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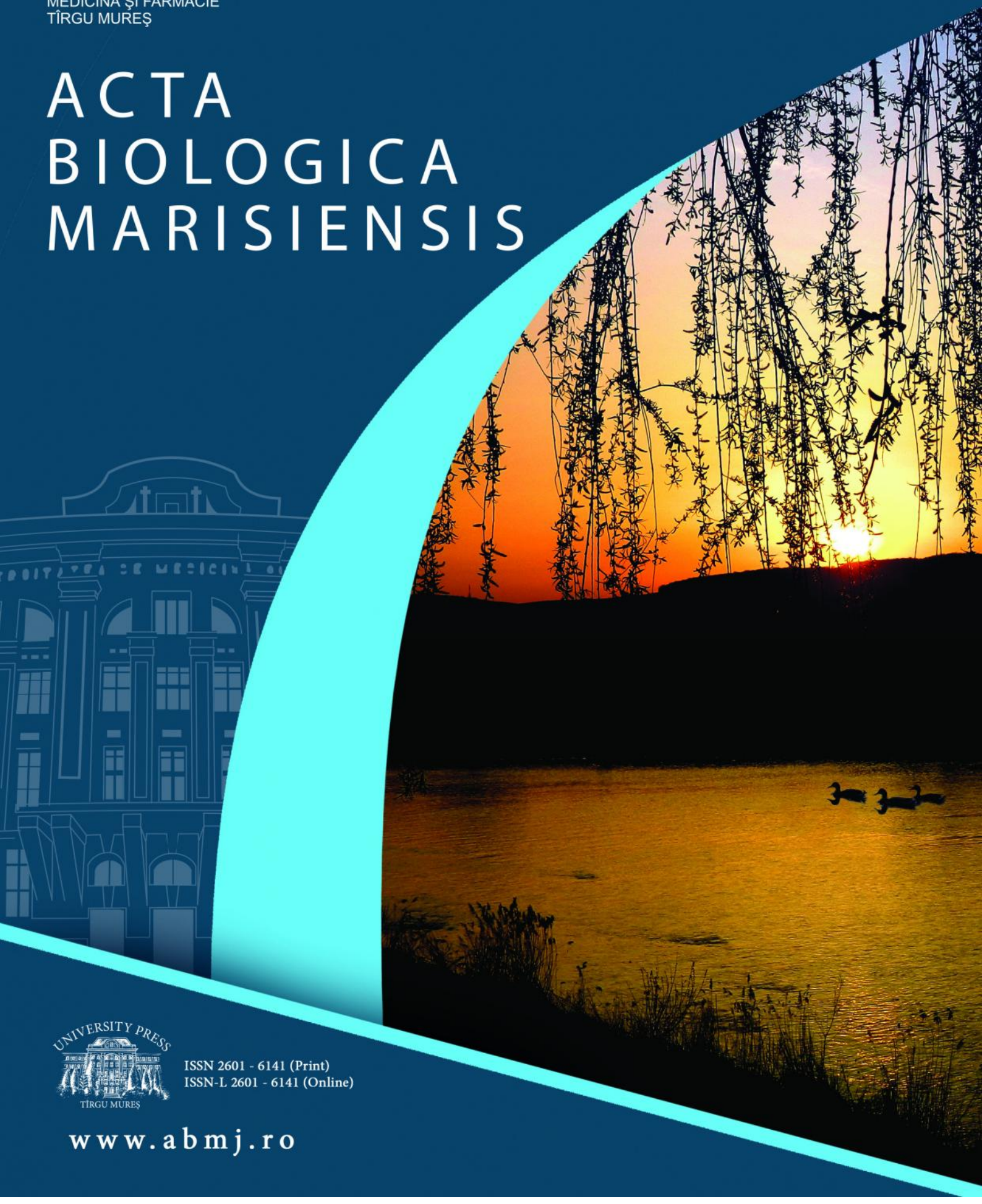
# ACTA BIOLOGICA MARISIENSIS



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# *ACTA BIOLOGICA MARISIENSIS*

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## SUPPLEMENTARY MATERIALS

**ORIGINAL PAPER**

# ANTIBACTERIAL ACTIVITY OF SPRUCE BARK (*PICEA ABIES* L.) EXTRACT AGAINST *ESCHERICHIA COLI*

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**Abstract:** The increase of antibiotic resistant bacteria in lasts years resulted in limited options for treatment of bacterial diseases. *Escherichia coli* is one of the most common Gram-negative bacterial pathogen and a cause of both community and hospital acquired infections. Medicinal plants are alternative rich sources of useful antibacterial agents. The antimicrobial activities of the spruce (*Picea abies* L.) bark polyphenolic extracts were examined for their capacity to inhibit the growth of *Escherichia coli*. Spruce bark extract was obtained by conventional aqueous extraction and with ultrasounds. The minimum inhibitory concentration was determined by microdilution method. The antibacterial effect of both extracts was strong against *Escherichia coli*. The antimicrobial effect of polyphenolic extracts on *Escherichia coli* was expressed at a concentration of 15 mg/ml. Antimicrobial activity of spruce bark compounds suggest a possible use of spruce bark in pharmaceutical preparations.

**Keywords:** antimicrobial agents, *Escherichia coli*, polyphenols, spruce bark, *Picea abies* L.

## 1. Introduction

The drug resistance in human pathogenic bacteria and many adverse effects of antibiotics has led to a search for new antimicrobial agents with plant origin. It is known that plants produce antimicrobial compounds. Thus, crude plant extracts have been used for a various purposes for a very long time (Moradi et al., 2016; Sharif et al., 2016). The antimicrobial activity of crude plant extracts was the basis of various applications, such as food preservation, pharmacy, natural therapies and medicine (Lis-Balchin and Deans 1997; Dorman and Deans, 2000; Sathasivampillai et al., 2017).

According to the classification system from "Vascular plants from Romania - Field Illustrative Determinator" (Sârbu et al., 2013), *Picea abies* (L.) H. Karst has the following systematic classification: Vegetable Kingdom, Pinophyta Division, Pinatae Class, Pinales Order, Pinaceae Family, *Picea* Genus, *abies* species. Norway spruce is very widespread in the Romanian forests. Spruce present great

economic importance, being used as construction wood, paper manufacturing, and in phytotherapy.

The literature data show many studies on volatile oil from spruce, with different antimicrobial activity and intensity depending on tested strains. The inhibitory effect was noticed against the Gram-positive and fungal strains (Radulescu et al., 2011; Chauhan and Dahiya, 2016). In the spruce bark alcoholic extract, was identified gallic acid, catechin and vanillic acid in high concentration (Ignat et al., 2013). The results of Ignat et al. (2013) showed that the spruce bark ethanol extracts exerted antibacterial activity against *Staphylococcus aureus* and for *Escherichia coli* and *Pseudomonas aeruginosa* being less susceptible.

The aim of this study was to evaluate the antimicrobial activity of *Picea abies* L. bark aqueous crude extracts against *E. coli*.

## 2. Materials and Methods

### 2.1 Plant/bacteria materials

Spruce (*Picea abies* L.) bark it is a waste product from a wood processing company (Vatra Dornei, Romania). The spruce bark was air-dried at room temperature and milled in a GRINDOMIX GM 2000 mill (0.5 mm diameter). The biomass was used without any pre-treatments.

To determine the antibacterial activity on *Escherichia coli*, ATCC 25922 strain was selected from the collection of Laboratory of Microbiology, Virology and Parasitology (Faculty of Medicine - University of Medicine and Pharmacy, Tîrgu Mureș).

### 2.2 Aqueous extraction

For extraction was used 20 g of ground spruce bark over which added 125 mL distilled water. The mixture was incubated for 45 min in a water bath at 85-90°C (Tanase et al., 2018a). Ultrasound assisted extraction was performed under ultrasounds action in accord with method previously described (Tanase et al., 2018a).

### 2.3 Minimum inhibitory concentration of *E. coli* (MIC)

To determine the MIC of the obtained extract against *E. coli*, the microdilution method was used, as previously described (Tanase et al., 2018a). Shortly, from fresh bacterial culture, a standard inoculum was prepared in liquid culture medium. One-hundred microliters of the tested extracts were mixed in the first well of the microplate with 100 µl of bacterial inoculum. We evaluated the MIC of the tested extracts in the first well with no bacterial growth. The MICs were calculated in mg/ml, by adjusting the obtained concentrations with the dilution factor and processed volumes.

*E. coli growth rate*

For *E. coli* growth rate determination, the method described in a previously paper was used (Tanase et al., 2018a). To determine if the tested solutions affect the *E. coli* growth rate, stock solutions of the both extracts was prepared. The concentration of the stock solution was adjusted to correspond to the MIC that was assessed by the microplate method. The total number of colony forming units/ml (CFU/ml) at time 0 (H0), 3 hours (H1), 6 hours (H2) was determined inoculating 50 µl from 1/100 diluted working solution and from 1/100 diluted control on Mueller Hinton agar plates. After 24 hours of incubation, the number of the colonies was counted using the colony counter "IUL Flash & Grow" and mathematically adjusted in order to be expressed as CFU/ml.

### 2.4 Statistical analysis

Using the calculated CFU/ml numbers from each time point, absolute growth curves were plotted. The following formula was used in

order to assess the growth rate ($r$) for the tested sample and control, after 6 hours of incubation. The statistical significance was assessed by GraphPad InStat 3 software, at a significance threshold value of $p<0.05$.

$$r = \frac{LN(CFU/ml \text{ for } H2 - CFU/ml \text{ for } H0}{\text{no. hours for } H2}$$

## 3. Results and discussions

### 3.1 Extract characterization

The previous results summarized that spruce bark extracts (EAM and USM) contain considerable quantities of bioactive aromatic compounds (EAM - 0.135 mg GAE/mL and USM 0.114 mg GAE/mL). The compounds identified in samples by HPLC were vanillic acid and taxifolin in small amounts (Tanase et al., 2018b).

### 3.2 Minimum inhibitory concentration of *E. coli* (MIC)

The results indicate that the two aqueous extracts (EAM and USM) of different concentrations have antibacterial activity against *E. coli*. The minimum inhibitory concentrations (MIC) of polyphenolic extracts required for growth inhibition of *E. coli* was 15 mg/mL both for EAM and for USM.

*E. coli growth rate*

As shown in **Table 1**, when the growth medium was enriched with both tested solutions (EAM and USM), the bacterial growth was significantly inhibited, compared with control. After three hours of incubation, the growth of *E. coli* was significantly reduced (**Table 1**, **Fig. 1** and **Fig. 2**). At H2 time, EAM inhibited the growth of *E. coli* **(Fig. 1**), while USM presented bactericidal effect (**Fig. 2**). As a research direction, it would be interesting to investigate which component of the USM extract induced the bactericidal effect on *E. coli*.

The biological activity of *P. abies* and other species of the Pinaceae family was previously tested on other strains. For the aqueous extract of *P. abies* bark, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used (Tanase et al., 2018b). For the spruce bark ethanol extracts, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were tested (Ignat et al., 2013). The ethereal extracts from *Pinus nigra*, *Pinus halepensis*, *Abies equi-trojani*, *Abies bornmulleriana*, *Abies cilicica*, *Abies nordmanniana*, *Cedrus libani* and *Picea orientalis* were examined against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* (Eryilmaz et al., 2015). The authors concluded that all the tested extracts, except *Abies bornmulleriana*, *Cedrus libani* and *Pinus halepensis* showed weak antibacterial activity against the various tested bacteria comparing with the standards.

From previous studies, it is known that spruce bark contains vanillic acid and taxifolin (Tanase et al., 2018). Moon et al., (2006) concluded that vanillic acid accelerated the death of *E. coli*. It was also found that taxifolin have antibacterial action against human pathogens inclusive *E. coli* (Asmi et al., 2017). Thus, the vanillic acid and taxifolin identified in the spruce bark crude extract can have a role in antibacterial activity against *E. coli*. The results of the present investigation suggest that the spruce bark crude extracts can be used as potential leads to discover new antibacterial agents to control *E. coli* bacterial infections.

