), three or more sclerenchyma bundels, secondary phloem (in form of a thin ring surrounding the secondary xylem), secondary xylem which occupies the largest area and fills the pith too. This structure had a lower degree of lignified cells than the plants collected later, in the flowering period, described by Ivănescu et al. (2015).

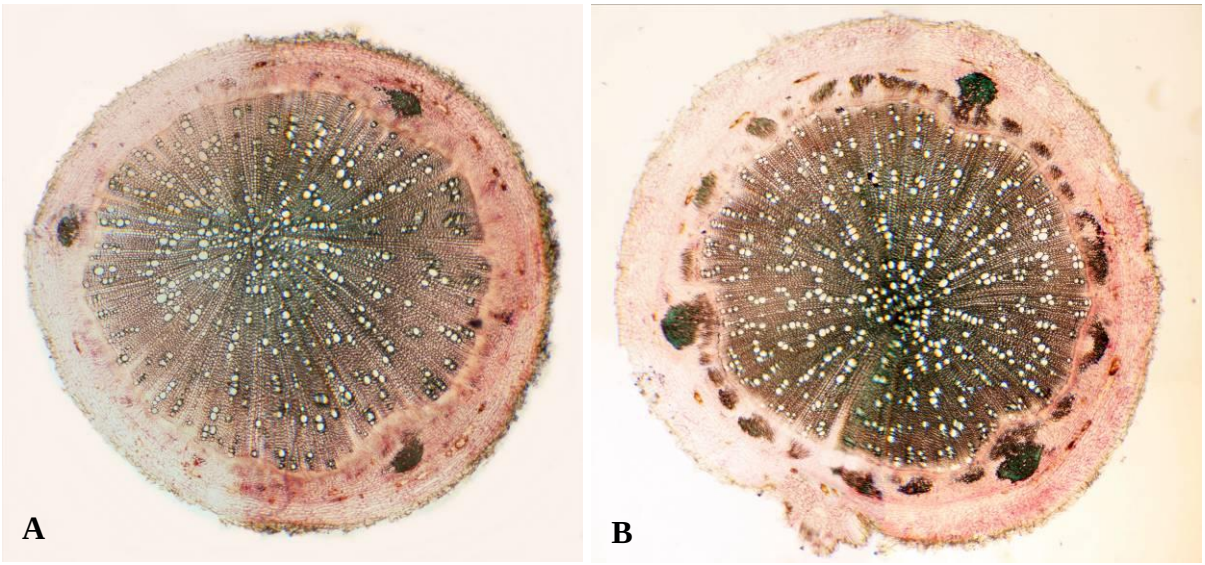

Fig. 1. *Artemisia annua* roots-general view (4x): **A.** Plant treated with *Rhizophagus irregularis*; **B.** Control plant (Photos: Erzsébet Domokos)

The cross section of stem (on middle part) was quasi-circular with more than 10 horns (**Fig. 3**). The stem presented a primary structure. From the external part of the stem to the internal part, the following tissues could be distinguished: epidermis with almost square or rectangular cells covered by cuticle, collenchyma layers under the epidermis, cortex without secretory cavities, collateral vascular bundles arranged in circle, and pith parenchyma (**Fig. 4.**). Glandular hairs, medifixed hairs, and stomata from the epidermis were less than on the leaf surface. These findings were in accordance with Ivănescu et al. (2015) and Tu (2017).

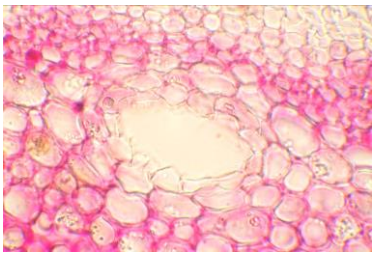

Fig. 2. Secretory duct in the cortex of *Artemisia annua* root-detail (40x) (Photo: Erzsébet Domokos)

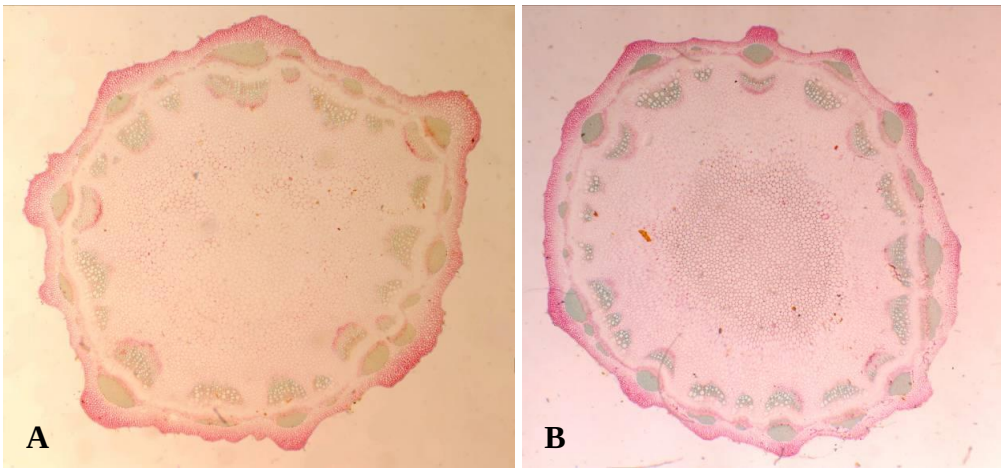

Fig. 3. *Artemisia annua* stems-general view (4x): **A.** Plant treated with *Rhizophagus irregularis*; **B**-control plant (Photos: Lilla Laura Csősz)

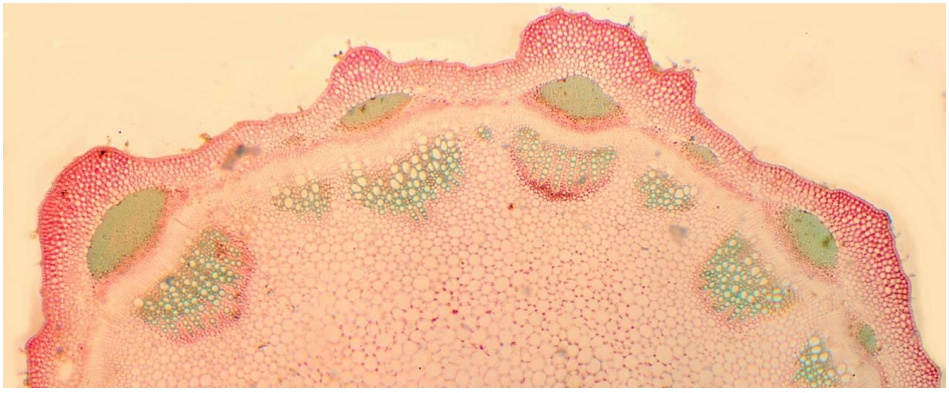

Fig. 4. *Artemisia annua* stem-detail (4x): epidermis, collenchyma layers, thin cortex, collateral vascular bundles, sclerenchyma cap covering the phloem, band of multi-layered fascicular and interfascicular cambium, pith (Photo: Lilla Laura Csősz)

The leaf transection (on ultimate lobe) presented the following structure (**Fig. 5.**): epidermis with cuticle, glandular hairs, medifixed hairs and stomata (**Fig. 6.**), isobilateral mesophyll, upper palisade parenchyma trough the midrib arranged in two layers (otherwise in one layer), collateral vascular bundle (surrounded by fundamental tissue) and lower palisade parenchyma arranged in one layer.

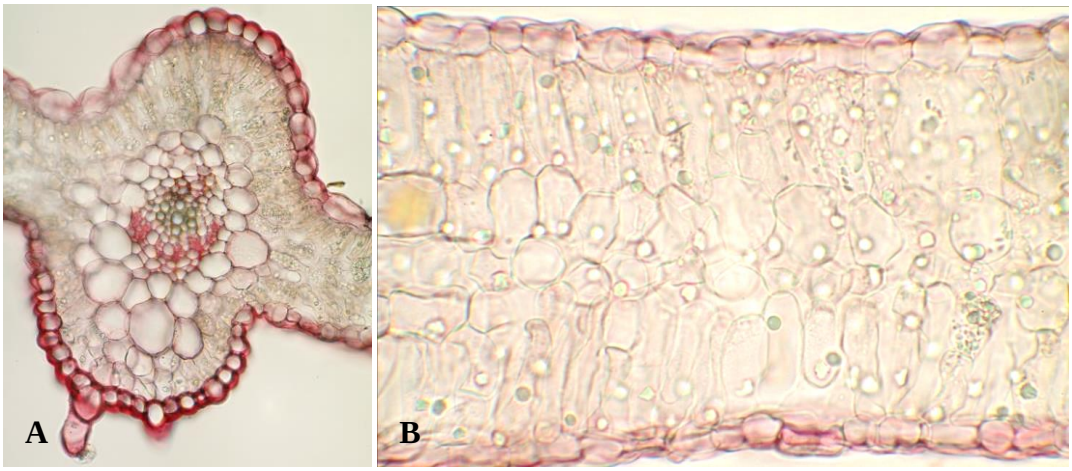

Fig. 5. *Artemisia annua* leaf: **A.** Detail of the midrib (40x); **B.** Detail of the mesophyll (40x) (Photos: Erzsébet Domokos)

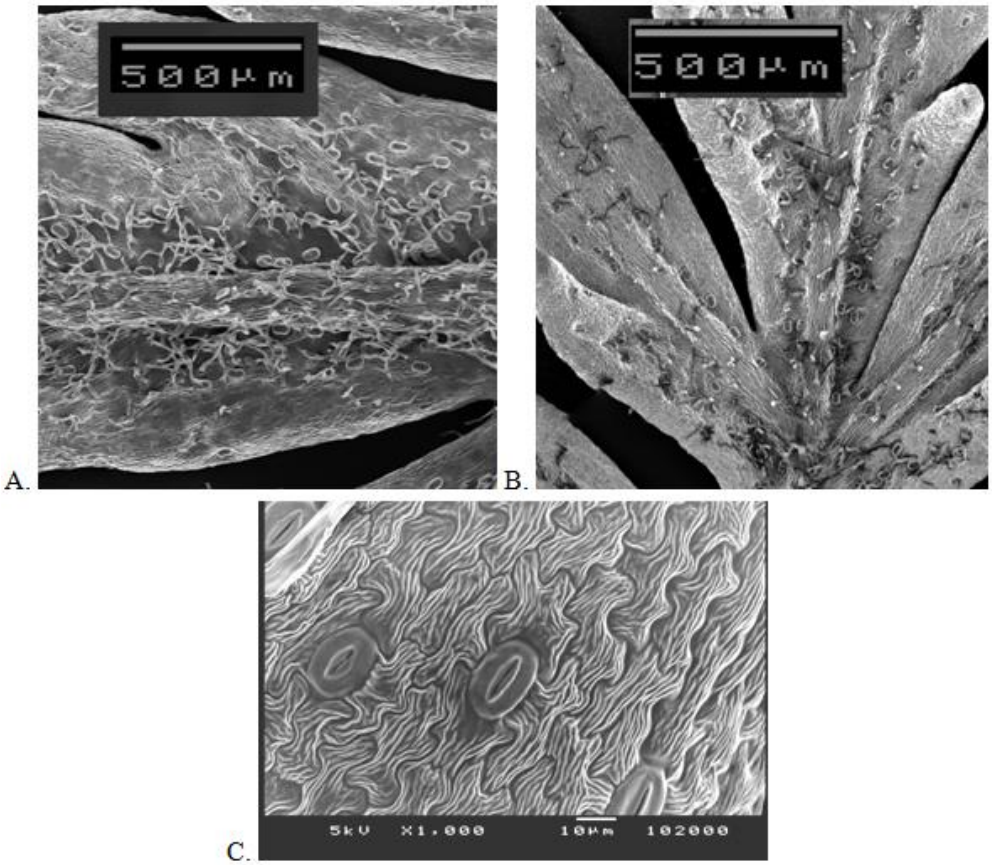

Fig. 6. Upper epidermis of *Artemisia annua* leafs (SEM, JEOL JSM-5200): **A.** Glandular hairs of plant treated with *Rhizophagus irregularis*; **B.** Glandular hairs of control plant; **C.** Epidermal cells with sinuately curved anticlinal wall and stomata (Photos: László Jakab-Farkas)

The measured and compared microscopic characteristics of the vegetative organs in case of treated and control plants were introduced in **Table 1**. In the roots of treated plants the secondary xylem occupied a significantly larger area than in control plants, while the sclerenchyma tissue occupied a smaller surface in treated plants root (**Fig. 1**). Zheng et al. (2005) found changes in the activity of enzymes responsible for cell lignification in roots of *Capsicum annum* plants inoculated with *Rhizophagus irregularis*. The fungi alleviated the activation of these enzymes (peroxidase-POD, polyphenol oxidase-PPO and phenylalanine ammonia-lyase-PAL) and acted themselves as protection agents against *Phytophthora capsici*. These findings could explain the lower percentage of lignified cells in roots of treated *Artemisia* plants.

In case of stem, plants inoculated with fungi had a higher percentage for vascular bundles than control plants, although the number of bundles didn't differ significantly (**Fig. 3.**).

According to Adolfsson et al. (2015) the inoculated (with *Rhizophagus irregularis*) *Medicago truncatula* plants presented significantly larger and thicker leafs than control plants. The number of palisade cells and chloroplasts were also significantly higher for treated plants as compared to controls, although the photosynthetic activity/leaf area was not influenced by the treatment. In our study no differences in leaf thickness were observed, but the area occupied by the palisade parenchyma was larger in case of the treated plants. Mycorrhized plants presented also a significantly higher glandular hair density on the upper epidermis than control plants (as published before in Domokos et al., 2018) (**Fig. 6.**). This is in accordance with other studies on *Artemisia annua* inoculated with different arbuscular micorrhyzal fungi (Kapoor et al., 2007; Mandal et al., 2015; Giri, 2017).

Table 1. The microscopic characteristics of vegetative organs in case of mycorrhized and control *Artemisia annua* plants

Vegetative organs	**Microscopic characteristics**	**Treated plants (mean ± SD)**	**Control plants (mean ± SD)**
Root		N = 20	N = 20
	Secondary xylem area (%)	44.374 ± 8.022 z = 3.509	37.169 ± 7.627 ***p* < 0.0001**
	Sclerenchyma area (%)	1.809 ± 1.071 z = 3.397	3.264 ± 0.802 ***p* < 0.0001**
	Number of secretory ducts	7.100 ± 3.905 z = 1.366	8.900 ± 3.160 *p* = 0.177
Stem	Vascular bundles area (%)	20.380 ± 3.849 z = 2.165	18.796 ± 4.252 ***p* = 0.029**
	Sclerenchyma cap area (%)	5.768 ± 1.991 z = 1.307	5.094 ± 1.241 *p* = 0.202
	Number of vascular bundles	11.000 ± 1.279 z = 1.839	11.917 ± 1.831 *p* = 0.076
Leaf	Palisade parenchyma area (%)	39.336 ± 10.253 z = 2.128	33.881 ± 7.973 ***p* = 0.032**
	Leaf lamina thickness (mm)	0.327 ± 0.045 z = 0.858	0.352 ± 0.069 *p* = 0.403
		N = 40	N = 40
	Glandular hair density/mm^2	32.640 ± 11.130 z = 4.234	21.769 ± 7.897 ***p* < 0.0001**

Note: bold values mean significant differences, where $p < 0.05$ (Wilcoxon signed rank test)

Conclusions

Rhizophagus irregularis can influence the development of vascular tissues in root and stem of *Artemisia* plants by increasing their surface in the organs. Mycorrhization also reduces the percentage of lignification in the cortex of the root, increases the percentage of palisade parenchyma in leaf and has a positive effect on foliar glandular hair density. Results of this experiment (published earlier) showed also that *R. irregularis* had a positive effect on the biomasses of roots and herba. Further investigations are necessary to find out the role of these histo-anatomic alterations in the growth and development of *Artemisia* plants.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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IDENTIFICATION OF THE HERBAL DRUG PRUNELLAE SPICA BASED ON MACROSCOPIC AND MICROSCOPIC CHARACTERISTICS

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Abstract: *Prunella vulgaris* L. belongs to the genus *Prunella*, Lamiaceae family, Nepetoideae subfamily. In Romania, the genus *Prunella* includes *Prunella vulgaris* L., *Prunella grandiflora* (L.) Jacq and *Prunella laciniata* L.. Amongst these, *Prunella vulgaris* is of particular importance, having numerous pharmacological actions. The purpose of this study is to analyze macroscopically and microscopically the main characters by which Prunellae spica can be identified and distinguished from other fruit-spikes from species of the Lamiaceae family.

Keywords: Prunellae spica, nutlets, histo-anatomical characteristics.

1. Introduction

Most species of the Lamiaceae family are aromatic plants that grow in many regions of the world. They are an essential source of phytochemical compounds that have beneficial effects in relieving certain conditions (Kozlowska et al., 2015). According to the new classification, the Lamiaceae family comprises seven subfamilies, about 230 genera and over 7.000 species with international distribution, but are rare or absent from high-altitudes and cold regions. Many species of this family are economically important due to the production of volatile oil (Tamaș, 1999; Dinç et al., 2009; Dınç and Dogu, 2012). The *Prunella* genus is a member of the Lamiaceae family, Nepetoideae subfamily. Among the species of this genus, *Prunella vulgaris* L., *Prunella grandiflora* (L.) Jacq and *Prunella laciniata* L. grow in the wild flora of Romania. *Prunella vulgaris* L., the species under study, grows in wetlands, plains, meadows, pastures, unpopulated areas, uninhabited lawns, both in the sun and the shade (Hodișan and Pop, 1976; Duke, 2001; Sârbu et al., 2013). According to the European Pharmacopoeia, 8th edition, the vegetable drug Prunellae spica represents the

fruit-spike of *Prunella vulgaris* L.. Phytochemical studies conducted so far on *Prunella vulgaris* L. have revealed the presence of triterpenoid, phenol carboxylic acids, flavonoids, triterpenoid saponins, and vitamins (Gu et al., 2007; Khare, 2007; Hon-Yeung and Qing-Feng, 2008).

The species *Prunella vulgaris* L. (selfheal) is widely used in traditional medicine in Asian countries, especially in China. In Romania, although is commonly spread in the wild flora, selfheal is rarely used in folk medicine.

The beneficial effects of *Prunella vulgaris* L. have been proven by pharmacological and/or clinical research, especially by Chinese researchers. Antiviral, antibacterial, anti-inflammatory, antioxidant, antihyperglycemic, hypolipidemic, antitumoral, hypotensive, sedative actions have been highlighted. The decoction of *Prunella vulgaris* L. has a broad antibacterial spectrum. *In vitro* experiments have shown that it has a moderate inhibitory activity on gram-positive bacteria (Psotova et al. 2003). The alcoholic extract of *Prunella vulgaris* L. reduces serum urea, creatinine and proteinuria in diabetic rats (Feng, 2000). In one study it was demonstrated that certain fractions of the ethanolic extract significantly reduced the cytotoxic effects of various cancer cell lines (Hwang, 2013).

The aerial part of *Prunella vulgaris* contains a high percentage of triterpenoids, the most important being the oleanolic acid and ursolic acid. Besides these compounds, important phenolic acids have also been identified in this herbal drug: rosmarinic acid, *p*-coumaric acid, caffeic acid, etc. A part of these compounds have antitumor activity, acting through several mechanisms (Huynh and Teel, 1999; Trochon et al., 2000; Raafat, 2016; Wang et al, 2019)

Studies on the action of aqueous and alcoholic extracts have highlighted the hypotensive effect on experimental animals. Studies on antihypertensive action are controversial. The compounds considered to be responsible for the antihypertensive activity are ursolic acid and oleanolic acid isolated from the methanolic extract (Mohsen and Ammar, 2009; Gu et al., 2013).

This study aimed to describe the macroscopic and microscopic characters by which Prunellae spica can be authenticated, considering the morphological resemblances with other species from Lamiaceae familly like *Ajuga decumbens* and *Ajuga ciliata*.

2. Materials and Methods

Prunella vulgaris L. was harvested from Mureș County, Romania, in dry weather during flowering and at the end of the flowering period when the fruit develops (**Fig. 1A**).

The dried herbal drug (the fruit-spike) was soaked in water to achieve the desired consistency. The nutlets were softened in hydroalcoholic solution (ethanol/water in a 1:2 ratio).

For the macroscopic analysis of the nutlets, a Jena-Zeiss stereomicroscope and the Nikon d7100 camera, 60mm lens, f22, shutter speed 350 iso 200 were used.

The microscopic analysis was performed with the MICROS-Austria microscope with a video camera.

Transverse sections of the softened samples were done with a single-edged razor blade. The sections and powder fragments have been cleared by boiling in 80% chloral hydrate solution for 5 minutes, followed by rinsing with water. Preparations were mounted with glycerin-gelatin (8% gelatin).

3. Results

The fruit-spike Prunellae spica (**Fig. 1B**) has a slightly flattened cylindrical shape, is 1.5-8 cm long and 0.8-1.5 cm wide, and has a light brown or reddish-brown color. It consists of 10 or more persistent whorls, each whorl being

delimited by two opposite, fan-shaped bracteoles, with acuminated tip, with reticulate venation, the outer surface being covered with numerous, conspicuous covering trichomes. Each bracteole is grown together at the base with three flowers made up of a persistent biconvex calyx, a corolla that usually lacks and four brown nutlets, small and obovate, with a white, sharp protuberance at the hilum. At the fruiting stage, the calyx is closed. The powdered herbal drug is reddish-brown or brown.