**Table 1.** Data presenting the growth rate of *E. coli* in presence of EAM and USM ($p<0.0001$)

| **Experimental variants** | **CFU/ml** | | | **Growth rate ($h^{-1}$)** | **Generation time (min)** |
|---|---|---|---|---|---|
| | **H0** | **H1** | **H2** | **r** | **g** |
| EAM | $1.4 \times 10^4$ | $2.8 \times 10^4$ | $3.1 \times 10^7$ | 1.28 | 32.42 |
| Control | $5.6 \times 10^4$ | $5.3 \times 10^6$ | $3.6 \times 10^8$ | 1.46 | 28.47 |
| USM | $2.8 \times 10^4$ | $2.1 \times 10^5$ | 0 | N/A | N/A |
| Control | $1.4 \times 10^4$ | $2.1 \times 10^6$ | $3.3 \times 10^8$ | 1.68 | 24.76 |

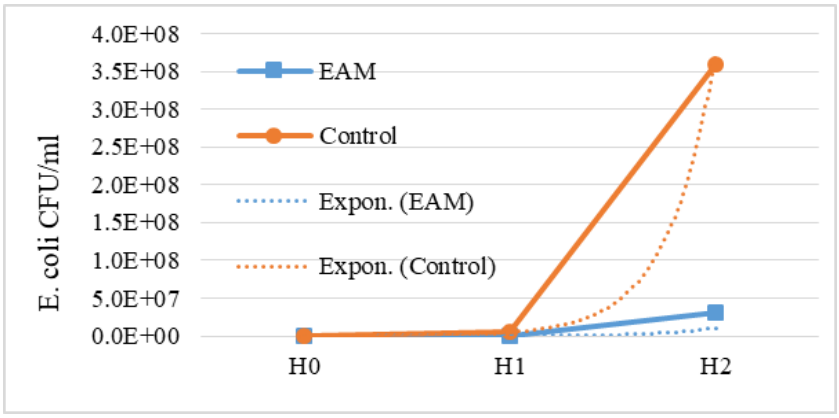


**Fig. 1.** The graphics representation of the growth rate for *Escherichia coli* in the presence of EAM comparing to the Control

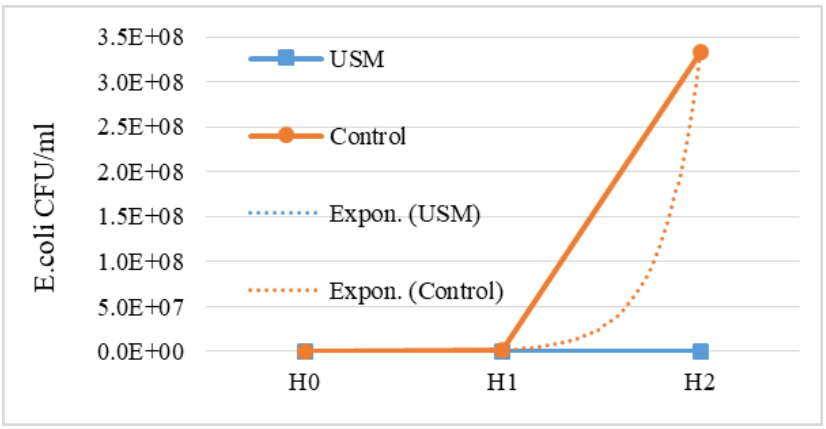


**Fig. 2.** The graphics representation of the growth rate for *Escherichia coli* in the presence of USM comparing to the Control

## Conclusions

The results of this study reveal that the spruce bark extract (obtained by hot water with or without ultrasounds) can be effective against Gram-negative bacteria such as *Escherichia coli*. Antimicrobial activity of this spruce bark extract, may suggest its use in pharmaceutical preparations. By demonstrating antimicrobial capacity of polyphenolic extracts, we can follow a new direction of research, namely reducing the pharmacological resistance of microorganisms to antibiotics, by using polyphenolic extracts.

## 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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**ORIGINAL PAPER**

# THE CHARACTERISTIC MEDICINAL PLANTS OF DIFFERENT VEGETATION TYPES FROM THE NIRAJ VALLEY, ROMANIA

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**Abstract:** In this study the medicinal plants of some representative vegetation types from a human-modified Eastern European landscapes were investigated. The studied territory is part of a Special Protection Area for bird species. The following sampling areas were included in the study: humid grasslands; mountain hay meadows; semi-natural dry grasslands; Sub-pannonic steppic grasslands; fringe communities of mesothermophilic forest edges; grey willow scrubs; thickets of willow; forests of white willow; hornbeam-beech, oak-hornbeam, and sessile oak forests; scrubs of blackthorn and hawthorn; Subcontinental peri-Pannonic scrubs. The ordering of medicinal plants on the basis of presence/absence data and the abundance data presented the grouping in the following typical communities: to the deciduous forests, to the coppices and scrubs, and to the grasslands and meadows. A total of 208 medicinal plants were found from which 37 species are included in the European Pharmacopoeia, and 13 in the Romanian Pharmacopoeia. The existing list of medicinal plants of the Niraj Valley in the scientific literature was completed with 33 taxa. The medicinal plants containing tannins (18.93%) were in higher percentage followed by those with essential oils (10.68%), flavonoids (10.68%), saponins (9.71%), alkaloids (7.77%), mucilages (6.80%), coumarins (5.34%). However rational (sustainable) exploitation of these natural resources is necessary.

**Keywords:** medicinal plants, Niraj River, Jaccard index, Bray-Curtis index, indicator value, traditional medicine, active principles.

## 1. Introduction

The Niraj River springs from the Gurghiu Mountains from an altitude of 1239 m, and it flows into the Mureș near Ungheni (close to the locality Vidrasău). The Niraj is a left tributary of the Mureș River. The natural course of the Niraj River has a length of 79 km. The drainage basin of the Niraj River covers a surface of 625 km$^2$ and hosts 66 localities, therefore the valley is a very dense populated area (Hajdu, 2010). The soils are represented by luvosol, regosol and faeziom, and in the meadow part of valley by aluviosol and hydromorphic soil (Josan, 1979; Florea and Munteanu, 2000, Blaga et al., 2005). The climate is moderate continental and the annual rainfall is 600 mm (Roșu, 1980). The woody vegetation is represented by: oak

(*Quercus petraea, Q. robur*) and hornbeam forests forming the association *Querceto-Carpinetum transsilvanicum*; beech and hornbeam forests forming the association *Fagetum transsilvanicum*; narrow strips of shrubs and coppices along the river with *Salix purpurea, S. triandra, S. caprea, S. fragilis, S. viminalis,* or with *Populus* sp., *Salix* sp. and *Alnus glutinosa*; hedges with *Crataegus monogyna, Prunus spinosa, Rosa canina*.

On the sunny slopes, the herbaceous vegetation is formed by the following species combinations: *Brachypodium pinnatum-Carex humilis, Brahypodium pinnatum-Dorycnium herbaceum, Bromus erectus, Festuca sulcata-Festuca pseudovina, Festuca sulcata-Agrostis tenuis.*

On the shaded slopes heyfields with *Agrostis tenuis, Agrostis tenuis-Festuca rubra, Trissetum flavescens-Festuca rubra, Cynosurus cristatus-Festuca rubra, Poa pratensis-Festuca pratensis, Arrhenatherum elatius* are present. The floodplain herbaceous vegetation is characterized by meso-hydrophilic and hydrophilic meadows with *Agrostis stolonifera, Deschampsia cespitosa, Alopecurus pratensis, Poa trivialis, Festuca pratensis, Carex gracilis, C. acutiformis* (Csűrös, 1963). Studies about the vegetation from the Niraj Valley have been carried out by a few researchers (Oroian and Giurgiu 2003; Kovács 2008; Sămărghiţan and Oroian 2011; Domokos, 2015; Oroian, et al. 2016).

The main goal of the paper was the study of the medicinal plants in some representative plant communities from the Niraj Valley. In order to fulfill the main goal the following aims were proposed: (1) inventory of plants used in traditional medicine and/or in phytotherapy from the studied phytocoenoses; (2) verifying the presence of medicinal plants in the European and Romanian Pharmacopoeia; (3) determination of characteristic medicinal plants for each studied association; (4) classification of herbs according to the dominant active principles in the plant; (5) notation of the plant product (drugs) used in traditional medicine and/or phytotherapy for each species.

## 2. Materials and Methods

The inventory of plants used in traditional medicine and/or in phytotherapy was possible on the base of 175 relevés made in the Niraj Valley. The field trips were conducted in the period 2012-2017. Other 13 relevés made by Kovács (2008) and Sămărghiţan and Oroian (2011), were also used for the study. For the nomenclature of the taxa the work of Sârbu et al. (2013) was used. The determination of characteristic medicinal plants for each of the studied association was made by Principal coordinate analysis (PcoA) and Indicator value analysis (IndVal, Dufrêne and Legendre, 1997). All statistics were made in R (Roberts and Oksanen 2006). The presence of medicinal plants was verified in the European Pharmacopoeia (2018) and Romanian Pharmacopoeia (1993). Information about the part of the plants used in the traditional medicine or phytotherapy, the dominant active principle and harvesting period of the plants was obtained from the work of Csedő (1980), Pârvu (2000), Oroian (2011), Eșianu (2016), Eșianu and Laczkó-Zöld (2016), and Muntean (2016).

## 3. Results and discussions

In the studied plant communities from the Niraj Valley a total of 652 plant taxa were found from which 208 are used in the traditional medicine and/or in phytotherapy. From these medicinal plants 37 species are included in the European Pharmacopoeia, and 13 in the Romanian Pharmacopoeia. The ordering of medicinal plants on the base of the abundance-dominance data (Bray-Curtis index, PC1: 15.06%, PC2: 6.88%) presented three

typical medicinal plant communities in the Niraj Valley: medicinal plants of the deciduous forests, medicinal plants of the coppices and scrubs, and medicinal plants of the grasslands (**Fig. 1**). The ordering of medicinal plants on the basis of the presence/absence data (Jaccard index, PC1: 14.07%, PC2: 6.15%) confirms that these are grouped in the three already mentioned communities. The similitude between the medicinal plants typical to coppices and scrubs and those of grassland increase when presence/absence data are taken into consideration (**Fig. 2**). The Indicator value analysis (IndVal) shows the fidelity and specificity of medicinal plants to the studied plant communities: thickets of willow (*Salicetum triandrae* Malcuit 1929); forests of white willow (*Salici-Populetum* Meijer-Drees 1936); Subcontinental peri-Pannonic scrubs (*Prunetum tenellae* Soó 1951); scrubs of blackthorn and hawthorn (*Pruno spinosae-Crataegetum* Soó 1931); grey willow scrubs (*Frangulo-Salicetum cinereae* Graebner *et* Hueck 1931); hornbeam-beech (*Carpino-Fagetum* Paucă 1941), oak-hornbeam (*Carpino-Quercetum petraeae* Borza 1941), and sessile oak (*Melico uniflorae-Quercetum petraeae* Gergely 1962) forests; semi-natural dry grasslands (*Carici humilis-Brachypodietum pinnati* Soó ex Pop *et al.* 2001); Sub-pannonic steppic grasslands (*Agrostio-Festucetum valesiacae* Borisavljevič *et al.* 1955; *Festuco sulcatae-Caricetum humilis praerossicum* Soó 1947); fringe communities of mesothermophilic forest edges (*Inulo ensifoliae-Peucedanetum cervariae* Kozl. 1925 em. Gils *et* Kovács 1977); humid grasslands (*Agrostio stoloniferae-Deschampsietum caespitosae* Újvárosi 1947); mountain hay meadows (*Poo-Trisetetum flavescentis* Knapp 1951 em. Oberd. 1983, *Festuco-Agrostetum capillaris* Horv. 1951).

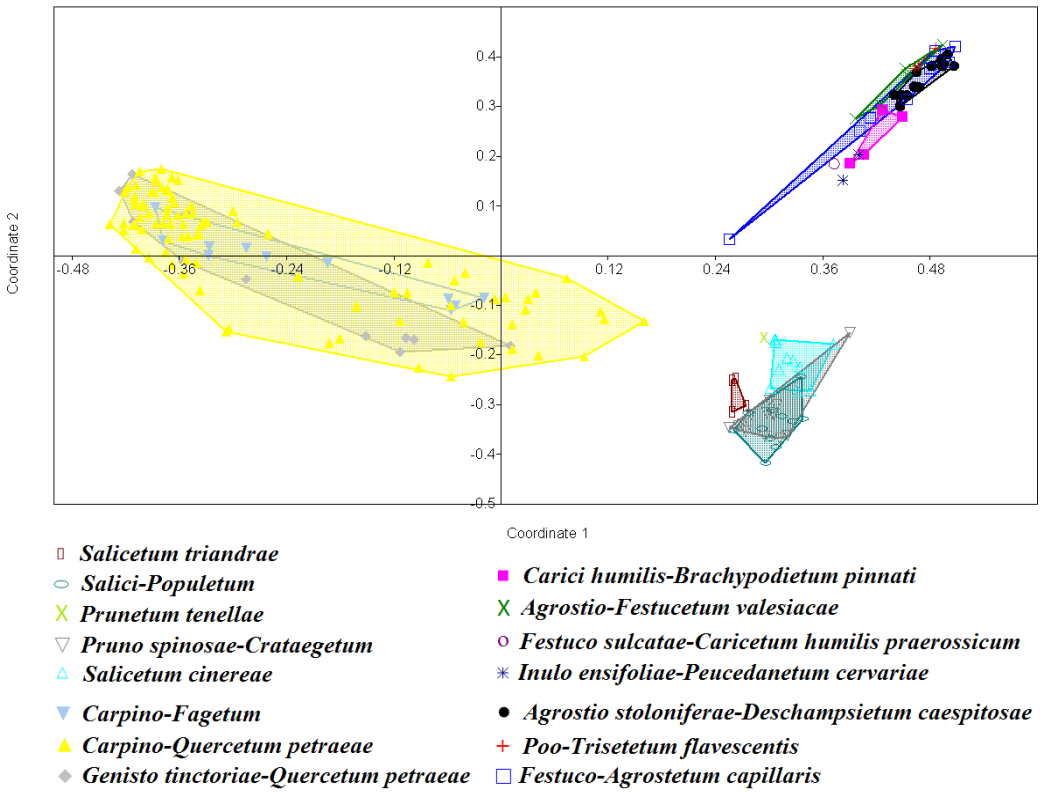


**Fig. 1.** Principal coordinate analysis (PcoA) of the medicinal plant communities from the Niraj Valley based on the Bray-Curtis index