3.1 Macroscopic analysis of the nutlets

The The nutlets are obovate, about 1-1.5 mm long, brown, smooth, glossy (**Fig. 2**). They are characterized by particular surface ornamentation: from the apical rounded region, three lighter colored stripes run down to the pyramidal abscission scar (**Fig. 3A, B, C, D**), delineating a dorsal convex side and two ventral, slightly flattened sides. After soaking in water, the nutlets produce mucilage (phenomenon called myxocarpy) and show a transparent, dense, layer of mucilage (**Fig. 4**).

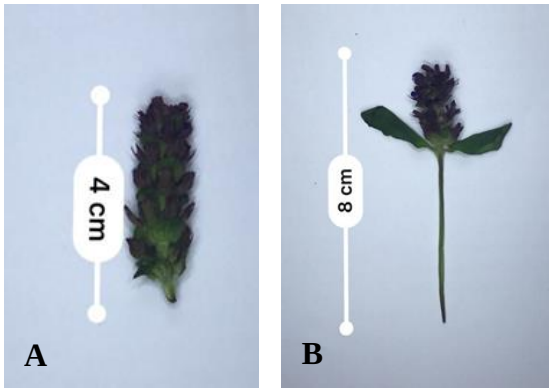

Fig. 1. Prunellae spica (A); *Prunella vulgaris* L. (B) (Photos: Alexandra Groșan)

Fig. 2. Nutlets viewed at the stereomicroscope (25x) (Photos: Alexandra Groșan)

Fig. 3. Nutlets photographed with a Nikon camera (f22, iso 600, 60 mm macro objective, flash): **A.** Three stripes form an Y-shaped mark in the apical region; **B.-C.** Nutlets in side view – stripe running down from the apix to the pyramidal abscission scar covered with white tissue (Photos: Alexandra Groșan)

Fig. 4. Nutlets viewed at the stereomicroscope, covered with a transparent, firm layer of mucilage (f22, iso 600, 60 mm macro objective, flash) (Photo: Alexandra Groșan)

3.2 Microscopic analysis of different parts of the fruit-spike

Bracteoles exhibit epidermal cells with sinuous anticlinal walls, glandular hairs with two secretory cells (**Fig. 5**) and covering trichomes with deposits of acicular crystals of calcium oxalate (**Fig. 6**). The venation is reticulate (**Fig. 7**). The calyx is bilabiate: the upper lip is broad, truncate, three-toothed (**Fig. 8**) while the lower lip is narrow and split up almost to the middle in 2 lanceolate, acuminate lobes. On the inner margins of the lobes there are only unicellular, bent covering trichomes (**Fig. 9, 12**) while on the surface and on the outer margins there are unicellular and multicellular covering trichomes (**Fig. 10**). The epidermal cells of the calyx have sinuous walls and diacytic stomata, one of the subsidiary cells being smaller (**Fig. 11, 13**). On the outer surface of the lower part of the calyx there are numerous glandular hairs with a monocellular stalk and a bicellular head (**Fig. 14**).

The base of the flower is covered with short, bi-cellular trichomes (**Fig. 15**). The style is bilobed with elongated, curved lobes (**Fig.16**). The endothecium of the anther shows characteristic thickenings (**Fig.17**). The outer epidermis of the petals is papillose, with conical shaped papillae and striated cuticle (**Fig. 18**) and shows glandular hairs with four secretory cells (**Fig. 20**). The filament has a short spur near the insertion site of the anther (**Fig.19**). The middle lobe of the lower lip is fringed and covered with papillae (**Fig. 21, 22**).

The transverse section of the wetted nutlet pericarp (**Fig. 23**) shows: epidermal cells containing mucilage, which become elongated when softened in water; a thin innermost cell layer, and a macrosclereid layer with sinuous anticlinal walls. Nutlets preparations show in surface view elongated macrosclereids (**Fig. 24, 25**), epidermal cells with straight walls (**Fig. 26**), and oil droplets in the endosperm (**Fig. 27**).

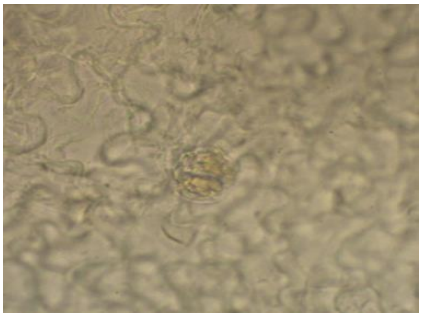

Fig. 5. Bracteole, surface view: epidermal cells with sinuous anticlinal walls; head with two secretory cells of a glandular hair (40x)

Fig. 6. Bracteole, surface view: covering trichome with a deposit of acicular crystals of calcium oxalate (40x)

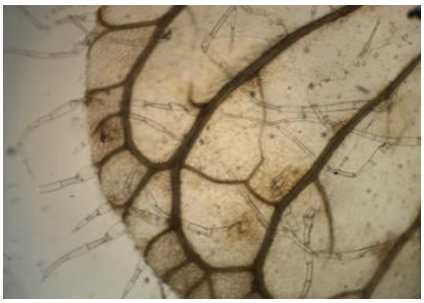

Fig. 7. Bracteole, surface view: reticulate venation, multicellular covering trichomes along the margin and on the surface (40x)

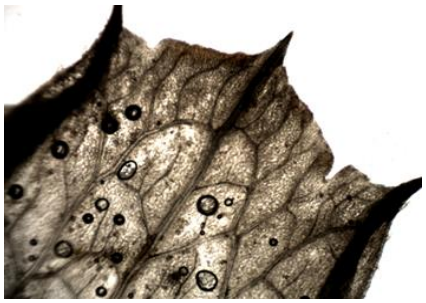

Fig. 8. Calyx, surface view: upper lip, three-toothed (40x)

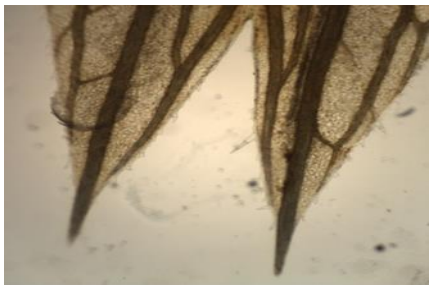

Fig. 9. Calyx, surface view: lower lip with two long acuminated lobes (40x)

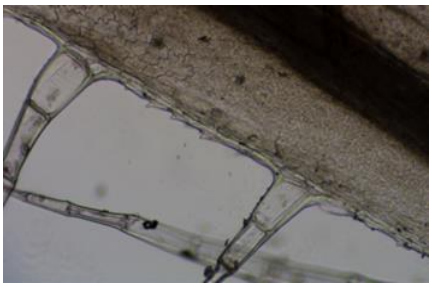

Fig. 10. Calyx, surface view: uni- and multicellular covering trichomes (40x)

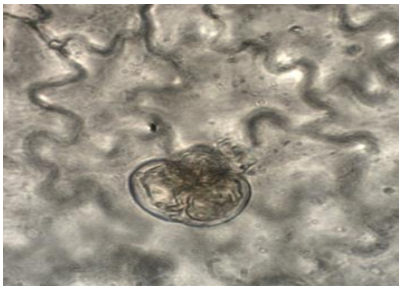

Fig. 11. Calyx, surface view: axially elongated epidermal cells with sinuous anticlinal walls; glandular hair with unicellular stalk and bicellular head (40x)

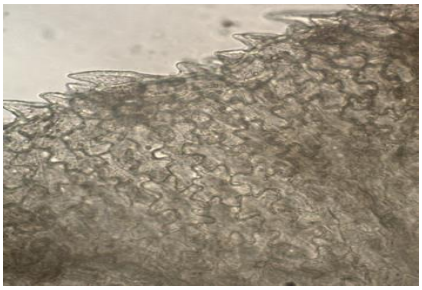

Fig. 12. Calyx, surface view: unicellular, bent covering trichomes along the margin between the lobes (40x)

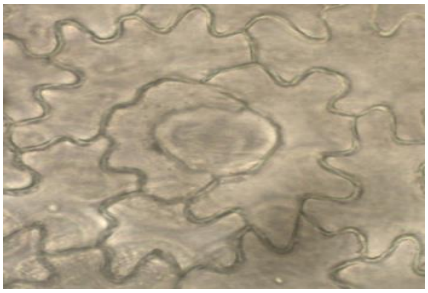

Fig. 13. Calyx, surface view: epidermal cells with sinuous anticlinal walls; diacytic stomata, onsubsidiaryar cell being smaller (40x)

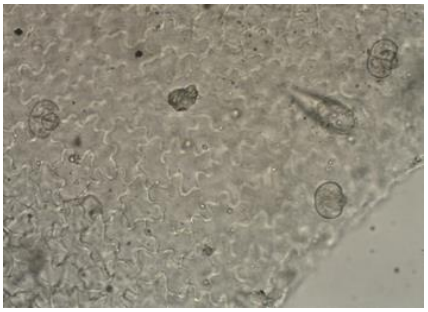

Fig. 14. Calyx, surface view: numerous glandular hairs at the lower region of the calyx (40x)

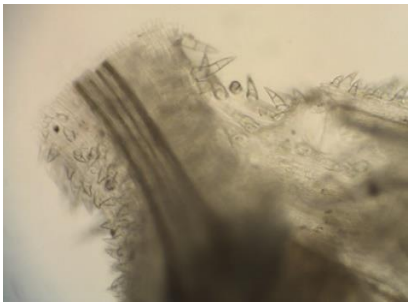

Fig. 15. Flower, surface view: uni- and bicellular trichomes at the base of the petal (40x)

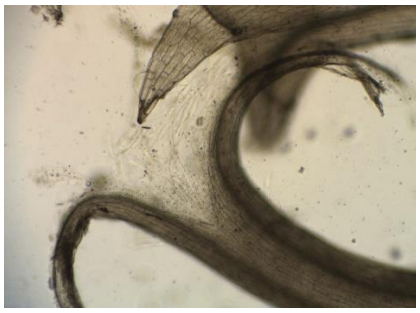

Fig. 16. Flower, surface view: bilobed style (40x)

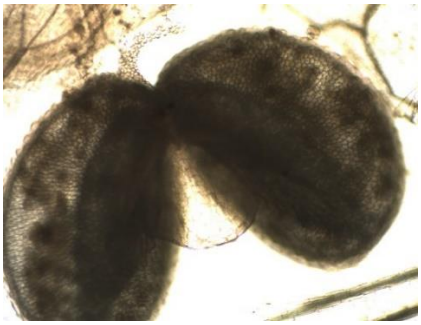

Fig. 17. Flower, surface view: anthers with thickened endothecium (40x)

Fig. 18. Flower, surface view: papillose epidermal cells with striated cuticle (40x)

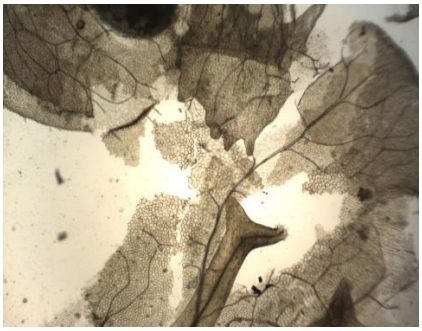

Fig. 19. Flower, surface view: fragments of petals; filament with a spur at the top (40x)

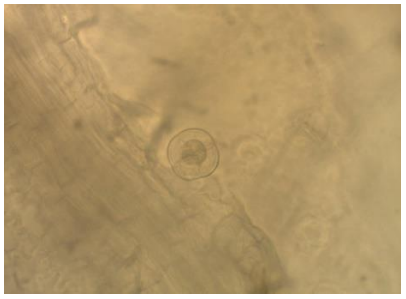

Fig. 20. Flower, surface view: glands with four secretory cells (40x)

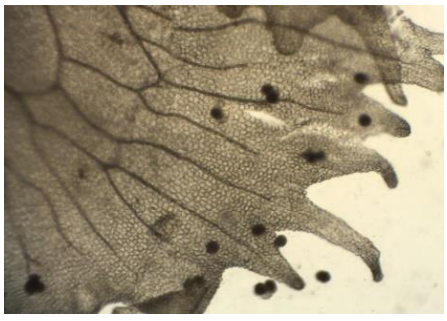

Fig. 21. Flower, surface view: fringed margin of the middle lobe of the lower lip (40x)

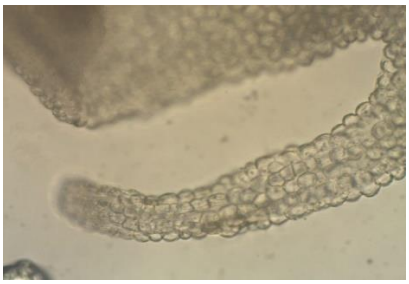

Fig. 22. Flower, surface view: fringe covered with papillae (40x)

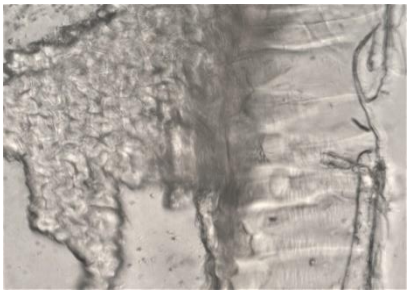

Fig. 23. Nutlet, transverse section: epidermal cells containing mucilage; macrosclereid layer showing sinuous anticlinal walls (10x)

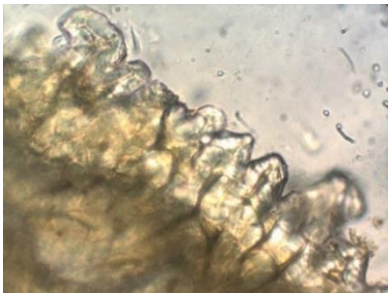

Fig. 24. Nutlet, surface view: elongated macrosclereids (10x)

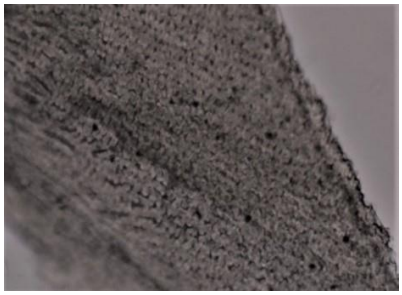

Fig. 25. Nutlet, surface view: macrosclereid layer showing sinuous anticlinal walls (10x)

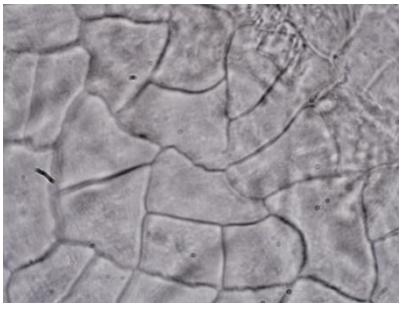

Fig. 26. Nutlet, surface view: epidermal cells with straight walls (10x)

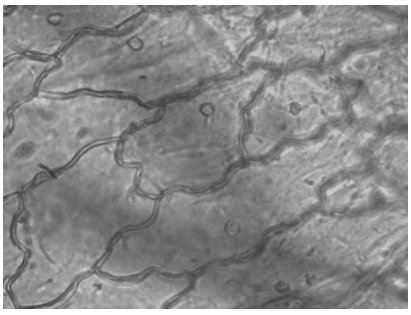

Fig. 27. Nutlet, surface view: endosperm with oil droplets (10x)

(Photos: Alexandra Groșan)

Discussions

The macroscopic and microscopic analysis of Prunellae spica has highlighted the main characters, which are important to identify this herbal drug and to distinguish it from similar fruit spikes coming from other Lamiaceae species (Moon, 2009).

The analyzed fruit-spike of Prunellae spica (**Fig. 1B**) has a slightly flattened cylindrical shape, is 1.5-8 cm long and 0.8-1.5 cm wide, and has a light brown or reddish-brown color. It consists of 10 or more persistent whorls, each whorl being delimited by two opposite, fan-shaped bracteoles, with acuminated tip, with reticulate venation, the outer surface being covered with numerous, conspicuous trichomes. Each bracteole is grown together at the base with three flowers made up of a persistent biconvex calyx, a corolla that usually lacks and four brown nutlets, small and obovate, with a white, sharp protuberance at the hilum. At the fruiting stage, the calyx is closed. The powdered herbal drug is reddish-brown or brown. All these characteristics are in accordance with those acknowledged by European Pharmacopoeia (Eur. Ph. 8.0, 2011).

Conclusions

The results of our histo-anatomical research confirm the very few and general data from the literature, but also bring new information related to macro- and microscopic characters with practical relevance to botanical identification of the herbal drug Prunellae spica. The nutlets can be easily identified by the Y-shaped mark in the apical region and the three stripes running down to the pyramidal abscission scar near the white hilum. Of the

microscopic characters, the following should be mentioned: glands with 4 secretory cells on petals; filament with a short conical spur at the top, near the anther; bilobed stigma; glandular hairs with unicellular stalk and bicellular gland on calyx and bracteoles; epidermal cells of calyx and bracteoles with sinuous anticlinal walls and diacytic stomata, one subsidiary cell being smaller.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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THERAPEUTIC ASPECTS OF CATECHIN AND ITS DERIVATIVES – AN UPDATE

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Abstract: Catechin and its derivatives are polyphenolic benzopyran compounds. The condensation of catechin units leads to the formation of condensed tannins. It is found in appreciable amount in green tea leaves, cocoa, red wines, beer, chocolate, etc. It possesses important antioxidant, antibacterial, antifungal, antidiabetic, anti-inflammatory, antiproliferative and antitumor properties. The present review outlines recent updates and perspectives of the effects of catechins and the pharmacodynamic mechanisms involved.

Keywords: catechin, antitumor, antioxidant, antibacterial, hypolipidemic.

1. Introduction

Catechins are flavanols which belong to polyphenolic compounds. Condensed or non-hydrolyzable tannins are formed by the condensation of catechin (epicatechin and a catechin epimer) (**Fig. 1**). Catechin, together with epicatechin and epigallocatechin gallate, are the main flavonoids which are found in the composition of green tea (Li et al., 2018). Many research results have highlighted that catechins have an important role in protection against degenerative diseases (Ide et al., 2018). Other studies have demonstrated an inverse reaction between catechin intake and the risk of cardiovascular diseases (Ikeda et al., 2018). It has been reported that catechins appear to produce greater antibacterial activity against Gram-positive bacteria than Gram-negative ones (Ajiboye et al., 2016; Gomes et al., 2018).

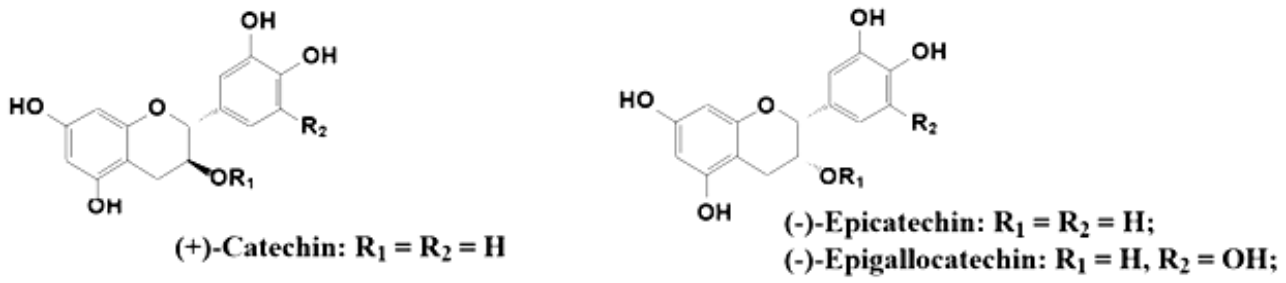

Fig. 1. Chemical structure of catechin and its derivatives

The present paper is a critical review of the recent literature on therapeutic aspects and pharmacodynamic mechanisms of catechin and its derivatives.

2. Bioavailability and biological effects of catechin and its derivatives

The bioavailability of catechin and its derivatives differs significantly depending on the form under which it is found (**Table 1**). When administered in esterified form with gallic acid, the absorption is much slower. Methylated forms were identified as a result of metabolization in the case of epigallocatechin. In another study, the plasma concentration of 4'-O-methyl-epigallocatechin was determined, whose value was 5 times higher in plasma and 3 times higher in urine than the concentration of epigallocatechin (Rothwell et al., 2018). Epigallocatechin gallate is the only flavonol form which is present in plasma in a significant percentage (48-55%).

The other catechins are detected as the glucoronidated or sulphated form. The main epicatechin metabolites are: epicatechin-3'-O-glucuronide, 4'-O-methylepicatechin-3'-O-glucuronide, 4'-O-methyl-epicatechin-5- or 7-O-glucuronide, 4'-O-methylepicatechine and epicatechin aglycone (Liao et al., 2018; Casanova et al., 2019). Metabolization leads to metabolites that can extend the beneficial effect of catechins, having a longer half-life (5 hours for epigallocatechin-3-gallate). In this case, renal excretion of catechins is very rapid (Manach et al., 2005; Ikeda et al., 2018).

Catechin influences molecular mechanisms involved in angiogenesis, extracellular matrix degradation, regulation of cell motility, and multiple resistance to cancers and associated disorders.

Based on epidemiological and experimental studies, a correlation between green tea consumption rich in catechins and cardiovascular health has been highlighted by several antioxidant, antihypertensive, anti-inflammatory, antithrombogenic, hypo-lipidemic, etc. effects (Rothwell et al., 2018). It was also shown that catechin and its derivatives, namely epigallocatechin gallate, would inhibit platelet aggregation. These effects were explained by the inhibition of cytoplasmic calcium growth (Lill et al., 2003; Watson et al., 2014). Catechins also have an important role in maintaining homeostasis (Matsui, 2015), their cardioprotective and antidiabetic effects have been demonstrated by several studies (Thielecke et Boschmann, 2009; Hashemipour et al. 2017).