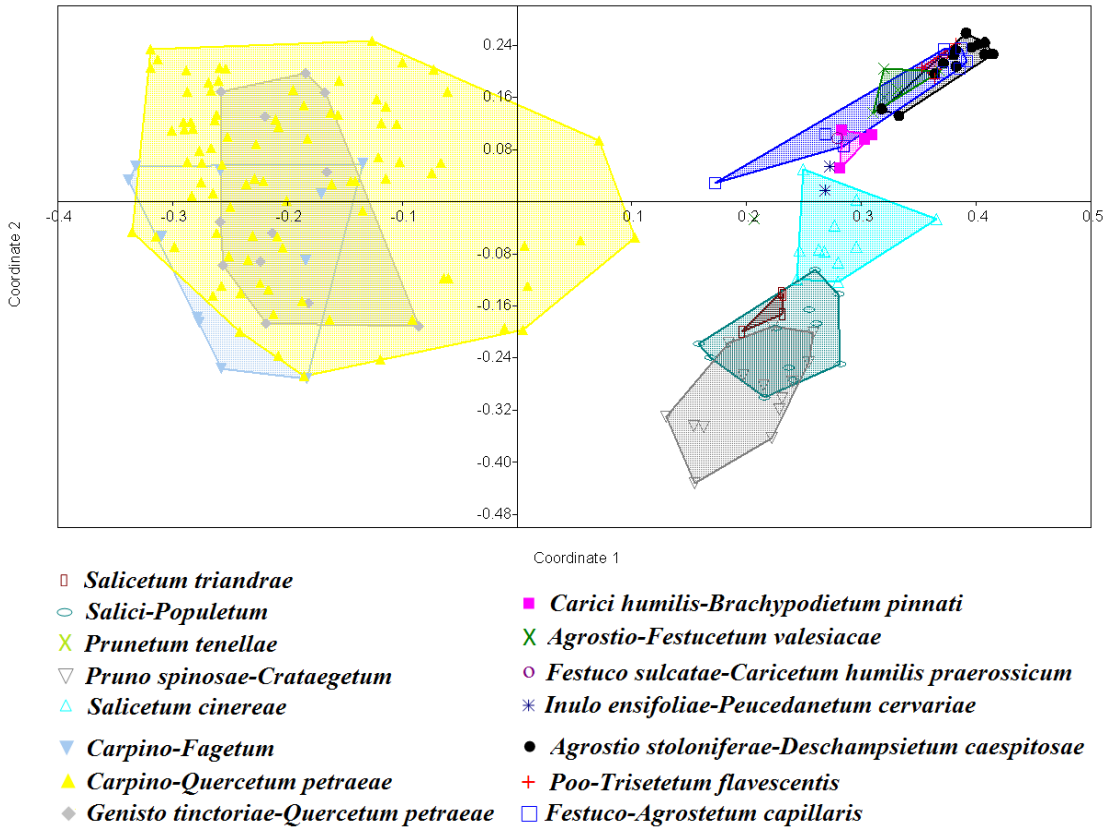


**Fig. 2.** Principal coordinate analysis (PcoA) of the medicinal plant communities from the Niraj Valley based on the Jaccard index

The indicator species value was calculated for identifying the characteristic medicinal plants of the different associations. The defined characteristic medicinal plants are those that are exclusively or almost exclusively in an association and have a high frequency and abundance-dominance value. The medicinal plants with a significant indicator value of the different plant associations are presented in **Table 1**.

According to our results the medicinal plant communities of the deciduous forests from the Niraj Valley are not differentiated by the type of forest. The associations *Carici humilis-Brachypodietum pinnati* and *Inulo ensifoliae-Peucedanetum cervariae* had the highest number of characteristic species (**Table 1**). The plant species with use in the traditional medicine and/or phytotherapy found in the studied associations from the Niraj Valley, their drugs, dominant active principles and harvesting periods are included in the Supplementary Material 1 (**Table 2**).

The medicinal plants containing tannins as the dominant active principle were in higher percentage (18.93%) followed by those with essential oils (10.68%), flavonoids (10.68%), saponins (9.71%), alkaloids (7.77%), mucilages (6.80%), and coumarins (5.34%) (**Fig. 2**).

**Table 1.** Plants used in traditional medicine and/or phytotherapy with significant indicator value from each studied association from the Niraj Valley

| | | | | |
|---|---|---|---|---|
| ***Salicetum triandrae*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Salix triandra* | Cortex | 0.97 | 0.001 |
| 2. | *Salix viminalis* | Cortex | 0.96 | 0.003 |
| 3. | *Tanacetum vulgare* | Flos | 0.92 | 0.009 |
| 4. | *Artemisia vulgaris* | Herba | 0.69 | 0.024 |
| 5. | *Rubus caesius* | Folium | 0.66 | 0.035 |
| ***Salici-Populetum*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Populus nigra* | Gemma | 0.91 | 0.005 |
| 2. | *Alnus glutinosa* | Cortex, folium, gemma | 0.89 | 0.004 |
| 3. | *Salix alba* | Cortex | 0.85 | 0.006 |
| 4. | *Sambucus nigra* | Flos | 0.68 | 0.021 |
| 5. | *Humulus lupulus* | Strobuli, glandulae | 0.61 | 0.036 |
| 6. | *Salix fragilis* | Cortex | 0.61 | 0.034 |
| 7. | *Saponaria officinalis* | Radix | 0.45 | 0.042 |
| ***Prunetum tenellae*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Prunus tenella* | Folium, semen | 1 | 0.01 |
| 2. | *Asparagus officinalis* | Rhizoma, radix | 0.96 | 0.013 |
| 3. | *Thalictrum minus* | Herba | 0.93 | 0.015 |
| 4. | *Potentilla recta* ssp. *recta* | Rhizoma | 0.67 | 0.019 |
| 5. | *Rosa canina* | Fructus | 0.6 | 0.028 |
| ***Pruno spinosae-Crataegetum*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Crataegus monogyna* | Folium, flos, fructus | 0.98 | 0.001 |
| 2. | *Prunus spinosa* | Flos, fructus | 0.61 | 0.04 |
| 3. | *Cornus sanguinea* | Cortex | 0.45 | 0.048 |
| 4. | *Pyrus pyraster* | Folium | 0.22 | 0.038 |
| ***Salicetum cinereae*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Salix cinerea* | Cortex | 1 | 0.001 |
| 2. | *Frangula alnus* | Cortex | 0.63 | 0.026 |
| 3. | *Rubus caesius* | Folium | 0.55 | 0.033 |
| 4. | *Lysimachia vulgaris* | Herba | 0.36 | 0.037 |
| 5. | *Iris sibirica* | Rhizoma | 0.27 | 0.043 |
| 6. | *Solanum dulcamara* | Stipes | 0.18 | 0.047 |
| ***Carpino-Fagetum* Paucă 1941, *Carpino-Quercetum petraeae* Borza 1941, *Melico uniflorae-Quercetum petraeae* Gergely 1962** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Fagus sylvatica* | Creosotum | 0.94 | 0.001 |
| 2. | *Quercus petraea* | Cortex | 0.71 | 0.033 |
| 3. | *Asarum europaeum* | Rhizoma | 0.67 | 0.038 |

| | | | | |
|---|---|---|---|---|
| 4. | *Convallaria majalis* | Herba | 0.62 | 0.035 |
| 5. | *Ajuga reptans* | Herba, folium, flos | 0.49 | 0.042 |
| 6. | *Galeobdolon luteum* | Herba | 0.46 | 0.011 |
| 7. | *Tilia cordata* | Flos | 0.42 | 0.001 |
| 8. | *Pulmonaria officinalis* | Folium | 0.36 | 0.026 |
| 9. | *Vinca minor* | Herba | 0.35 | 0.032 |
| ***Carici humilis-Brachypodietum pinnati*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Peucedanum oreoselinum* | Rhizoma | 0.75 | 0.019 |
| 2. | *Solidago virgaurea* ssp. *virgaurea* | Summitates | 0.75 | 0.038 |
| 3. | *Euphrasia rostkoviana* ssp. *rostkoviana* | Herba | 0.75 | 0.023 |
| 4. | *Elymus repens* | Rhizoma | 0.68 | 0.034 |
| 5. | *Hypericum perforatum* | Herba | 0.63 | 0.021 |
| 6. | *Anthyllis vulneraria* ssp. *vulneraria* | Herba, flos | 0.5 | 0.022 |
| 7. | *Polygala comosa* | Herba | 0.5 | 0.022 |
| 8. | *Heracleum sphondylium* | Radix, herba | 0.5 | 0.02 |
| 9. | *Linum catharticum* | Semen | 0.5 | 0.014 |
| ***Agrostio-Festucetum valesiacae*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Achillea millefolium* var. *collina* | Herba | 0.4 | 0.041 |
| ***Festuco sulcatae-Caricetum humilis praerossicum*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Stachys germanica* | Herba | 1 | 0.018 |
| 2. | *Trifolium campestre* | Herba | 1 | 0.01 |
| 3. | *Calluna vulgaris* | Herba | 1 | 0.011 |
| 4. | *Orchis morio* | Tuber | 0.7 | 0.048 |
| 5. | *Senecio jacobaea* | Herba | 0.67 | 0.021 |
| 6. | *Helianthemum nummularium* | Folium | 0.67 | 0.022 |
| 7. | *Teucrium chamaedrys* | Herba | 0.58 | 0.041 |
| ***Inulo ensifoliae-Peucedanetum cervariae*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Genista sagittalis* | Herba | 1 | 0.001 |
| 2. | *Nepeta nuda* | Semen | 0.8 | 0.024 |
| 3. | *Vincetoxicum hirundinaria* ssp. *hirundinaria* | Radix | 0.77 | 0.005 |
| 4. | *Origanum vulgare* ssp. *vulgare* | Herba | 0.71 | 0.02 |
| 5. | *Rosa gallica* | Petalum | 0.5 | 0.027 |
| 6. | *Veronica teucrium* ssp. *teucrium* | Herba | 0.5 | 0.029 |
| 7. | *Veronica orchidea* | Herba | 0.5 | 0.031 |
| 8. | *Digitalis grandiflora* | Folium | 0.5 | 0.02 |
| 9. | *Melilotus officinalis* | Herba, flos | 0.38 | 0.049 |
| ***Agrostio stoloniferae-Deschampsietum caespitosae*** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Sanguisorba officinalis* | Herba | 0.91 | 0.001 |
| 2. | *Filipendula ulmaria* | Herba | 0.68 | 0.036 |

| | | | | |
|---|---|---|---|---|
| 3. | *Polygonum bistorta* | Rhizoma | 0.59 | 0.02 |
| 4. | *Oenanthe aquatica* | Fructus | 0.48 | 0.045 |
| ***Poo-Trisetetum flavescentis* Knapp 1951 em. Oberd. 1983, *Festuco-Agrostetum capillaris* Horv. 1951** | | | | |
| | **Plant taxa** | **Drugs** | **IndVal [a]** | ***p* Value [b]** |
| 1. | *Convolvulus arvensis* | Herba | 0.67 | 0.024 |
| 2. | *Potentilla argentea* ssp. *argentea* | Rhizoma | 0.33 | 0.047 |

Note: a - Indicator value analysis; b - significance criterion

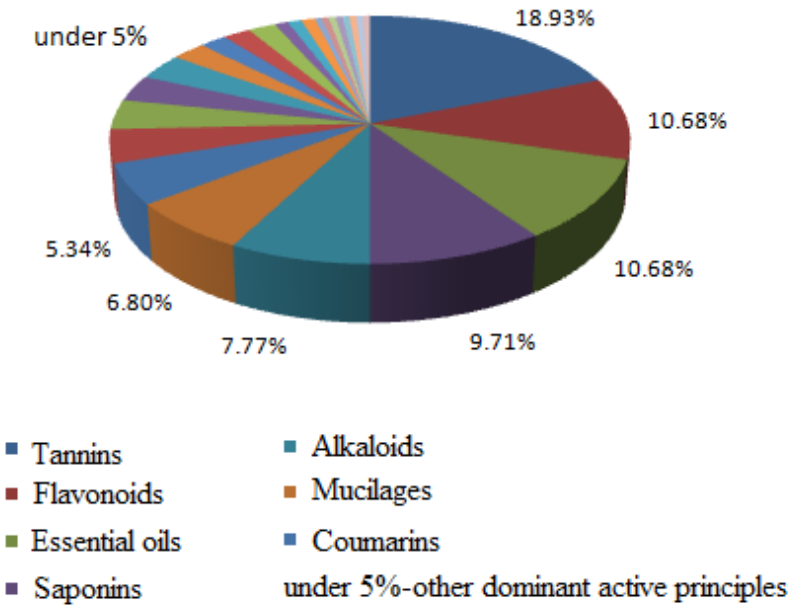


**Fig. 3.** Spectrum of the dominant active principles present in the medicinal plants of some representative plant communities of the Niraj Valley

## Conclusions

The existing list of medicinal plants from the Niraj Valley in the scientific literature (Oroian and Giurgiu, 2003) was completed with 33 new taxa. From the 208 taxa, 37 plants are included in the European Pharmacopoeia (from which 4 taxa are reported for the first time in the Niraj Valley) and 13 in the Romanian Pharmacopoeia. The studied habitats provide shelter for 18 endangered, rare and vulnerable plant taxa, some of which are also medicinal plants (e.g. *Narcissus poeticus* ssp. *radiiflorus*, *Achillea ptarmica*, *Orchis laxiflora* ssp. *elegans*, *Orchis militaris*, *Orchis morio* ssp. *morio*, *Prunus tenella*, and *Iris sibirica*). However rational (sustainable) exploitation of these natural resources is necessary.

## 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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**ORIGINAL PAPER**

# COMPARATIVE PHYTOCHEMICAL RESEARCH OF *SOLIDAGO* GENUS: *S. GRAMINIFOLIA*. NOTE I. FLAVONOIDS

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**Abstract:** *Solidago graminifolia* L. Salisb. is one of the latest species appeared in Romania. Due to the interest for the *Solidago* species as medicinal plants, we researched its phytochemical composition in comparison with the other three species present in Romania: *Solidago virgaurea* L., *Solidago canadensis* L., *Solidago gigantea* Aiton. Starting from the chemotaxonomic value of flavonoids, and their valuable biological properties, we wanted to analyze these substances from *S. graminifolia* compared to other *Solidago* species in Romanian flora. The studied species have a high content of flavonoids (3.44-5.21%). The flavonoid substances identified in the indigenous species of *Solidago* have a high chemotaxonomic value, each species having a charateristic chromatographic profile, therefore their analysis is useful in the case of adulterations. The qualitative analysis of flavonoids was performed by TLC and HPLC-MS, mean while the quantitative determination was achieved by spectrophotometric method and individual fractions separated by HPLC-MS.

**Keywords:** *Solidago virgaurea* L., *Solidago canadensis* L., *Solidago gigantea* Aiton, *Solidago graminifolia* L. Salisb., flavonoids.

## 1. Introduction

In Romania Flora, vol. IX (1964), there are mentioned only three *Solidago* species (Asteraceae family), one from the spontaneous flora (*S. virgaurea* L., Golden rod) and two adventitious species, initially cultivated as ornamental plants, originating from North America, then escaped as subspontaneous: *S. canadensis* L. and *S. gigantea* Aiton. (Early Golden rod) (**Fig. 1**). Flora Europaea (Tutin et al., 2010) presents five species of *Solidago*, four of which are found in the Romania flora. Concerning *S. altissima* L., it is not recognized as a distinct species but only as a variety of *S. canadensis* (*S canadensis* var. *scabra* Torrey & A. Gray) and therefore Sârbu et al. (2013) mentioned it in the Observation section.

In 1975, Negrean (Sârbu et al., 2011) reported for the first time in the Flora of

Romania the fourth species of *Solidago*, *S. graminifolia* (L.) Salisb. (syn. *Euthamia graminifolia* (L.) Nutt.) in the area of Maramureș county, a species originating also from North America which was introduced in Europe as an ornamental plant, then become adventitious, but with a lower colonization rate than the other two adventitious species mentioned before. *S. graminifolia* can be distinguished by the marginal flowers of the flower-heads with short ligule (0.8-1 mm), the linear lanceolate leaves which are scabrous (rough) on the edges, and numerous flower-heads arranged in corymbose panicles (Tutin et al., 2010; Sârbu et al., 2013).

In 2012, a second area in the country was reported for *S. graminifolia* in Cluj county (place Ciucea, leg. Tămaș M.) (**Fig. 2**).

The interest for the *Solidago* species as medicinal plants (Ciulei et al., 1993; Grigorescu et al., 2001) led us to carry out phytochemical research studies also on this last species appeared in the Romanian flora in comparison with the other three species. Starting from the chemotaxonomic value of flavonoids (Tămaș, 1986), and their valuable biological properties, we wanted to analyze these substances from *S. graminifolia* compared to other *Solidago* species in Romanian flora. In addition to the flavonoid substances from the *Solidago* species, we have also studied triterpenoid saponins (Tămaș and Roșca, 1988), phenolic compounds (Dobjanschi et al., 2005) and volatile oils (Dobjanschi, 2006). A botanical and chemical complex study was conducted on these species by Dobjanschi (2006) and a pharmacological study by Voștinaru (2007). There have also been performed researches focused on the diuretic action (Tămaș and Toader, 1989), anti-inflammatory action (Pîrvu et al., 2000) and hypotensive action (Rácz-Kotilla et al., 1977). In the European Pharmacopoeia 9.0 (9.4-2018) it is mentioned in the monograph of *Solidaginis virgaureae herba* a flavonoid content of 0.5-1.5 % expressed in hyperoside, and for highlighting the substitutions with *S. canadensis* and *S. gigantea*, it is mentioned thin layer chromatography for flavonoids determination where should not be present the orange fluorescence band characteristic for quercitroside (Ph.Eu.9.0). In Ph.Eu.9.0 is also official the monograph *Solidaginis herba*, which presents the blooming aerial parts of *S. gigantea* and *S. canadensis* for which a minimum content of 2.5 % of flavonoids expressed in the hyperoside is envisaged.

**Fig. 2.** *Solidago graminifolia* L. Salisb. (original photo made in Ciucea, Cluj county, 2016)

Among the flavonoid substances in the *Solidago* species, there have been mentioned glycosides of quercetol and kaempferol, including rutoside, hyperoside, quercitroside, isoquercitroside, astragaloside, isorhamnetin, nicotiflorina (Bisset and Wichtl, 1994), and the pharmacological properties include diuretic and saluretic action, anti-inflammatory, kidney stone lysis, spasmolytic and disinfectant of urinary tracts (Weiss and Fintelmann, 2000).

In our country, *Virgaureae herba* is used in phytotherapy and is included in the Plafar Nomenclature (1990). It is also official in the European Pharmacopoeia 6.0 (2007), and the *Solidago virgaurea* extracts are present in the composition of some pharmaceutical products such as: Rhoival (pulv.), Prostaforton (tablets), Uralyt (tablets, caps.), Cystinol (sol.), Nieron (caps.), and others (Rote Liste, 1988). There are few phytochemical and pharmacological data recorded on *S. graminifolia.* Thus, Kalemba et al. (1994) analyzed the extracted volatile oil and identified 80 components, the main ones being β-felandren (23%), sabinen (18%) and β-pinen (10%). Derda et al. (2008) tested some plant extracts, including *S. graminifolia* for its amoebicide activity.

## 2. Materials and Methods

### 2.1. Materials

The plant material (herba) was harvested at the beginning of August 2016 during flowering from Ciucea (Cluj county) from a ruderal area on the bank of a tributary river (Surduca) of Crişul Repede river (Cluj County), dried in the shade and then ground to a fine powder (IV sieve, Romanian Pharmacopoeia 10$^{th}$ Edition-FR X). A voucher specimen (no.143.3.1.1) of *S. graminifolia* was deposited in the Pharmaceutical Botany Discipline, Faculty of Pharmacy, University of Medicine and Pharmacy Iuliu Haţieganu Cluj-Napoca.

### 2.2. Extraction method

A 2 % extract in methyl alcohol was prepared for the chemical analysis. Thus, to 1 g of vegetable powder is added 50 ml of methyl alcohol and the mixture is kept in boiling in the water bath for 30 minutes in a reflux condensed flask. After cooling, it is filtered and then methyl alcohol is added in a 50 ml volumetric flask.

### 2.3. The quantitative determination of total flavonoids

It was performed according to the spectrophotometric technique indicated by FR X for *Cynarae folium* monograph and the expression of rutoside content (g %) by using a calibration curve for this substance.

### 2.4. The qualitative analysis of flavonoids

It was performed by thin layer chromatography (TLC) using silica gel G plates (Merck) with a layer of 0.25 mm, mobile phase consisting in formic acid-water-ethyl acetate (6:9:90), reference substances (Karl Roth GmbH Karlsrue, Germany) such as: rutoside, hyperoside, quercitroside, isoquercitroside, chlorogenic acid and caffeic acid, 0.1 % solutions in methyl alcohol, revealed with NEU-PEG reagents under UV light 365 nm (Jork et al., 1990).

### 2.5. High performance liquid chromatography (HPLC)

It was employed for both qualitative and quantitative analysis for the flavonoid fractions which were separated and identified by the technique indicated by Fodorea and Vlase (2005). The separated fractions were identified by using reference substances (Karl Roth GmbH Karlsrue, Germany) and confirmed by mass spectrum assays (MS). An HPLC apparatus coupled with an HP 1100 mass spectrometer with binary pump series, HP 1100

autosempler, HP 1100 thermostat, HP 1100 UV detector, and an Agilent Ion Trap 1100 VL mass spectrometer were used.

## 3. Results and discussions

### 3.1. The quantitative determination of total flavonoids

The total flavonoid content determined by spectrophotometric method and expressed as rutoside (g %) was 4.06 % for *S. virgaurea*, 5.21 % for *S. canadensis*, 5.20 % for *S. gigantea* and 3.44 % for *S. graminifolia*. It results that the analyzed *Solidago* species are rich in flavonoid substances, the higher content of total flavonoids in two species, more than 5%, can be correlated with their higher proportion of flowers (inflorescences) compared to the lower number of flowers in the herba product of *S. graminifolia* and *S. virgaurea* species.

### 3.2. The qualitative analysis of flavonids

From the TLC analysis (**Fig. 3**) it results that the richest species in flavonoid fractions are *S. gigantea* and *S. canadensis* in which 4-5 fractions with yellow fluorescence, one with green fluorescence and four with blue fluorescence were highlighted. It can also be noticed that each *Solidago* species has a characteristic chromatographic profile. Thus, *S. graminifolia* is the only species that does not contain rutoside (Rf 0.18, **Fig. 3**-1), being present in all the other three analyzed species. *S. virgaurea* does not have quercitroside (Rf 0.65, **Fig. 3**-2), *S. canadensis* is the only one containing high concentration of isoquercitroside (Rf 0.55). Hyperoside (Rf 0.47, **Fig. 3**-3), caffeic acid (Rf 0.85) and chlorogenic acid (Rf 0.40, **Fig. 3**-4) are common substances for all analyzed species.

Therefore, the TLC analysis for flavonoids of *Solidago* species can be used for their chemical characterization and as identification criteria for substitutions between species even when referring to their extracts. Thus, for *S. graminifolia* it is characteristic the presence of hyperoside and quercitroside and the absence of rutoside, and for *S. virgaurea* the presence of rutoside and hyperoside, but also the absence of quercitroside, meanwhile for *S. canadensis*, rutoside, hyperoside and quercitroside are present, and isoqueritroside occurs in significant quantities only in *S. gigantea*.

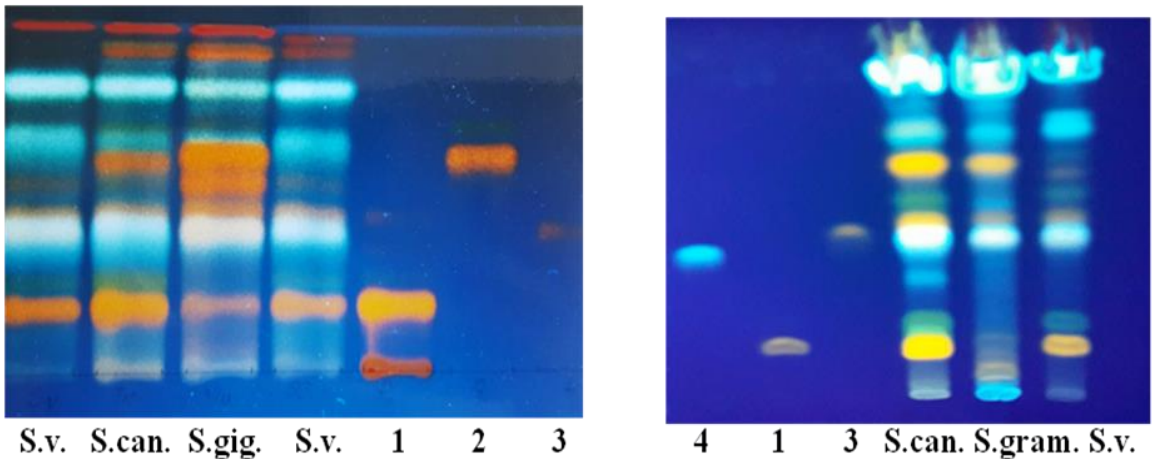


**Fig. 3.** TLC chromatograms of *Solidago* species (S.v. = *S. virgaurea*; S.can. = *S. canadensis*; S.gig. = *S. gigantea*; S.gram. = *S. graminifolia*; 1 = rutoside; 2 = quercitroside; 3 = hyperoside; 4 = chlorogenic acid)

### 3.3. High performance liquid chromatography

From the HPLC-MS analysis obtained for *S. graminifolia* and compared to those obtained for the other species (**Figures 4-7**), it can be noticed that both the content of the separated flavonoid fractions and their number is lower for this species (**Tables 1-4**). Thus, rutoside is absent only in *S. graminifolia*, being present in all three other analyzed species, the highest rutoside content being present in *S. canadensis*. Another flavonoid fraction, quercitroside, is absent in *S. virgaurea*, but it is present in the other species which is a differentiation and evidence of the substitutions for *S. virgaurea*. Hyperoside is present in all analyzed species but in variable amounts, as well as isoquercitroside, the latter being present in the highest concentration in *S. gigantea*, and the free aglycons quercetol and kaempferol are present only in traces. In addition to the HPLC-MS quantification of each fraction separated by this technique, the sensitivity of this method is higher, so that some fractions that could not be detected by TLC appeared in HPLC-MS chromatograms, of course, in small amounts.

By the presence of quercitroside in *S. graminifolia*, this species appears to be closer to *S. canadensis* and *S. gigantea*.