3. Antiallergic properties

New studies have been conducted on the anti-inflammatory and anti-allergic effects of catechin (Hussein et al., 2015). The pharmacological effects of catechin in mice with allergic rhinitis were determined by performing haematoxylin and eosin staining and Giemsa staining of the nasal tissues essential in observing the allergic symptoms. The results showed that catechin, at 75, 150 or 300 mg/kg bodyweight, reduced the allergic symptoms in mice with allergic rhinitis, such as sneezing and nasal rubbing. Catechin could reduce interleukin-5, interleukin-13 and ovalbumin E serum concentrations and restore T helper type 2 / T helper type 1 cell balance. Catechin has efficiently decreased the inflammation in allergic rhinitis. The mechanism of action would be that catechin inhibited the expression of TSLP (lymphatic stromal lymphopoietin) in epithelial cells by influencing the NF-κB / TSLP pathway (Pan et al., 2018).

4. Cardiovascular effects

Recent evidence demonstrates that catechins can be key mediators in cardiovascular health through mechanisms underlying blood pressure reduction,

vasodilation, and atherosclerosis (Mangels et al, 2017).

The prevalence of coronary heart disease in Asian people is demonstrated to be very low due to their increased tea consumption (Shahid et al., 2016; Li et al., 2018). Also, recent studies have demonstrated that, due to their antioxidant effect, they have the ability to reduce cytotoxicity produced by amiodarone (commonly used antiarrhythmic drug) in human lung fibroblast cells (Cooper et al., 2005; Santos et al., 2017).

5. Antioxidant and anti-tumor effect

Clinical trials conducted so far have shown the beneficial effects of catechin due to its antioxidant action. The ability of catechins to cross the blood-brain barrier has led to increased interest related to their antioxidant properties beneficial for the prevention and treatment of neurodegenerative diseases.

Catechin and other catechins in green tea block carcinogenesis and help modulate signal transduction pathways related to proliferation, transformation, inflammation and metastasis of cells. It also has a chemopreventive potential (Grzesik et al, 2018; Yang et al., 2018; Baranowska et al., 2018) and catechin nanohybrids significantly improve the anti-tumour effect by inducing apoptosis of WM266 human melanoma versus free catechin (Di Leo et al., 2017). Also, the loading of catechin with PLGA (poly (1-lactide-co-glycolide)) fibers had a high effect of reducing the reactive oxygen species, so processing of catechin in controlled release forms could allow future localized applications of great importance in the fields of tissue engineering and wound healing (Ghitescu et al., 2018).

Oxidative stress plays a central role in the degeneration of neurons by activating intracellular signaling cascades, which have a role in autoimmune apoptosis and extracellular modulation (Suryavanshi et al, 2017, Bhatt et al., 2012) processes. Generation of ROS in neuronal cells activates inflammatory mediators like TNF-α, COX-2, NF-κβ as well as proapoptotic mediators such as Bcl-2 and caspase-9 (Suryavanshi et al, 2017; Kimura-Ohba et al., 2016). Catechin directly or indirectly decreases neuronal damage by reducing oxidative stress, scavenging ROS and improving antioxidant enzymes (Shai et al 2015; Xiang et al., 2016).

6. Lipid-lowering effects

Studies in rats have shown that catechin in green tea can reduce the risk of cardiovascular disease (CD). The effect has been attributed to the antioxidant and anti-inflammatory properties of catechin. Also it is suggested that catechin reduces the risk of cardiovascular disease by lowering cholesterol and triglyceride levels (Cooper et al., 2005). In vitro and in vivo studies show that catechins in green tea inhibit intestinal absorption of dietary lipids, by interfering with lipid digestion and their solubilization (the critical steps involved in the intestinal absorption of dietary fats, cholesterol and other lipids). Based on the information available so far, it is clear that green tea and its catechins effectively reduce the intestinal absorption of lipids (Ikeda et al., 1992). The mechanism of action of catechin is based on the fact that it inhibits the absorption of lipids. This effect appears to be associated with its ability to form complexes with lipids and lipolytic enzymes, thereby interfering with emulsification, hydrolysis, micellar solubilization processes and subsequent absorption of lipids. These mechanisms are not fully clarified and further studies are needed to define mechanisms underlying lipid absorption inhibition (Shishikura et al.; 2006; Koo et al., 2007).

7. Antibacterial and antiviral effect

Polyphenols are among the most abundant compounds in the plant kingdom. They have been reported to be associated with a number of organoleptic properties of drinks and foods.

Table 1. The effect of catechin and its derivates

Compound	Effect	Reference
epicatechin gallate epigallocatechin-3-gallate	beneficial role in muscles regeneration	Kim AR et al.,2017
epigallocatechin, epicatechin, epigallocatechin-3-gallate, epicatechin-3-gallate	antioxidant cardioprotective	Ikeda et al., 2018
epigallocatechin gallate	antibacterial	Miyamoto et al., 2017
epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, gallocatechin gallate	bactericidal effects on oral bacteria, *Aggregatibacter actinomycetemcomitans*	Chang et al., 2019
epicatechin epicatechin gallate epigallocatechin epigallocatechin gallate	antioxidant	Grzesik et al., 2018
catechin	antioxidant, anti-inflammatory, beneficial effect in the management of diabetic autonomic neuropathy in rats	Addepalli V et Suryavanshi SV, 2018
catechin	antibacterial	Gomes et al., 2018
catechin	antioxidant	Caro et al., 2019
catechin	hepatoprotective	Akinmoladum et al., 2018
catechin	cytotoxic	Di Leo et al., 2017
catechin	anti-microbial	Chunmei et al., 2010
epigallocatechin-3-gallate	antioxidant,	Roychoudhur et al., 2017
epigallocatechin-3-gallate	antibacterial	Founier-Larente et al., 2016
catechin	antioxidant, antibacterial	Akiboye etal., 2016
catechin	antibacterial	Diaz-Gomez et al., 2013
catechin	antibacterial	Diaz-Gomez et al., 2014
catechin	antibacterial antioxidant	Li et al., 2018
catechin	antibacterial	Zhang et al., 2016
catechin	antifungal	Saito et al., 2013s
epigallocatechin gallate	antibacterial	Nakayama et al., 2013

Several authors have highlighted the antibacterial effect of polyphenols against bacteria that can cause gastrointestinal diseases.

Several studies associate catechin with different antibiotics. It has been observed that the combination of antibiotics like catechin-imipenem, catechin-erythromycin, catechin-tetracycline would have a synergistic inhibition effect against *Eschericia coli*, which suggests that polyphenols may be considered promising alternatives for the treatment of bacterial and viral infections, thus the catechin can be used for the prophylaxis of influenza and A (H1N1) infection (Gomes et al., 2018; You et al., 2018; Diaz-Gomez et al., 2013). Other studies demonstrate that black tea consumption would influence the incidence of Helicobacter pylori infection due to its catechin content (Boyanova et al., 2015; Naveeda et al., 2018; Diaz-Gomez et al., 2013).

8. Antidiabetic effect

Certain studies have pointed out that catechin would have a significant potential to reduce blood glucose, body weight and body mass index (BMI) in both elderly and obese subjects by stimulating thermogenesis (Hashemipour et al., 2017; Pastoriza et al., 2017; Addepalli et al., 2018). Catechins control plasma glucose levels by modulating the glucose transport system (Grzesik et al., 2018).

9. Immunostimulatory effect

Catechin also intervenes in immune function modulation through humoral and cellular mechanisms. It has been emphasized that catechin decreases cyclophosphamide-induced myelosuppression (Ganeshpurkar et al., 2018).

Conclusions

Catechin and its derivatives represent a class of phenolic compounds with a very wide therapeutic potential. At present, not all the mechanisms of action are fully known, but studies have shown that these compounds would be worthwhile investigating.

Numerous studies have shown positive effects of green tea and tea-based products due to their content of catechin and its derivatives. These compounds would also be beneficial for the improvement of degenerative, metabolic and cardiovascular diseases as well as the quality of life in elderly population. Studies on cell lines highlight the effects of catechin on cancer chemopreventive activity. However, catechins and their derivatives must be given full attention due to numerous effects, for the development of new, more efficient and more stable drug structures. There are currently no food supplements with catechin or its derivatives in pure form, but most of the green tea extract supplements are based on its effects and its derivatives.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BIOCHEMICAL CHANGES OCCURING IN NEONATES WITH SEPSIS

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Abstract: This retrospective study aims to analyze the relationship between biochemical changes occurring in newborns with sepsis proven by positive blood culture (BC) and possible correlations with 1 min Apgar score, 5 min Apgar score, gestational age (GA), and birth weight (BW). We included in the study all infants ≤7 days of life with positive BC that were admitted to the Neonatal Intensive Care Unit (NICU) and Neonatology Department (ND) of the County Emergency Clinical Hospital of Târgu Mureş, a tertiary level hospital, between 2014-2018. The analyzed parameters are: day of life for blood sampling (0-7 days of life), gender, Apgar score (1 and 5 minute), GA, BW, urea, creatinine, total bilirubin, direct bilirubin, aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), c-reactive protein (CPR), bacteria involved, empiric antibiotics administered before blood sampling, temperature of the newborn on the day of BC. We found there is a statistically significant negative correlation between 1 and 5 min Apgar score and creatinine, between GA and urea and also between BW, GA and Direct Bilirubin. We found a statistically significant positive correlation between BW, GA and GPT.

Keywords: neonate, septicemia, bacteremia, biochemistry, CRP.

1. Introduction

According to World Health Organization (WHO) in 2017, globally 2.5 million children died in the first month of life, approximately 7000 newborn deaths every day with about 1 million dying on the first day and close to 1 million dying within the next 6 days.

The most common causes are: preterm birth, intrapartum-related complications (birth asphyxia, lack of breathing at birth), infections and birth defects. The vast majority of newborn deaths take place in low and middle-income countries (WHO, 2018). Annual neonatal mortality rates (NMRs, the probability of dying during the first 28 days of life) vary widely across the world, but west and Central Africa and South Asia had the highest NMRs while Western Europe has the lowest NMRs in 2017 (Hug et al., 2019).

Neonatal sepsis is defined as a systemic infection (positive culture of blood, urine or cerebrospinal fluid) occurring in infants at ≤ 28 days of life. According to the time of onset of the disease, neonatal sepsis may be classified in early onset (EOS, defined as a positive culture during ≤3 days of life) and late onset (LOS, a positive culture >3 days of life) (Simonsen et al., 2014).

2. Materials and Methods

The study was approved by the Ethics Committee of the County Emergency Clinical Hospital of Târgu Mureș and it follows the Helsinki Declaration principles.

A 5 years retrospective study, from 2014-2018, was performed in June 2019, to evaluate the relationship between biochemical changes occurring in newborns with sepsis proven by positive blood culture (BC) and possible correlations with 1 min Apgar score, 5 min Apgar score, gestational age (GA), birth weight (BW).