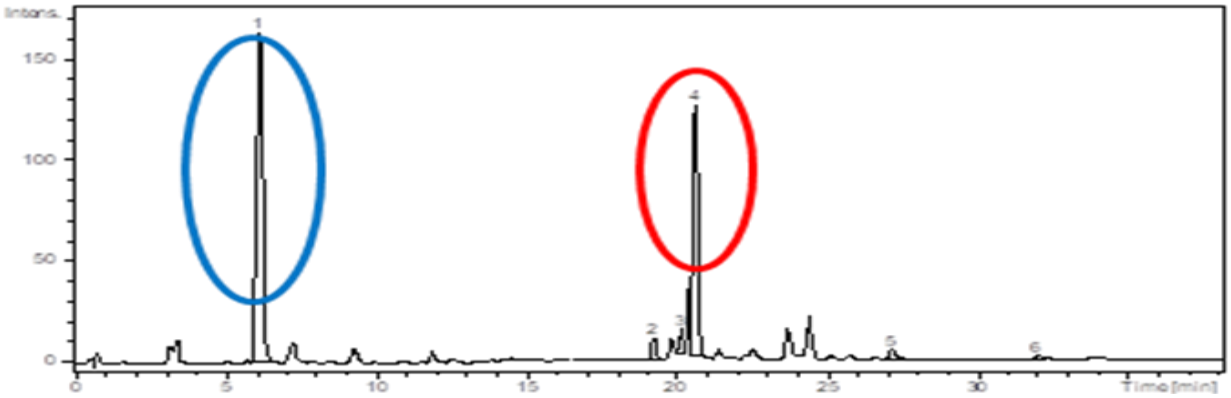


**Fig. 4.** HPLC chromatogram obtained for *Solidago virgaurea* extract

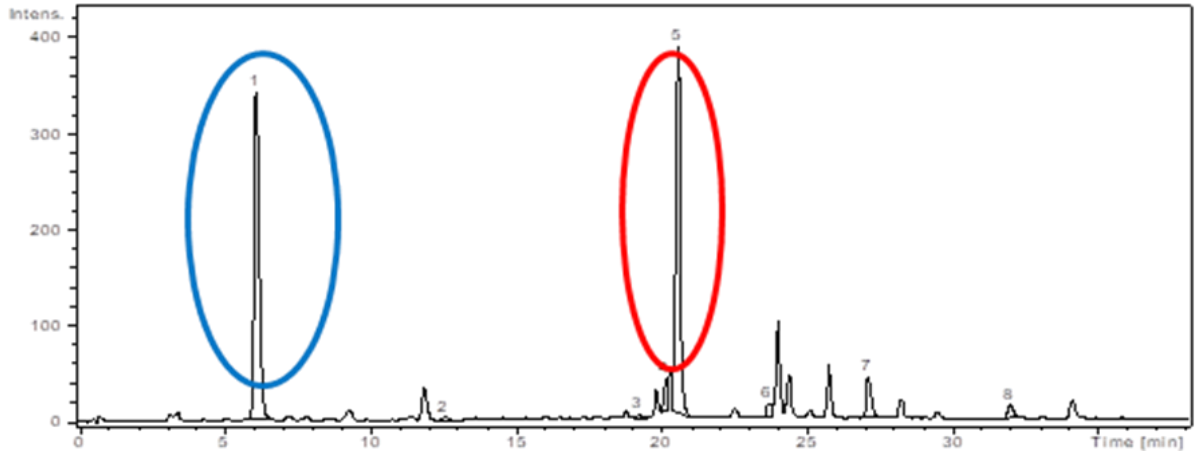


**Fig. 5.** HPLC chromatogram obtained for *Solidago canadensis* extract

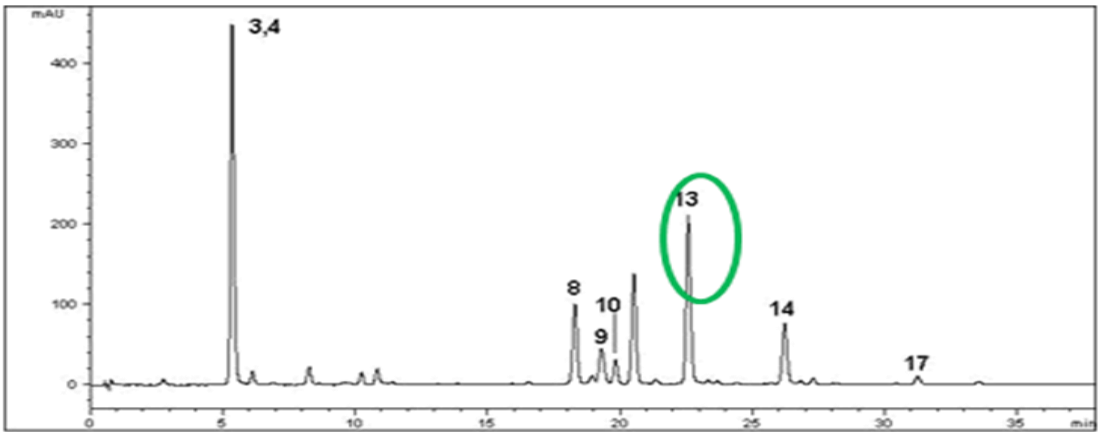


**Fig. 6.** HPLC chromatogram obtained for *Solidago gigantea* extract

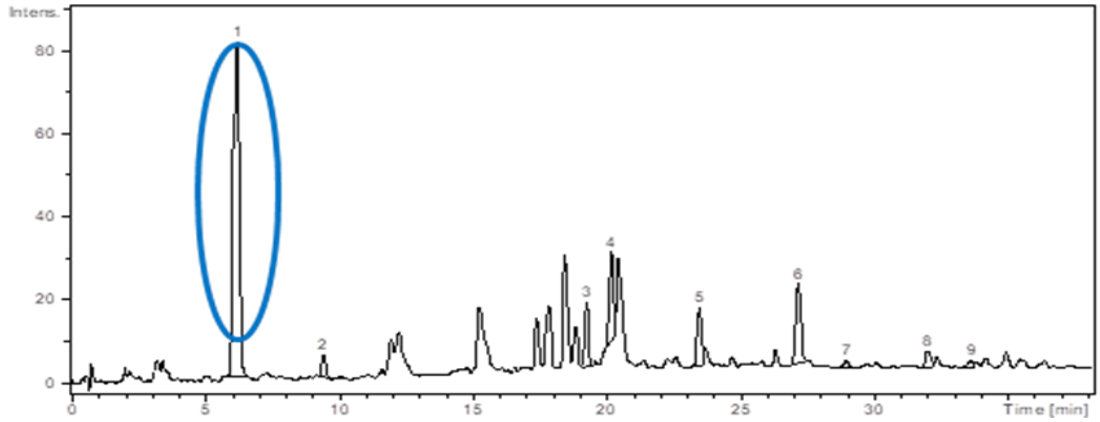


**Fig. 7.** HPLC chromatogram obtained for *Solidago graminifolia* extract

**Table 1.** HPLC-MS results obtained from *Solidago virgaurea* extract

| Number on chromatogram | Compound | UV identification | MS qualitative identification | Quantity (µg/pharmaceutical product) |
|---|---|---|---|---|
| 2 | Hyperoside | YES | YES | 11.288 |
| 3 | Isoquercitrin | YES | YES | 16.994 |
| 4 | Rutoside | YES | YES | 200.521 |
| | Quercitrin | NO | YES | - |
| 5 | Quercetol | YES | YES | 3.587 |
| 6 | Kaempferol | YES | YES | 1.212 |

**Table 2.** HPLC-MS results obtained from *Solidago canadensis* extract

| Number on chromatogram | Compound | UV Identification | MS qualitative identification | Quantity (µg/pharmaceutical product) |
|---|---|---|---|---|
| 3 | Hyperoside | YES | YES | 5.379 |
| 4 | Isoquercitrin | YES | YES | 47.507 |
| 5 | Rutoside | YES | YES | 694.113 |
| 6 | Quercitrin | YES | YES | 40.374 |
| 7 | Quercetol | YES | YES | 32.106 |
| 8 | Kaempferol | YES | YES | 12.554 |

**Table 3.** HPLC-MS results obtained from *Solidago gigantea* extract

| Number on chromatogram | Compound | UV identification | MS qualitative identification | Quantity (µg/pharmaceutical product) |
|---|---|---|---|---|
| 8 | Hyperoside | YES | YES | 120.000 |
| 9 | Isoquercitrin | YES | YES | 82.490 |
| 10 | Rutoside | YES | YES | 45.540 |
| 13 | Quercitrin | YES | YES | 450.560 |
| 17 | Kaempferol | YES | YES | 8.520 |

**Table 4.** HPLC-MS results obtained from *Solidago graminifolia* extract

| Number on chromatogram | Compound | UV identification | MS qualitative identification | Quantity (µg/pharmaceutical product) |
|---|---|---|---|---|
| 3 | Hyperoside | YES | YES | 17.507 |
| 4 | Isoquercitrin | YES | YES | 33.946 |
| | Rutoside | NO | YES | - |
| 5 | Quercitrin | YES | YES | 31.587 |
| 6 | Quercetol | YES | YES | 14.323 |
| 8 | Kaempferol | YES | YES | 4.130 |
| 9 | Apigenin | YES | YES | 2.442 |

## Conclusions

For the first time, a qualitative and quantitative study of flavonoid substances from a new *Solidago* species, recently reported in Romania flora, *S. graminifolia*, was carried out, compared to the other three *Solidago* species from Romania flora.

In *S. graminifolia* species, both the number of flavonoid fractions identified by TLC and HPLC methods, and the content of these substances are lower compared to the other three analyzed species. Through its chromatographic profile, *S. graminifolia* appears closer to *S. canadensis* and *S. gigantea*.

From the qualitative analysis of the four *Solidago* species, we found that rutoside is absent in the case of *S. graminifolia* and quercitroside is absent in *S. virgaurea*.

The qualitative analyzes of the flavonoid substances from *Solidago* species are useful for identifying the possible substitutions between species, the chromatographic profile being a characteristic for each species.

## 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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**ORIGINAL PAPER**

# TOXICITY ASSESSMENT OF *NEPHROLEPIS EXALTATA* (L.) SCHOTT, FAM. NEPHROLEPIDACEAE

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**Abstract:** The fern *Nephrolepis exaltata* (L.) Schott, fam. Nephrolepidaceae, has little traditional medicinal use. In order to estimate its potential safety, in the present study we have investigated the phytotoxicity (on *Triticum aestivum* L.) and toxicity on brine shrimp of extracts from plants grown hydroponically. The species identity was confirmed by macroscopic and microscopic examinations on rhizomes, rachises, pinnae and runners, using bright field and fluorescent microscopy. Biological assays were performed on aqueous and ethanol solutions of the fronds. The brine shrimp lethality assay was performed on *Artemia franciscana* Kellog and a phytobiological bioassay on *Triticum aestivum* L. Lethality, root elongation and karyokinetic film modifications were evaluated, and $LC_{50}$ and $IC_{50}$ values were calculated. The microscopic analysis revealed the main histo-anatomic elements: polystelic structure and hypodermis (rhizome, rachis, runners), trichomes (rachis), homogenous structure, trichomes and diacytic/anisocytic stomata (leaves). The ethanol and aqueous extracts showed low cytotoxic effects on both *Triticum aestivum* roots and *Artemia franciscana* nauplii.

**Keywords:** *Nephrolepis exaltata*, microscopy, *Triticum* bioassay, *Artemia* bioassay, hydroponics.

## 1. Introduction

The fern *Nephrolepis exaltata* (L.) Schott, Nephrolepidaceae, has been studied for its soil phytoremediation properties (Sultana et al., 2014), the effects of its volatile oil (El-Tantawy et al., 2016), its possible hormonal and cytotoxic effects on human cancer cells (Bobach et al., 2014), and its air purifying capabilities (Wolverton and Wolverton, 1993).

The genus name comes from the Greek words *nephros* meaning a kidney and *lepis* meaning a scale, in reference to its kidney-shaped indusia. The specific epithet (*exaltata*) means very tall or lofty.

The species, *Nephrolepis exaltata* (L.) Schott, is a perennial plant, terrestrial or epiphytic in its native state (both forests and open habitats) (Nauman, 1993), mostly found in the tropics as it prefers relatively high temperatures and high humidity, but no direct sunlight. Originally it is from the south of the USA, Central and South America, but it has become naturalized in different parts of the

world such as the Canary Islands, Africa, Asia, India, Polynesia and New Zealand (Large and Farrington, 2016). Its fronds are alternatively pinnate with deltate-oblong, falcate to different degrees and minutely serrated pinnae, with different degrees of inequality, a truncated to auriculated base, having deltate or acute, acroscopic lobes (Nauman, 1993; Hovenkamp and Miyamoto, 2005). Its indusia are kidney to U-shaped attached at a sinus, described as "narrow or broad" by Nauman (1993), although in a more recent revision it is claimed that typically the species has a "wide" sinus (Hovenkamp and Miyamoto 2005). It propagates usually asexually through long thin and green runners (but also through spores) and is a common houseplant commercially found in florist shops. It closely resembles *Nephrolepis cordifolia* (L.) C. Presl, but presents no tubers (Nauman, 1993; Hovenkamp and Miyamoto, 2005) and its fronds are not erect, but arching. Because of its sword-like fronds it received the name sword fern (Langeland, 2001).

The phytochemistry of *Nephrolepis exaltata* (L.) Schott has previously been investigated qualitatively only, and the following chemical constituents have been found: saponins, flavonoids, tannins and reducing sugars (Oloyede et al., 2014).

The species is traditionally used in the island of Fiji to treat women's menstrual disorders (Cambie and Ash, 1994).

The more studied species, *Nephrolepis cordifolia* (L.) C. Presl, has the following folkloric uses: in India it is used to stop the bleeding of wounds, as a treatment for coughs, for stomach and intestinal disorders (Singh and Upadhyay, 2014).