Data were collected of all infants ≤7 days of life with positive BC that were admitted to the Neonatal Intensive Care Unit (NICU) and Neonatology Department (ND) of the County Emergency Clinical Hospital of Târgu Mureș, a tertiary level hospital.

The data were obtained by accessing the H3 electronic medical database and the laboratory records. Tracked parameters:

- general data: day of life that the blood sampling was performed (0-7 days of life), gender, Apgar score (1 and 5 minute), GA, BW
- biochemical data: urea, creatinine, total bilirubin, direct bilirubin, aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), c-reactive protein (CPR)
- microbiological data: positive BC, bacteria involved, empiric antibiotics administered before blood sampling, temperature at blood sampling for BC.

We set a maximum of 2 days between the biochemical analyses and the date of blood sampling for the BC.

Patients excluded:

- Microorganism considered to be contaminants: *Methylobacterium* spp., *Streptococcus mitis, Streptococcus oralis, Micrococcus luteus, Ochrobactrum anthropi* and the association over 3 types of germs;
- A positive BC with coagulase-negative staphylococci (CoNS) and when in the comments of the microbiologist it was specified contamination, possible contamination or skin flora.

The data analysis included descriptive statistics elements (frequency, percentage, confidence interval 95%, mean, median, standard deviation) and inferential statistics. The D'Agostino & Pearson test was applied to determine the distribution of the analyzed data series. The Pearson correlation coefficient, respectively Spearman, was calculated. The significance threshold chosen for p was 0.05. The statistical analysis was performed using the GraphPad Prism 7 utility, the Trial variant.

3. Results and discussions

We identified 694 BC performed on first 7 days of life on neonates, which of 88 (12.68%) are positive, 26 (3.74%) contaminated and 62 (8.94%) true positive BC. In our group 24 (38.70%) neonates are from the NICU and 38 (61.30%) are from the ND. 13 (20.96%) of newborns with sepsis died in hospital. **Table 1** contains the description of the studied group.

In our group, 32 (51.61%) of neonates had EOS and 30 (48.39%) had LOS. The bacteria being identified and the antibiotics used, if it were the case, are presented in **Table 2**. The most common empirical choices of antibiotics for the treatment of neonatal sepsis were Aminoglycoside (in EOS 5, 71.42%; in LOS 7, 22.58%) and Penicillin (in EOS 4, 57.14%; in LOS 12, 38.70%) and for both categories.

Table 1. The description of the studied group

Parameter (unit of measure, number of values	*Mean±SD[a]*	*Median*	*Normal range[e]*
Age (days, 62)	3.5±2.31	3	
Gestational age (weeks, 62)	35.13±4.44	36	
Birth weight (grams, 62)	2482±1050	2530	
1 min APGAR score (62)	7.22±2.28	8	
5 min APGAR score (62)	8.06±1.83	9	
Temperature at blood collection (°C, 62)	36.98±0.65	37	
Urea (mg/dL, 47)	56.25±39.37	43.98	9-14
Creatinine (mg/dL, 50)	0.78±0.39	0.66	0.17-0.85
Total Bilirubin (mg/dL, 48)	7.24±6.08	6.11	0-12.6
Direct Bilirubin (mg/dL, 43)	0.80±0.53	0.57	0-0.6
GOT[b] (U/L, 52)	60.97±48.48	48	0-110
GPT[c] (U/L, 50)	35.34±48.96	16	0-60
CRP[d] (mg/L, 50)	82.10±92.98	53.71	0-5

Note: a - Standard Deviation; b - aspartate aminotransferase; c - alanine aminotransferase; d - c-reactive protein; e - normal clinical biochemistry reference ranges for neonates in Clinical Laboratory of County Emergency Clinical Hospital of Târgu Mureş (data from the manufacturer and the literature)

Table 2. The bacteria identified and the antibiotics used

Parameter		*Frequency*	*Percentage*	*Confidence interval (95%)*
Gender	Female	23	37.10%	25.16%-50.31%
	Male	39	62.90%	49.69%-74.84%
Positive bacteria	*Streptococcus*	3	4.84%	1.01%-13.50%
	Staphylococcus	20	32.26%	20.94%-45.34%
	Stenotrophomonas maltophilia	2	3.23%	0.39%-11.17%
	Escherichia coli	10	16.13%	8.02%-27.67%
	Enterococcus	9	14.52%	6.86%-25.78%
	Listeria	2	3.23%	0.39%-11.17%
	Klebsiella	7	11.29%	4.66%-21.89%
	Candida	7	11.29%	4.66%-21.89%
	Serratia	2	3.23%	0.39%-11.17%
	Acinetobacter	2	3.23%	0.39%-11.17%
Empiric antibiotics administered before blood sampling	Aminoglycoside	12	50.00%	29.12%-70.88%
	Penicillin	16	66.67%	44.68%-84.37%
	Carbapenem	5	20.83%	7.13%-42.15%
	Cephalosporin	3	12.50%	2.66%-32.36%
	Polymyxin	4	16.67%	4.74%-37.38%
	Fluoroquinolone	1	4.17%	0.11%-21.12%
Blood sampling on treatment	No	38	61.29%	48.07%-73.40%
	Yes	24	38.71%	26.60%-51.93%

Of the 24 newborns receiving antibiotic empirical therapy, 16 (66.66%) of them had associations of drug classes (14, 58.33% received association with 2 classes and 2, 8.33% association with 3 classes). The most common association of drug classes was Penicillin with Aminoglycosides (7, 43.75%).

In **Table 3** correlations between 1 min Apgar score, 5 min Apgar score, GA, BW and biochemical parameters are presented.

Acute kidney failure (AKF) is a common clinical problem in NICUs. According to Mathur et al. (2006), in India, renal failure occurred in 26% neonates with sepsis and Low birth weight is an important risk factor for the development of AKF, a significantly higher number of babies with AKF weighed less than 2500 gm. The mortality was three times higher in neonates with AKF. In Turkey, Agras et al. (2004) found a frequency of 3.4% AKF in the NICU, the premature newborns constituting 31.1% of the cases. The most common condition that contributed to AKF that they found was asphyxia (40.0%) followed by sepsis/metabolic disease (22.2%) and feeding problems (17.8%). In another study, also conducted in Turkey, the prevalence of neonatal AKF was 8.4%. The common cause of AKI was respiratory distress syndrome, followed by sepsis, asphyxia, dehydration, congenital anomalies of the urinary tract, congenital heart disease, and medication. In that case, the overall mortality rate was 23.8% (Bolat et al., 2013). In Egypt, 40.7% of the AKI cases were born after full-term pregnancy while 59.3% were pre-term babies. The predisposing factors for AKI were sepsis (63%), respiratory distress syndrome (55.6%), mechanical ventilation (51.9%), peri-natal asphyxia (18.5%), dehydration (14.8%), surgical operation (11.1%), congenital heart disease (7.4%), sub-galeal hematoma (3.7%), polycythemia (3.7%) and intra-ventricular hemorrhage (3.7%) (Youssef et al., 2015). Although the prevalence and mortality rate are different depending on the hospital, the causes remain roughly the same and sepsis is found everywhere. We found a statistically significant negative correlation between a high 1 and 5 min Apgar score and a low value of creatinine. We have also found a statistically significant negative correlation between a high GA and a low value of urea.

Hepatic pathology is common among newborns with sepsis. Jaundice is a well-known complication of sepsis or nonbacterial infection. Sepsis and bacterial infection are responsible for up to 20% of cases of jaundice in patients of all ages in a community hospital setting (Whitehead et al., 2001). Sepsis is more likely to manifest with jaundice in infants and children than in adults. Various mechanisms that can lead to hyperbilirubinemia alone during systemic infection are hemolysis, hepatic dysfunction, cholestasis (Chand and Sanyal, 2007). We found that a high BW and a high GA is significantly negative correlated with a low value of Direct Bilirubin. Another cause of neonatal jaundice is urinary tract infection (UTI). Shahian et al. (2012) found 12.5% of the asymptomatic jaundice neonates with the onset of unconjugated hyperbilirubinemia in the first week of life, and suggested that urine culture should be considered as a part of the diagnostic evaluation of jaundice neonates >3 days of life with an unexplained etiology (Shahian et al., 2012). On the other hand, Oswari et al. (2013) found that serum gamma-glutamyltransferase (GGT) and AST values can be used to predict the prognosis of patients with sepsis-associated cholestasis (Oswari et al., 2013). Our results show that there is a positive statistical correlation between BW, GA and GPT, a high BW or a high GA is correlated with a high GPT value.

Table 3. Correlations between independent variables (1 min Apgar score, 5 min Apgar score, Gestational age, Birth weight) and biochemical parameters

1 min Apgar score			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.08372	-0.3698 to 0.2169	0.5758
Creatinine	-0.3012	-0.5407 to -0.01638	0.0336*
Total Bilirubin	0.1412	-0.1574 to 0.4161	0.3385
Direct Bilirubin	0.02178	-0.2889 to 0.3283	0.8897
GOT	0.02761	-0.2550 to 0.3058	0.8460
GPT	0.2574	-0.03110 to 0.5063	0.0712
CRP	-0.1415	-0.4110 to 0.1508	0.3271
5 min Apgar score			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.1393	-0.4174 to 0.1626	0.3503
Creatinine	-0.2826	-0.5262 to 0.003939	0.0468*
Total Bilirubin	0.1140	-0.1843 to 0.3930	0.4404
Direct Bilirubin	-0.1084	-0.4037 to 0.2072	0.4888
GOT	0.01448	-0.2672 to 0.2939	0.9189
GPT	0.2335	-0.05643 to 0.4872	0.1026
CRP	-0.1801	-0.4435 to 0.1118	0.2106
Birth weight			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.2740	-0.5266 to 0.02312	0.0624
Creatinine	-0.008219	-0.2937 to 0.2786	0.9548
Total Bilirubin	-0.007929	-0.2994 to 0.2849	0.9573
Direct Bilirubin	-0.5542	-0.7369 to -0.2961	0.0001*
GOT	0.09467	-0.1910 to 0.3656	0.5044
GPT	0.5324	0.2905 to 0.7104	0.0001*
CRP	-0.2504	-0.5007 to 0.03856	0.0795
Gestational age			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.2938	-0.5420 to 0.001547	0.0450*
Creatinine	-0.06826	-0.3477 to 0.2223	0.6376
Total Bilirubin	-0.01890	-0.3093 to 0.2747	0.8985
Direct Bilirubin	-0.4443	-0.6622 to -0.1571	0.0028*
GOT	0.1060	-0.1800 to 0.3754	0.4546
GPT	0.4558	0.1950 to 0.6563	0.0009*
CRP	-0.1143	-0.3878 to 0.1777	0.4292

Note: a - correlation coefficient; b - significance criterion * - significant values where $p \leq 0.05$

CRP is an acute phase reactant, a protein synthesized and secreted by the liver in response to inflammatory cytokines, specifically IL-6 (Satar and Özlü, 2012) and is commonly used for bacterial sepsis detection in neonates. Still it is not useful as an early phase infection marker and it lacks specificity (Ng and Lam, 2006). All neonates in our study had a high CRP level, the mean being 8.21 mg/dl. In their study, Zhou et al. (2016) have found a CRP level >0.8 mg/dl in neonates (39.1%) with positive blood culture results and 45.3% of them died within 7 days after birth, a higher prevalence than us (20.96%) (Zhou et al., 2016). Also, Mannan et al. (2010) found that CRP was raised in 72% of cases of neonates with positive blood culture and only in 4% of control cases, and their study concluded that CRP is the most sensitive method (93%) in culture proven sepsis, 79% in suspected sepsis and its positive predictive value in suspected sepsis amounts to 88%.