The plants in this study were cultivated in a hydroponic deep water culture system as opposed to regular soil. Hydroponics is a method of growing plants using no soil and a nutrient solution that contains all the macro and micro-elements needed for the plants to grow. Deep water culture is one of the simplest methods of cultivating plants in a pure hydroponic medium, as the plants have their roots permanently submerged in an aerated solution which provides dissolved oxygen to the roots. This hydroponic setup has the advantages of eliminating the possible diseases related to soil pathogens, the mineral composition and the pH of the solution can be fully controlled in order to better suit the plants nutrient needs, thus eliminating potential nutrient deficiencies. Also, there are no water pumps that can become clogged in time. Plants usually grow faster in hydroponics than in geoponics. There are, in counterpart, a few downsides: a regular maintenance is needed, as well a constant control of the possible pH fluctuations; the water temperature is hard to maintain consistently as it directly influences dissolved oxygen concentrations. The water must be constantly changed in order to prevent growth of possible algae and other potentially pathogenic microorganisms (anaerobic bacteria and fungi). If not, the roots can become subject to rotting, regularly induced by the parasite *Pythium* spp. (Owen-Going et al., 2003). Plants grown hydroponically can more easily be handled and the roots examined, compared to plants grown in soil. Also, the nutrient solution can be used to test substances on the plants during scientific experiments.

Our objectives in this study were to check the identity of the species and establish its specific histo-anatomical elements of rhizomes, rachises, pinnae and runners through microscopic analysis of cross-sections and surface preparations using bright field and fluorescent microscopy. In order to estimate its potential safety for human use, in the present study we have investigated its phytotoxicity on *Triticum aestivum* L. and its lethality on the invertebrate brine shrimp, *Artemia franciscana* Kellog.

## 2. Materials and Methods

### 2.1 Hydroponic growth conditions

The plants were cultivated in an in-house laboratory hydroponic deep water growth system. They were obtained commercially (Dedeman, Bucharest, Romania, imported from the Netherlands), potted, having at the time of purchasing about 30 cm in height and 12 cm in diameter. The roots were cleaned of earth, washed and planted in net pots filled with hydrocorn. The grow tent used was a HL 100 V2.0 (HOMEbox, Berlin, Germany). The water was constantly aerated using a Hailea ACO 9602 air pump that had an output of 7.2L of air per minute. The tubing had 4 mm in diameter and the airstone was a round ceramic 150 mm Hailea airstone (pepika.ro). The plants had a 12 hour day/night light cycle and were grown under a 250W growth spectre MH lamp (GIB lighting, Germany). The photosynthetic active radiation (PAR) was measured with a MQ-500 full-spectrum quantum sensor (Apogee Instruments, terra-preta.ro) and had 10 µmol $m^{-2}$ $s^{-1}$ used to simulate household conditions. Inside the tent the average day temperature was 26.39ºC (s.d.1.18) and the average relative humidity 65.32% (s.d.4.41). The hydroponic solution was made using Flora Series (General Hydroponics, USA), was kept at a concentration used for cuttings or seedlings, had an average pH of 6.51 (s.d.0.10) and an average temperature of 24.22ºC (s.d.1.24). Measurements were made using an ADWA AD31 total dissolved solids (TDS) meter (ADWA, Hungary) and a Testo 206-pH1 pH meter (Testo, Germany).

### 2.2 Macroscopic and microscopic examination

The species' identity was confirmed by macroscopic and microscopic examinations on rhizomes, rachises, pinnae and runners using bright field and fluorescent microscopy. We used a Labophot-2 microscope equipped with a Nikon digital camera; an Euromex Oxion microscope equipped with a CMEX 5 camera; an Optika B-383FL fluorescence microscope equipped with a Nikon digital camera. We have examined surface preparations from the pinnae, clarified with NaOH 5% and manually obtained cross-sections from rhizomes, rachises, pinnae and runners clarified with Javel water and stained with iodine green and carmine alum double stain, FABIL (Dinu et al., 2013) and calcofluor (Herth and Schnepf, 1980).

### 2.3 Toxicity assay

For both the phytotoxicity and the lethality assays, the extracts were prepared as follows: 5g of dried and pulverized fronds were extracted with 50ml ethanol 96% at reflux (about 70ºC, for 30 minutes): after cooling, the solution was filtered in a 50 ml volumetric flask and filled to the mark with solvent in order to obtain a 10% ethanol extractive solution. This solution was then evaporated on a hot plate in order to obtain a dry extract which was then suspended in 50ml of distilled water using an ultrasonic bath. The 10% aqueous extractive solution was obtained from leaves in a similar manner, using a temperature of 100ºC, for 30 minutes.

#### 2.3.1 Phytotoxicity assay

The assessment of the phytotoxicity was done on embryonic wheat roots (*Triticum aestivum* L. - the Constantinescu method) (Dinu et al., 2012). The ecological Spelta wheat was obtained commercially from Solaris Plant. We examined the influence on the embryonic root elongation and the modifications suffered by the mitotic film in root tips.

The wheat caryopses were germinated in laboratory conditions (kept for 24h in distilled water at room temperature in Linhardt dishes). After germination 11 caryopses with a 1 cm root tip were collected in separate Petri dishes

(ten to be used for the root elongation assay, one for the acetic orcein staining and microscopic investigation). For both extracts five samples were prepared: for the ethanol extracts: E1 (10%), E2 (5%), E3 (1%), E4 (0.5%), E5 (0.1%) and for the aqueous extracts: A1 (10%), A2 (5%), A3 (1%), A4 (0.5%), A5 (0.1%) (the concentration is expressed as grams of dried plant material for 100 ml of solvent). The control sample (M) consisted of distilled water. The root elongation was observed at the same time of day for three consecutive days. For the microscopic study of the nuclei from the embryonic root, we used the acetic orcein stain and squash method. The mitosis was observed under the 100X objective lens after immersion in cedar oil. Root elongation and karyokinetic film modifications were evaluated (Dinu et al., 2012).

2.3.2 Lethality assay

The brine shrimp lethality assay was performed on *Artemia franciscana* Kellog. The cysts were commercially obtained from Ocean Star International (USA), repackaged by S.K. Trading (Thailand), originating from Great Salt Lake (USA). Artificial seawater was prepared dissolving a commercial salt mixture (Coral Marine, Grotech) in distilled water, by sonication, at a concentration of 33.5 g/L. The hatching was initiated about 48 hours before the initiation of the test, as it takes cca 36-48 hours. The test was carried out in a 24 (6 x 4) multiwell test plate, the extract solutions of different concentrations being placed in triplicate in the wells. The concentrations used were 10%, 5%, 1%, 0.5%, 0.25%, 0.125%, and 0.0625% for both the aqueous and the ethanol extracts. As a control, artificial seawater was used.

The hatched nauplii were transferred to the wells with a micropipette, counting between 10 and 20 nauplii per well. All dead and alive nauplii at 24h and 48h were counted and recorded (Ancuceanu et al., 2016).

### 2.4 Statistical analysis

The statistical comparisons between multiple groups for the concentration and the type of extract for the *Triticum* assay were done by using robust and parametrical models of multiple regression (equivalent to bidirectional ANOVA models). The statistics were performed with the R packs “car”, “robustbase” and “robust” (Fox and Weisberg, 2011; Maechler et al., 2017; Wang et al., 2017) on the values measured at 48h.We have evaluated the lethality and calculated the $IC_{50}$ and $LC_{50}$ by using non-linear regression (4-parameter logistic models, as implemented in the R pack “dr4pl”) (Landis et al., 2018).

## 3. Results and discussions

### 3.1 Macroscopic and microscopic examinations

The identity of the species has been confirmed by macroscopic findings consistent with the literature data: the sessile pinnae can be recognized macroscopically by their pale green color and by their deltoid shapes, with unequal bases, the anterior part being auriculated, the edges slightly serrated, the indusia kidney shaped, the spores ellipsoidal or spherical with an uneven surface. (Kramer et al., 1990; Nauman, 1993; Ancuceanu, 2013). Based on our microscopic examination, the following histological characteristics (see Fajuke et al., 2018 for surface preparations) have been highlighted:

Similarly to other fern species (Dinu et al., 2013; Dinu and Ancuceanu, 2016), the rhizomes show a thick lignified hypodermis, cortical parenchyma and a polystelic structure with hadrocentric vascular bundles of the meristele type.

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The runners are covered in trichomes, and show an internal structure similar to the rhizome, but with less lignification. The rachis is covered with few pluricellular uniseriate trichomes and internally shows a lignified epidermis with cuticle and a pluristratified lignified hypodermis, a large hadrocentric vascular bundle in the stele encircled by an endodermis which shows Casparian strips. Internally they show a homogenous structure, hadrocentric vascular bundle, lobed cell epidermis (puzzle-shaped), stomata with 2 to 3 diacytic or anisocytic accessory cells, pluricellular uniseriate trichomes with a rounded tip. The clarified and stained cross sections are shown in **Fig. 1**, the surface preparations are shown in **Fig. 2** and the observations for the surface preparations are similar with those found in the literature (Fajuke et al., 2018; page 23).

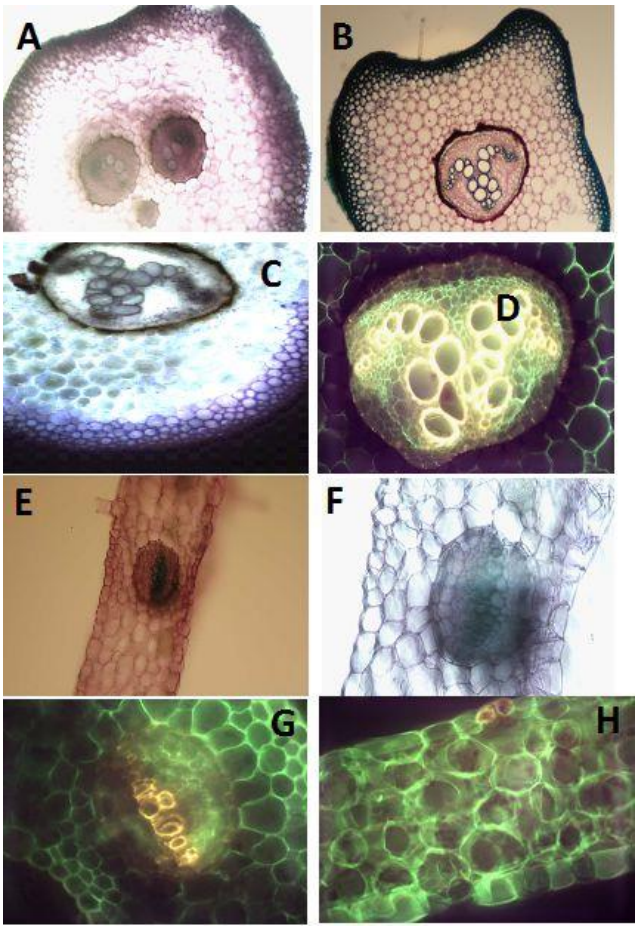


**Fig. 1.** Cross sections of the rhizome, rachis, and pinna of *Nephrolepis exaltata*:
**A**. Rhizome - polystelic structure, lignified pluristratified hypodermis, hadrocentric vascular bundles (meristeles) (rhizome, ob. 4x, ig+ca); **B.** Rachis - epidermis and lignified pluristratified hypodermis, pluricellular uniseriate trichome, hadrocentric vascular bundle (ob. 4x, ig+ca);
**C.** Rachis - epidermis and pluristratified lignified hypodermis, hadrocentric vascular bundle (ob. 4x, f); **D.** Rachis - hadrocentric vascular bundle in the stele, endodermis with Casparian strips (ob. 10x, c); **E.** Pinna - homogenous inner structure, basal trichome cell, hadrocentric vascular bundle, Casparian strips on the endodermis (ob. 4x, ig+ca); **F.** and **G.** Pinna - hadrocentric vascular bundle, Casparian strips present on the endodermis (ob. 10x, f and c); **H.** Pinna - Epidermis with cuticle, stomata, substomatal cavity, homogenous inner structure (ob. 10x, c). Abbreviations: ig – iodine green; ca – carmine alum; f – FABIL; c – calcofluor.

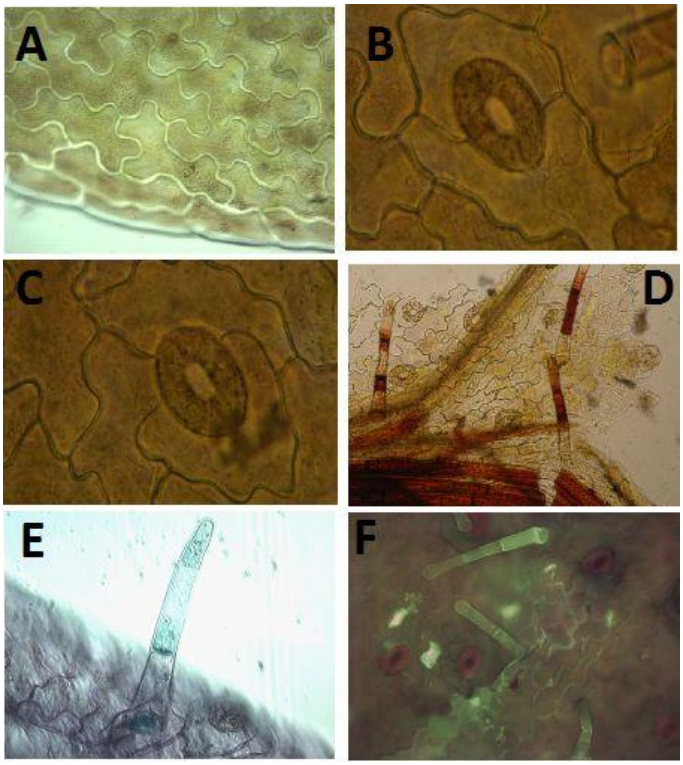


**Fig. 2.** Surface preparations of the *Nephrolepis exaltata* pinnae, clarified with NaOH 5%: **A.** Epidermis with cuticle, lobed cells (ob. 10x, no staining); **B.** Diacytic stoma (ob. 40x, no staining); **C.** Anisocytic stoma (ob. 40x, no staining); **D.** Stomata, uniseriate pluricellular trichomes, lobed cells (ob. 10X, no staining); **E.** Uniseriate pluricellular trichome (ob. 40x, FABIL); **F.** Stomata, uniseriate pluricellular, trichomes, lobed cells (ob. 10x, calcofluor).

### 3.2 The phytotoxicity assay

The analyzed extracts have a moderate dose-dependent mitoinhibitory effect, ($p<0.0001$) on the *Triticum aestivum* embryonic tips, producing a mitodepressive and stathmokinetic effect. No statistically significant difference was seen between the aqueous and the ethanol extracts with respect to the inhibitory effect on the root elongation ($p>0.64$) both in the parametric models of multiple regression and on the robust regression models. The $IC_{50}$ for the aqueous extract was of 0.93% (95%CI 0.70-17.96%). Strong inhibition of root elongation occurs only at high concentrations (10%, 5%), which suggests that the phytotoxic effect of the extract is relatively low. The results for the *Triticum* assay are presented in **Fig. 3**.

The microscopic examination of the acetic orcein and squashed wheat root tips revealed the following for both the ethanol and the aqueous extracts: At high concentrations (10%) the mito-depressive effect was pronounced: elongated prophases, interphases characterized by nuclei with hypertrophied nucleoli, quasi-normal metaphases, anaphases, very frequent telophases with slight tropokinesis. At 1% and 0.5%, interphases with 2-3 hypertrophied nucleoli, metaphases showing tropokinesis, anaphases with bridges, frequent telophases with bridges and slight tropokinesis; metaphases and telophases are predominant.

At 0.1%, numerous divisions have been observed; interphase cells with nuclei having two to four hypertrophied nucleoli, numerous prophases, metaphases in tropokinesis and numerous telophases (normal, in tropokinesis, and with a simple bridge indicating delayed chromosomes) (**Fig. 4**).

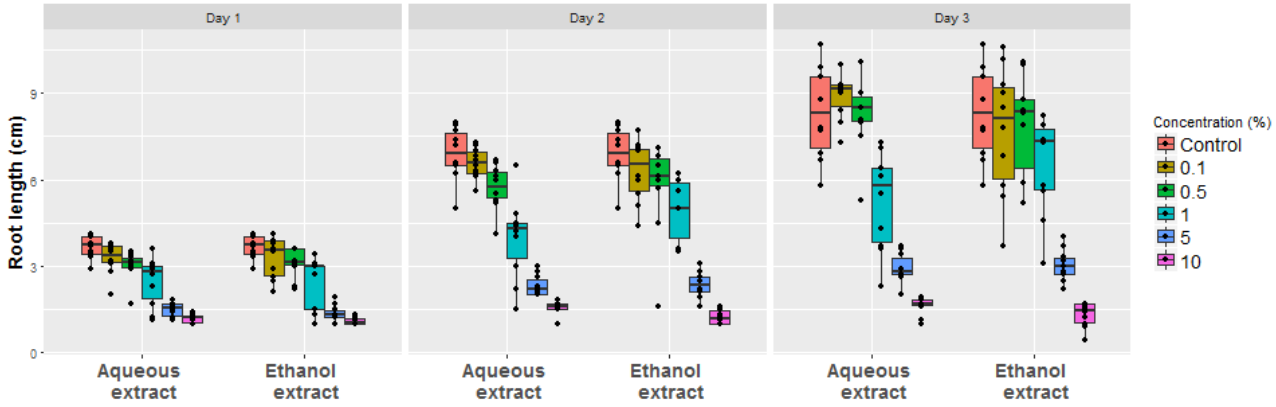


**Fig. 3.** Boxplot and point type graphs illustrating the root elongation (*T. aestivum* L.) variation according to the *Nephrolepis exaltata* extract, concentration of extract and day of measurement

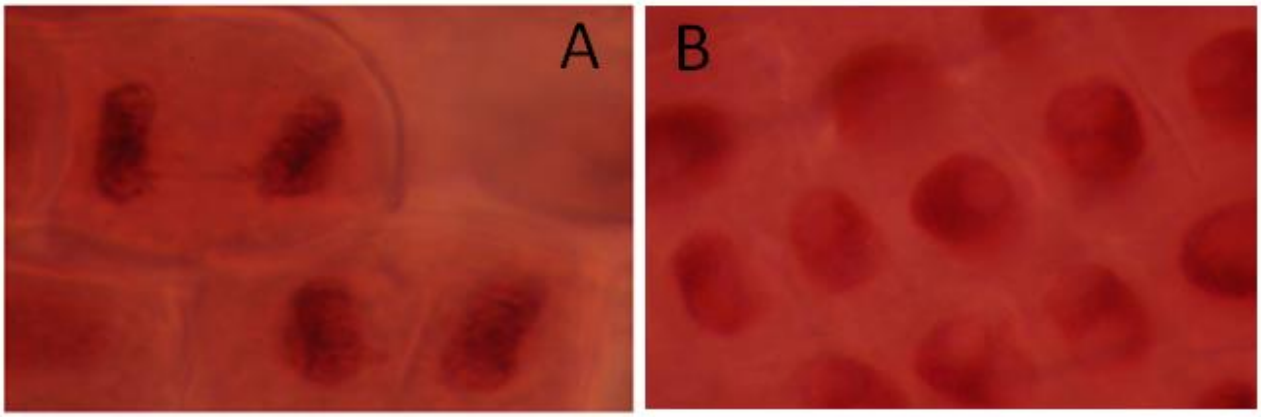


**Fig. 4.** Acetic orcein stained nuclei of the embryonic root tips of *Triticum aestivum* tested with aqueous and ethanol extract of *Nephrolepis exaltata*: **A.** Telophase in tropokinesis and simple bridge. **B.** Nuclei with hypertrophied nucleoli (ob. 100x).

### 3.3 The lethality assay

In the *Artemia* assay, $LC_{50}$ for the aqueous extract was 1.093% (95% CI 0.869-1.374%), and the $LC_{50}$ for the ethanol extract was 0.686% (95% CI 0.400-1.172%). In the literature, $LC_{50}$ values higher than 0.05% have been stated to be "practically non-toxic" (Moshi et al, 2010). The lower margin of the 95% CI of the values computed by us for the two extracts are much higher than the 0.05%, and thus one should conclude that the extracts of *N. exaltata* are virtually devoid of acute toxicity. The concentration-lethality curves for the two extracts are shown in **Fig. 5**.

*Nephrolepis exaltata* (L.) Schott was previously considered to belong to the families Oleandraceae, Davalliaceae or Lomariopsidaceae and has recently somewhat provisionally classified to its own family, Nephrolepidaceae due to uncertainty in its phylogenetic placement, (Kramer, 1990) until the accumulation of further data. Our microscopic data is consistent with the close relationship between the genus Nephrolepis and these families, although they are not very specific. Oleandraceae, although the anatomy has variations, are usually characterized by a peripheral sclerified sheet, parenchyma and a dictyostele. Similar features are observable in *N. exaltata* (Hovenkamp and Ho, 2012). However, the dictyostele seen in the rhizome of *N. exaltata* seems quite different in morphology from the one seen in a published image for *Oleandra musifolia* (Nopun, 2016).

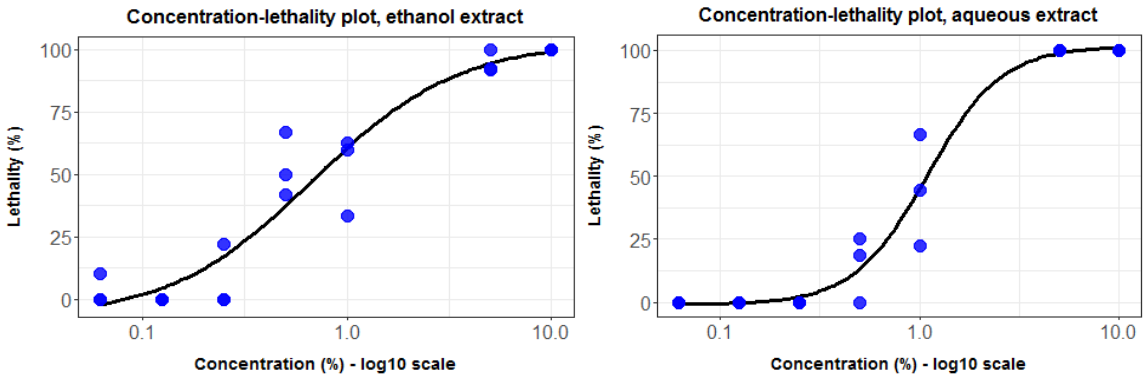


**Fig. 5.** Dose-response graphs and modeling for the *Artemia* assay with *Nephrolepis exaltata* extracts

A dictyostelic structure of the long-creeping rhizomes has also been described for the Davalliaceae species (Smith et al., 2006). The dictyosteles of Lomariopsidaceae have elongated ventral meristeles, (Chen et al., 2017) unlike those seen in our *Nephrolepis* sections.

The low inhibitory effect seen in the *Triticum* test, as well as the low lethality observed in the *Artemia* bioassay suggests that the plant may be safely used for therapeutic purposes. There is little ethnopharmacological knowledge on the use of this species. However, data from Fiji and India show that it is traditionally used (the rhizome) for the treatment of menstrual disorders, women's sterility and as an aid in childbirth (Cambie and Ash, 1994; Singh and Singh, 2012). In vitro data on prostate cancer cell lines LNCaP and PC-3 indicated that fractions from leaves might have potential antiandrogenic effects (Bobach et al., 2014). Such folk medicine data together with the apparent low toxicity seen in our experiments indicate that further investigation on both its chemical composition and pharmacological properties of extracts prepared from rhizomes and leaves are of interest and should be carried out.

## Conclusions

In this study we have characterized the microscopic histo-anatomical elements of identification of the species by using cross-sections of plant parts and surface preparations. The ethanol and aqueous extracts of *Nephrolepis exaltata* (L.) Schott have reduced toxic effects both on the roots of *Triticum aestivum* L. and on the *Artemia franciscana* Kellog nauplii. Further research is needed to elucidate fully the chemical composition of this fern and its potential pharmacologic activity.

## 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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**ORIGINAL PAPER**

# PHARMACO-BOTANICAL MAPPING AND EVALUATION OF THE MEDICINAL FLORA – POTENCIAL ALONG THE NIRAJ AND TÂRNAVA MICĂ RIVERS

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**Abstract:** This study offers the partial results of the botanical studies, botanical cartography, and the evaluation of the medicinal flora potential in the ROSCI0297 Natura 2000 area (Dealurile Târnavei Mici – Bicheș). From March 4th, 2016 to March 3th, 2017, in a 1000 ha sample area and a 34 days long fieldwork the specific location of 101 officially applied medicinal plant species was identified and mapping was achieved. Simultaneously, 26 medicinal plant analogues, 300 other valuable taxa (e. g. plants under protection, rare orchids etc.), and the cartography of invasive plants were documented. Another important result of the first year is the identification, listing and analysis of the reshaping, influential and impairing factors of the vegetation and habitats.

**Keywords:** medicinal plants, spontaneous flora, botanical cartography, potential, Niraj and Târnava Mică rivers

## 1. Introduction

Publicated studies (Csűrös 1963; Oroian and Giurgiu, 2003; Kovács, 2008; Domokos, 2015; Oroian et al., 2016; Arany et al., 2017; Nagy, 2017; Nagy 2017a; Nagy 2017b; Nagy 2017c; Nagy, 2018; Nagy 2018a) and studies in form of manuscript (Molnár, 1997; Veress, 2002; Jenei, 2005; Fazakas, 2007; Nagy, 2017d) about the vegetation along the Niraj and Târnava Mică rivers have been carried out by a few researchers.

Many of the ecosystem services of medicinal plants in Transylvania are still untapped. The studied area still has the capacity to provide these species: a recent study proves that 300 tons of mushrooms and medicinal plants can be legally harvested annually (Arany et al., 2017). Nonetheless, the areas' flora is not nearly exploited enough botanically and/or pharmaco-botanically. The fact that most of the medicinal plants still come from the spontaneous flora also contributed to this research. Moreover, the improvement and selection of medicinal plants is also based on spontaneous flora.

The subject of this work is the botanical study, the pharmaco-botanical mapping, and the evaluation of the (semi-)spontaneous flora potential in the territory between the Târnava Mică and Niraj rivers.

The aim was to carry out a comprehensive six-year study that can lay the foundation of a sustainable development in the territory.

## 2. Materials and Methods

### 2.1 What do we define by the notion of medicinal plant?

The first problem confronted at the beginning of the research: what do we define by the notion of medicinal plant? A definition for medicinal plants was created: first the unjustified knowledge belonging to popular medicine and those studied by ethno-pharmaco-botany were excluded. Only scientifically proven knowledge was considered (**Fig. 1**). Only those species were considered medicinal plants that are beneficial for medicinal purposes based on the active substance they contain. The circle of medicinal plants was restricted by studying only Vascular plants (Tracheophyta), because since their appearance in the (semi) spontaneous flora of the Carpathian Basin there has been reliable information published on them. Moreover, their effects or drugs/drug preparations can be found in at least one scientific description (pharmacopoeia, monographs, standards, etc.). Several specialized publications were synthesized from the group of those mentioned above since they offer limited information (for example, „Farmacopeea Română X$^{th}$ edition" contains only 25 species of spontaneous medicinal plants).

With these relatively objective methods, it was possible to compile a database containing 300 species of medicinal plants which can be found in the (semi)spontaneous flora of the Carpathian Basin. These 300 species have the potential to offer 336 drugs and drug preparations. This number is considered as the total number of medicinal plants, which was compared with the medicinal flora of the studied area, observing their differences as well.