Hofer et al. (2012) found that a growing body of evidence suggests a link between GA and CRP kinetics with lower baseline CRP values and a lower CRP response to infection in preterm compared to term newborns. All correlations between all independent variables that we studied (1 and 5 min Apgar score, GA, BW) and CRP are negatively correlated, a high value of independent variable is associated with a low CRP value, but it is not statistically significant. Hofer et al. (2012) conclude that CRP has the best diagnostic accuracy when combined with another infection marker like PCT, IL-6, and IL-8, that provides a higher sensitivity during the early phases of sepsis.

The gold standard for diagnostic sepsis is BC but the CRP is also particularly useful for monitoring the response to treatment and guiding antibiotic therapy. The highest level of CRP concentrations is detected during the first day of illness but because sustained pro-inflammatory action of IL-6, production could be detected until 24 hours after treatment was started. In their study, Janković et al. (2001) found that in the case of non-adequate initial antibiotic therapy of neonatal sepsis, CRP level increases further during the second day, but if the treatment is appropriate in the second day there is a significant decrease of CRP levels. CPR level can be taken as indication for replacement of initial antibiotics during the second day of treatment of sepsis neonates. The pathogens that are involved in neonatal sepsis are different depending on the type of neonatal sepsis, EOS or LOS, and the country's degree of development. Organisms associated with EOS are Group B *Streptococcus* (GBS, in special *Streptococcus agalactiae*), *Escherichia coli* (*E. coli*) which together account for about 70% of cases, and *Streptococcus viridans*. In LOS, organisms associated are CoNS, *Staphylococcus aureus*, *Candida albicans* and *Klebsiella pneumoniae* (Shah and Padbury, 2014; Cortese et al., 2016; Resende et al., 2015). In developed countries, in EOS are dominant GBS and E. coli, and in LOS are CoNS and GBS followed by *Staphylococcus aureus* (Hyde et al., 2002; Vergnano et al., 2005). In developing countries, the pathogens associated with EOS are *E. coli*, GBS, *Enterobacter*, *Enterococcus*, *Listeria* and with LOS *Pseudomonas* spp., *Salmonella*, *Seratia*. On both, EOS and LOS, are more associated *Klebsiella*, *Acinetobacter*, *Staphylococcus aureus* and also CoNS (Vergnano et al., 2005).

The appropriate empirical antibiotic selection during neonatal sepsis is based on the likely etiologic pathogens based on epidemiologic surveillance. Cortese et al. (2016) found that for EOS, the recommended empiric therapy as 1st line is Ampicillin and an Aminoglycoside, and for LOS is Vancomycin and an Aminoglycoside. Also in our study the most used antibiotic was Aminoglycoside followed by Penicillin but for both of type of sepsis.

Conclusions

There is a statistically significant negative correlation between 1 and 5 min Apgar score and creatinine, between Gestational Age and urea, and also between Birth Weight, Gestational Age and Direct Bilirubin. The statistically significant positive correlation is between Birth Weight, Gestational Age and alanine aminotransferase.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BOTANICAL SURVEY OF MEDICINAL PLANTS USED IN THE TRADITIONAL TREATMENT OF HUMAN DISEASE IN MONTAIN HAY MEADOWS FROM GURGHIULUI MOUNTAINS

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Abstract: The aim of this study was to identify the medicinal and aromatic plants from mountain hay meadows (6520 - Natura 2000 habitat) of Gurghiului Mountains and to analyze the correlation of these herbs with their therapeutic compounds as well as the human diseases on which they can be used on therapeutic purpose. The area covered by this study was the Gurghiului Mountains. Regarding the vegetation, this area is characterized by the predominance of forest ecosystems, along with semi-natural mountainous grasslands. The floristic inventory for the studied area included numerous medicinal plants with therapeutic chemical compounds. These medicinal plants were grouped in this study according to the dominant active principles used in phytotherapy. Two plant associations were identified: *Festuco rubrae-Agrostietum capillaris* Horvat 1951 and *Poo-Trisetetum flavescentis* Knapp ex Oberdorfer 1957. This survey demonstrates that the medicinal plant area in the Gurghiului Mountains is a promising economic resource for developing this region, but it needs planned exploitation.

Keywords: grasslands, habitats, medicinal plants.

1. Introduction

Today is increasing interest in the health benefits of medicinal plants. This is with good reason as they might offer a natural safeguard against the development of certain conditions and be a putative treatment for some diseases (Tahraoui et al., 2007). Ethnobotanical studies have become increasingly valuable in the development of health care and conservation programs (Nadembega et al., 2011; Wright et al, 2007; Tahraoui et al., 2007). The green pharmaceuticals are receiving extraordinary importance and popularity. Ethnobotany and ethnopharmacology have contributed to the discovery of many important plant-derived drugs.

Vegetal product research can be guided by ethnopharmacological knowledge. In the same time, it can make a contribution to drug innovation by providing novel chemical structures and/or mechanisms of action. Both plant-derived drugs and crude plants have to take the same pharmaco-economic hurdle that

has become important for new synthetic drug (De Smet, 1997).

An increased interest worldwide for the estimation of therapeutic potential of herbal medicine, prompted us to study grasslands with plants showing medicinal potential, especially herbs used in a variety of human disorders. Sustainable use of wild populations of medicinal plants requires robust assessment of the distribution and abundance of target species (Nkomo et al., 2014). In different countries, many medicinal plants are widely distributed and used across regions. However, relatively few are cultivated. Thus, the conservation of these plants requires efforts that are directed to key habitats, including secondary forests, disturbed areas and agrolandscapes (Aguilar-Støen and Moe, 2007).

Medicinal plants growing in semi-natural and natural ecosystems are a valuable commodity because they are a cheap resource; the quality of spontaneous herbs is seldom superior to those cultivated and their consumers acceptance is higher.

Our study represents an inventory on medicinal plants identified in the mountain hay meadows from the area of the Gurghiului Mountains. The collected data represent the preliminary information required in view of a future phytochemical investigation on the most used plants.

2. Materials and Methods

2.1 Study area

Gurghiului volcanic mountains are on the western edge of the Eastern Carpathians Center (Mureș County). They fall into the group of the youngest mountains in Romania. By their geographical location they fall in the temperate mountains, wet and cool climate. This climate together with edaphic conditions is responsible for the richness and diversity of flora existing in the study area.

2.2 Botanical survey

The survey was carried out during the year 2014-2015 and subsequent data analysis that was completed in 2016. The study of medicinal plants was done in Natura 2000 habitat, 6520 – Mountain hay meadows, comprising semi-natural mountain meadows. This study of medicinal plants was carried out based on our own research in the field by using classic techniques, procedures promoted in literature, and some statistical analysis (Oroian, 1998; Sămărghiţan et Oroian 1999; Tămaș, 1999; Oroian et Sămărghiţan, 2000; Sămărghiţan, 2005; Oroian, 2011; Coldea, 2012; Rácz et al, 2012).

The type of habitat has been coded in accordance with existing interpretations of habitats in Romanian manuals (Cristea et al., 2004; Gafta et Mountford, 2008). Habitat structure characterization was done using phytosociological surveys. The inventory of the medicinal species was based on the active principles contained therein, and data obtained from bibliographic information (Istudor, 1998; Palade, 1998; Sămărghiţan et Oroian 1999; Tămaș, 1999; Oroian et Sămărghiţan, 2000; Istudor, 2001; Palade et al, 2003; Cristea et al., 2004; Doniță et al. 2005; Aguilar-Støen et Moes, 2007; Gafta et Mountford, 2008; Stănescu et al., 2002; Stănescu et al.2002b; Yberrt et al., 2013; Council Directive 92/43/EEC; Romanian Pharmacopoeia; European Pharmacopoeia).

3. Results and discussions

3.1 Medicinal plants recorded

Two plant associations were identified: *Festuco rubrae-Agrostietum capillaris* Horvat 1951 and *Poo-Trisetetum flavescentis* Knapp ex Oberdorfer 1957.

They were classified according to Coldea (2012) as follows: Cls. Molinio-Arrhenatheretea, Ord. Arrhenatheretalia, All.

Arrhenatherion Koch 1926, Ass. *Poo-Trisetetum flavescentis* Knapp 1951 em. Oberdorfer 1983; All. Cynosurion R.Tx.1947, Ass. *Festuco- Agrostetum capillaris* Horvat 1951.

The phytocoenosis of these two associations belong to **6520** – Mountain hay meadows habitat of community interest listed in Annex I of Habitats Directive (Council Directive 92/43/EEC). The flora of these associations included many medicinal plants. We mention that the phytosociological surveys were recorded at different altitudes ranging between 504-1255 m, and 29 surveys were processed. A part of the plants identified in these surveys were medicinal species. Thus, in the *Festuco rubrae-Agrostietum capillaris* association 74 taxa out of 148 identified, contained certain therapeutic chemical compounds, while in the *Poo-Trisetetum flavescentis* association 57 taxa out of 141 identified, contained certain therapeutic chemical compounds.

The most common herbs, whose presence in phytosociologic surveys is very high (**81-100%**) are: *Achillea millefolium*, *Plantago lanceolata*, *Prunella vulgaris* and *Trifolium pratense*, followed by those with high frequency (**61-80**%): *Alchemilla xanthochlora*, *Carum carvi*, *Equisetum arvense*, *Euphrasia rostkoviana*, *Pimpinella saxifraga*, *Plantago media*, *Rumex acetosella*, *Thymus pulegioides*, *Veronica chamaedrys*, *Viola tricolor* etc. The following species have an average frequency between **41-60**%: *Daucus carota*, *Fragaria vesca*, *Galium mollugo*, *Galium verum*, *Mentha longifolia*, *Polygala vulgaris*, *Potentilla erecta* etc.

3.2 Therapeutic uses of medicinal plants

The medicinal plants were gathered according to the dominant active principles for which they are used in traditional medicine or phytotherapy. From the total medicinal species recorded in inventory, the most numerous species contain: tannins (16,66% of the species), essential oils (12,22% of the species), coumarins (11,11% of the species), flavonoids (10% of the species), saponins (8,88% of the species), alkaloids and mucilage (6,66% each), iridoids, bitter compounds and organic acids, vitamins and provitamins (4,44% each) (Stănescu et al., 2002; Stănescu et al.2002b; Stănescu et al. 2004; Wright et al., 2007; Eşianu et Ștefănescu, 2016) etc. (**Table 1**).

Table 1. Checklist of medicinal species used in traditional medicine and phytotherapy according to the dominant active principles

The dominant active principles	Species	Medicinal vegetal products
MUCILAGE	*Anchusa officinalis*	Flos et folium
	Platago lanceolata	Folium
	Plantago major	Folium
	Plantago media	Folium
	Tussilago farfara	Folium
	Verbascum lychnitis	Flos
PHENOLIC GLYCOSIDES	*Filipendula ulmaria*	Flos
	Populus tremula	Gemma
	Salix alba	Cortex
ANTHRAQUINONE DERIVATIVES	*Rumex acetosa*	Herba
	Rumex acetosella	Herba
	Rumex crispus	Rhizoma
NAPHTODIANTHRONES	*Hypericum maculatum*	Herba

	Hypericum perforatum	Herba
CARDIAC GLYCOSIDES	*Digitalis grandiflora*	Folium
SAPONINS	*Bellis perennis*	Flos
	Equisetum arvense	Herba
	Ononis arvensis	Radix
	Polygala comosa	Herba
	Polygala vulgaris	Herba
	Primula veris	Rhizoma cum radicibus
	Trifolium pratense	Flos
	Viola tricolor	Herba
FLAVONOIDS	*Crataegus monogyna*	Folium, fructus et flos
	Eupatorium cannabinum	Rhizoma et radix
	Linaria vulgaris	Herba
	Pilosella officinarum	Herba
	Prunella vulgaris	Herba
	Trifolium repens	Herba
	Veronica chamaedrys	Herba
	Veronica officinalis	Herba
	Vincetoxicum hirundinaria	Radix
	Viola tricolor	Herba
COUMARINS	*Cruciata glabra*	Herba
	Cruciata laevipez	Herba
	Galium mollugo	Herba
	Galium verum	Herba
	Heracleum sphondylium	Radix, folium et fructus
	Medicago falcata	Herba
	Medicago lupulina	Herba
	Medicago sativa	Herba
	Melilotus officinalis	Flos et herba
	Pastinaca sativa	Radix
	Pimpinella saxifraga	Radix
TANNINS	*Agrimonia eupatoria*	Herba
	Alchemilla xanthochlora	Herba
	Anthyllis vulneraria	Flos
	Fragaria vesca	Folium
	Fragaria viridis	Folium
	Geranium robertianum	Herba
	Geum urbanum	Rhizoma
	Lysimachia nummularia	Herba
	Lythrum salicaria	Herba
	Polygonum bistorta	Rhizoma
	Potentilla argentea	Rhizoma
	Potentilla erecta	Rhizoma
	Potentilla recta	Rhizoma
	Potentilla reptans	Rhizoma
	Salix alba	Cortex
DEPSIDES	*Cichorium intybus*	Herba et radix
ESSENTIAL OILS	*Achillea millefolium*	Flos
	Carum carvi	Fructus
	Juniperus communis	Psudo-fructus
	Mentha longifolia	Folium
	Origanum vulgare	Herba
	Petasites hybridus	Rhizoma

	Picea abies	Turiones
	Thymus glabrescens	Herba
	Thymus pulcherrimus	Herba
	Thymus pulegioides	Herba
ALLANTOIN	*Symphytum officinale*	Radix
GLYCORESINS	*Convolvulus arvensis*	Herba
IRIDOIDS	*Ajuga reptans*	Herba
	Euphrasia rostkoviana	Herba
	Lamium album	Herba
	Stachys germanica	Herba
	Stachys officinalis	Herba
ALKALOIDS	*Clematis vitalba*	Folium
	Colchicum autumnale	Semen
	Echium vulgare	Herba
	Genista tinctoria	Herba
	Senecio jacobaea	Herba
	Veratrum album	Rhizoma
BITTER COMPOUNDS	*Centaurium erythraea*	Herba
	Gentiana asclepiadea	Radix
	Glechoma hederacea	Herba
	Taraxacum officinale	Radix et herba
BITTER-AROMATIC COMPOUNDS	*Artemisia vulgaris*	Herba
ORGANIC ACIDS, VITAMINS and PROVITAMINS	*Daucus carota*	Radix
	Rosa canina	Fructus
	Rubus idaeus	Folium
	Urtica dioica	Folium

Inexhaustible green treasure of Gurghiului Mountains can be an important source of active ingredients for achieving herbal extracts used in various diseases. The most numerous herbs are used in disorders of the digestive system (37 sp.), respiratory system (18 sp.), skin disorders (15 sp.), muscular and skeletal systems (10 sp.) genitourinary system (8 sp.), in gynecological disorders (4 sp.), cardiovascular, CNS disorders and geriatrics (2 sp. each) (**Table 2**).

As shown in **Table 2**, the majority of plants were reported to be used for more than one type of disease.

Table 2. Medicinal plant species used in various disorders

PHYTOTHERAPY FOR DIGESTIVE SYSTEM DISORDERS	Phytotherapy of mouth ▪ gingivitis, ▪ stomatitis, thrush, ▪ periodontitis, ▪ dental abscesses, ▪ tonsillitis	*Achillea millefolium, Agrimonia eupatoria, Centaurium erythraea, Geum urbanum, Lysimachia nummularia, Lythrum salicaria, Polygonum bistorta, Potentilla* sp., *Thymus* sp.
	Hyperacid gastritis and ulcer disease	*Equisetum arvense, Hypericum* sp.,*Medicago sativa, Melilotus officinalis, Plantago* sp., *Rubus idaeus, Symphytum officinale*
	Gastric hypoacidity - dyspepsia, anorexia	*Artemisia vulgaris, Centaurium erythrea, Euphrasia rostkoviana, Gentiana asclepiadea*

	Acute and chronic liver disease	*Achillea millefolium*, *Hypericum* sp., *Taraxacum officinalis*, *Thymus* sp.
	Functional disorders of the gallbladder and biliary tract	*Achillea millefolium*, *Agrimonia eupatoria*, *Cichorium intybus*, *Eupatorium cannabinum*, *Hypericum* sp., *Mentha longifolia*, *Pastinaca sativa*, *Petasites hybridus*, *Taraxacum officinale*
	Phytotherapy in constipation	*Cichorium intybus*, *Convolvulus arvense*, *Rumex* sp.
	Phytotherapy in diarrhea	*Achillea millefolium*, *Agrimonia eupatoria*, *Geum urbanum*, *Lythrum salicaria*
	Vomiting - nausea	*Mentha longifolia*
	Abdominal colic	*Achillea millefolium*, *Mentha longifolia*
	Flatulence (bloating)	*Carum carvi*, *Mentha longifolia*
	Helminthiasis - anthelmintic plant	*Achillea millefolium*, *Gentiana asclepiadea*, *Rosa canina*, *Thymus* sp.
PHYTOTHERAPY FOR CARDIOVASCULAR SYSTEM DISORDERS	Heart failure	*Digitalis grandiflora*
	Cardiac neurosis	*Crataegus monogyna*
	Angina pectoris	*Crataegus monogyna*
PHYTOTHERAPY FOR RESPIRATORY SYSTEM DISORDERS	Immuno-stimulatory plant	*Achillea millefolium*, *Equisetum arvense*, *Hypericum* sp., *Rosa canina*
	Central and peripheral antitussives	*Plantago* sp., *Tussilago farfara*, *Verbascum lychnitis*
	Expectorant	*Primula veris*, *Picea abies*, *Polygala* sp., *Viola tricolor*
	Asthma	*Ajuga reptans*, *Origanum vulgare*, *Petasites hybridus*
PHYTOTHERAPY FOR GENITOURINARY SYSTEM DISORDERS	Diuretic	*Equisetum arvense*, *Juniperus communis*, *Lamium album*, *Ononis arvensis*, *Taraxacum officinale*, *Viola tricolor*, *Urtica dioica*
	Urolithiasis	*Equisetum arvense*, *Rosa canina*, *Urtica dioica*
PHYTOTHERAPY FOR GYNECOLOGICAL DISORDERS	Menopausal Disorders	*Genista tinctoria*, *Medicago* sp.
	Dysmenorrhea	*Achillea millefolium*, *Artemisia vulgaris*
PHYTOTHERAPY FOR SKIN DISORDERS	Acne	*Taraxacum officinale*, *Viola tricolor*
	Eczema	*Achillea millefolium*, *Taraxacum officinale*,*Viola tricolor*
	Dermatomycosis	*Achillea millefolium*, *Populus tremula*, *Thymus* sp.
	Alopecia (hair loss)	*Urtica dioica*
	Wounds	*Equisetum arvense*, *Hypericum* sp., *Populus tremula*, *Plantago* sp., *Symphytum officinale*
	Light burns	*Hypericum* sp., *Populus tremula*
	Bruises	*Achillea millefolium*, *Symphytum officinale*

PHYTOTHERAPY FOR LOCOMOTORY SYSTEM DISORDERS	Plant products with anti-inflammatory / analgesic, anti-rheumatic and hyperemic action	*Hypericum* sp., *Juniperus communis*, *Medicago sativa*, *Mentha longifolia*, *Picea abies*, *Populus tremula*, *Salix alba*, *Taraxacum officinale*, *Urtica dioica*
PHYTOTHERAPY FOR CNS SYSTEM DISORDERS	Sleep disturbances; Nervousness; Depression.	*Hypericum* sp.
PHYTOTHERAPY IN GERIATRY		*Crataegus monogyna*, *Urtica dioica*

There is major interest in the health benefits of herbs and botanicals (Foote et Cohen, 1998). In the same time, there are an increasing number of papers claiming that plants or plant-derived active principles may function as agent against many human diseases. Most of these researches have determined the level of clinical support for the traditional use of common or folklore medicines. Many plant species are known as sources of treating human ailments, this study documents the plants from Gurghiului Mountains, used in Romania by traditional healers for the treatment of different human disease.

Our study confirms that wild medicinal plants and natural products obtained from these are still a major source of medicine for the people living in the studied area.

Conclusions

The medicinal plants generally have significant less adverse effects compared with synthesized substances and also people have a better tolerance to these plants than synthetic drugs. In this paper we summarize information on medicinal and aromatic plants with current information in the international literature and highlight the current state of ethnopharmacological, phytochemical and clinical research on some of the more widely used and better known species. Mountain hay meadows from Gurghiului Mountains can be an important source of active substances for achieving herbal extracts used in various diseases, but it can also provide a comparative basis for future similar floristic research to be carried out in the Eastern Carpathians.

The most numerous herbs identified in study area are those used for: disorders of the digestive, respiratory, dermatological disorders, musculoskeletal and urogenital systems. Further experimental investigation of these medicinal and aromatic plants may possibly offer effective and alternative affordable management of some human disease.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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