The enumeration provides a complete and detailed synthesis of the Carpathian Basin spontaneous medicinal flora as well as the drugs and drug preparations obtained from them: it synthesizes the data of three pharmacopoeias (Ph.Hg. VIII., 2010; Ph.Eur. IX., 2017; Ph.Ro X., 1993), three monographs (ESCOP–European Scientific Cooperative on Phytotherapy, EMA–European Medicines Agency, WHO–World Health Organization) and two drug standards (Hungarian–www.mszt.hu and Romanian–www.asro.ro), as well as two specialized books (Bernáth, 2000; Muntean, 2007).

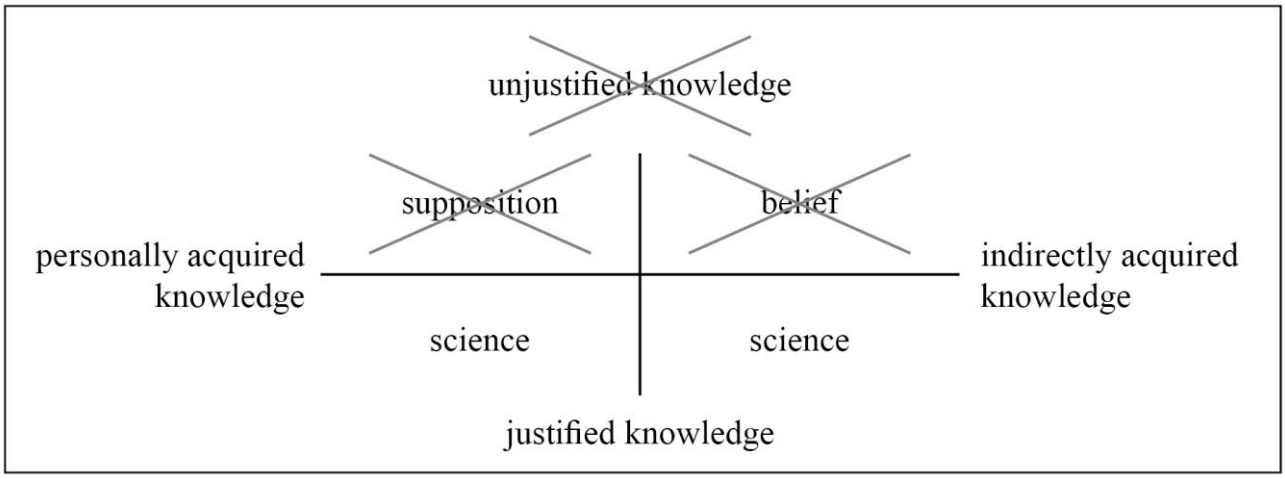


**Fig. 1.** Delimitation and diminution of the notion of medicinal plants, based on the type of knowledge regarding their efficacy

The nomenclature of the taxa was made on the basis of the Catalogue of Life, which is the most comprehensive and authoritative global index of species currently available (www.catalogueoflife.org). Regarding the endangered plant species, different studies were used to identify them (Simon, 2000; Dihoru and Negrean, 2009; Bartha, 2012).

### 2.2 Delimitation of the researched area

In the delimitation of the researched area the following criteria was considered: the vast majority of habitat types found in this territory (forests, meadows, etc.), but also urban areas, as well as secondary habitats and agricultural lands, in order to document the medicinal plants that are becoming wild or are resistant to human activities.

For the exact delimitation of the researched area a GPS device was used (Garmin GPSmap 64s), as well as satellite imagery and aerial images. An area of 1000 ha was delimited which was divided into 37 cartographic files, that was further divided into 1000 units of 1 ha (**Fig. 2**).

Then the most important features of the files and delimitated subunits (coordinates, dimensions, surface, altitude, level difference, exposure, and occasionally soil type, erosion degree) were recorded.

In the first year of the research, the fieldwork took place between 4 March 2016 and 3 March 2017 and required 34 active days of fieldwork.


**Fig. 2.** The location of the research area (control test site) and sample area (mapping location)

## 3. Results and disscusions

In the points to follow, the partial results will be presented obtained in the first year regarding the (semi)spontaneous medicinal flora of the Carpathian Basin and the territory between the Târnava Mică and Niraj rivers.

### 3.1 Results regarding the total (semi) spontaneous flora of the Carpathian Basin

The 300 medicinal plants listed in the database represent the total number of medicinal plants in the Carpathian Basin, while the 336 drugs/drug preparations represent the total number of drugs/drug preparations in this territory. All these medicinal plants are classified as follows: 5 classes (Equisetopsida – 0.33%;Gnetopsida – 0.33%; Liliopsida – 2.99%; Magnoliopsida – 94.68%; Pinopsida – 1.66%), 32 orders, 64 families (**Table 1** – in the Supplementary Material 2) and 159 genera. Regarding the number of the medicinal plants, the richest class is represented by angiosperms (Magnoliopsida), the richest order by Rosales, the richest family by Rosaceae and the richest genus by *Rosa*.

Regarding the geographical spreading (Simon, 2000), the documented medicinal plants of the Carpathian Basin can be classified in 24 different groups. 24.82% of these (101 species) belong to the Eurasian flora (**Fig. 3**).

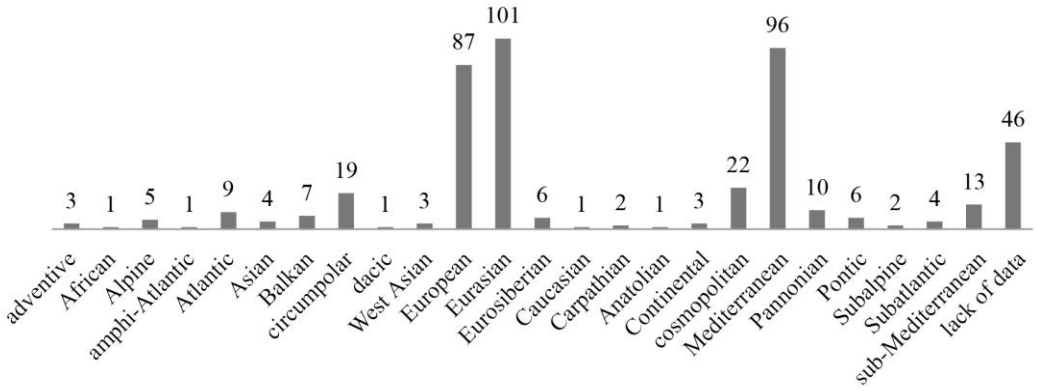


**Fig. 3.** The number of medicinal plant species from the Carpathian Basin with reference to the geographical spreading

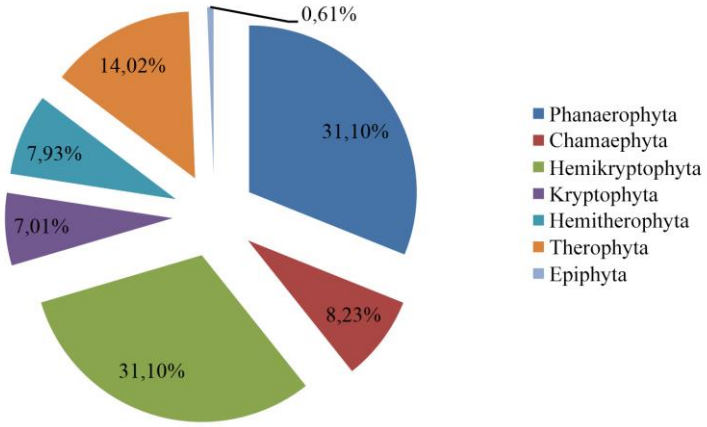


**Fig. 4.** The status of medicinal plant species from the Carpathian Basin with reference to the biological group aspect

Based on a biological group aspect (Raunkiaer, 1907; Raunkiaer, 1934; Ujvárosi, 1952; Hunyadi, 2011), the medicinal plants of the Carpathian Basin are mostly woody plants from the phanerophytes and herbaceous and perennial plants from the hemicryptophytes, both groups comprising of 102 species with a proportion of 31.10% (**Fig. 4**).

### 3.2 Results regarding the total (semi-) spontaneous flora of the territory between Târnava Mică and Niraj rivers

During the first year, in the studied area 101 species of (semi)spontaneous medicinal plants were documented, which number constitutes as the 33.66% of the medicinal plants flora of the Carpathian Basin (**Fig. 5**).

These medicinal plants are classified as follows: 4 classes (Equisetopsida – 0.99%; Liliopsida – 3.96%; Magnoliopsida – 94.05%; Pinopsida – 0.99%), 26 orders, 44 families (**Table 1** – in the Supplementary Material) and 88 genera. Regarding the number of the medicinal plants, the richest class is made up of the Magnoliopsida, the richest order of the Asterales, the richest family of the Asteraceae and the richest genus of the *Galium*, *Plantago* and *Quercus*. The percentage division of the (semi)spontaneous flora of the studied territory according to order, reveals that the *Fabales* represent themselves with 7 medicinal taxa, which constitutes the 6,93% of the medicinal plants in the studied territory, and 77.77% of the medicinal plants that belong to the *Fabales* order in the Carpathian Basin (a total of 9). This points out that the area is very rich in medicinal plants belonging to the *Fabeles* order.

In terms of geographical spreading, the documented medicinal plants in the studied area can be classified in 16 different groups. The highest percentage (30.43%) is made of Eurasian flora, meaning 49 species **Fig. 6**).

In terms of bioform, the majority of the (semi)spontaneous medicinal flora in the studied area are herbaceous and perennial hemikryptophytes (53 species – 41.09%) (**Fig. 7**).

In the studied area, medicinal plants were found in the following proportions: 48,51% natural condition; 48,51% disturbed/degraded condition and 2,97% lack of data. 14.29% of the medicinal plants (7 taxa: *Arnica montana* L.; *Menyanthes trifoliata* L.; *Valeriana officinalis* L.; *Adonis vernalis* L.; *Helleborus purpurascens* Waldst. & Kit.; *Hepatica nobilis* Schreb.; *Hippophae rhamnoides* L.) found in their natural condition are endangered species protected by local, regional, national or international laws. If we classify these 101 plants according to Social Behavior Types (Borhidi 1995), we notice that the number of those species found in their natural conditions is 39.81% (**Fig. 8**). This fact shows that the habitat's risk factors (erosion, environmental pollution, and deforestation) are in fact only transformative factors concerning the number and abundance of the medicinal plants. In regard to medicinal flora, the area has a 1.33 degree of degradation, since the species that indicate the degradation are definetely in majority. In terms of relative „temperature figures" (T value/index), the majority (48 species – 47.52%) are species characteristic of the montane mesophilous broad-leaved forest belt (**Fig. 9**).

In terms of relative „moisture figures" (W value/index), the majority consist of plants of semi-humid habitats, under intermediate conditions (31 species – 30.10%) (**Fig. 10**). In terms of relative soil „reaction figures" (R value/index), the majority is species characteristic of mostly neutral soils but also in acid basic ones generally widely tolerant, more or less indifferent plants (38 species – 38.00%) (**Fig. 11**).

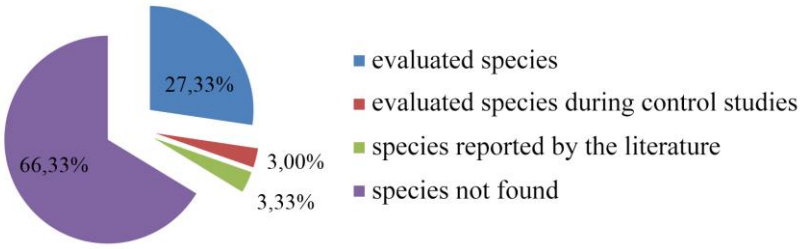


**Fig. 5.** The status of the medicinal plants from the studied area with reference to the Carpathian Basin medicinal flora

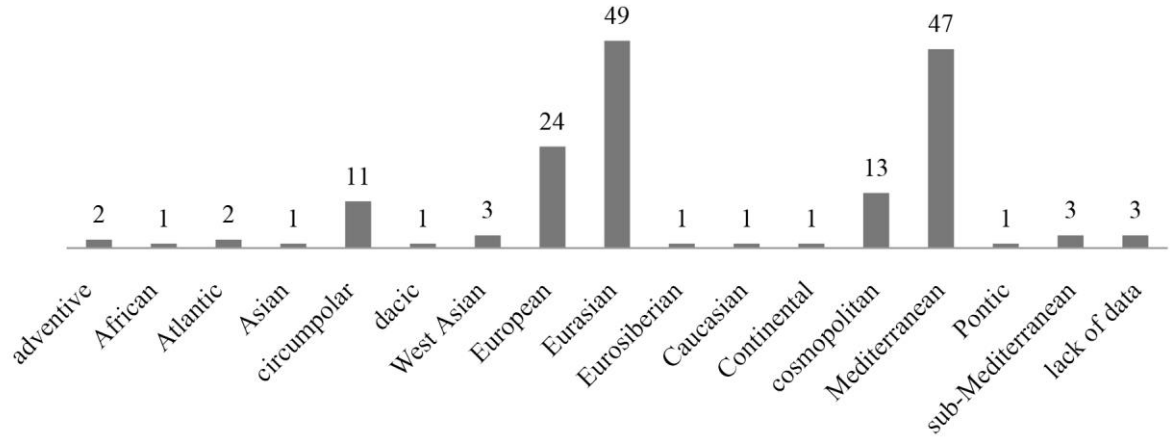


**Fig. 6.** The number of medicinal plant species from the studied area with reference to the geographical spreading

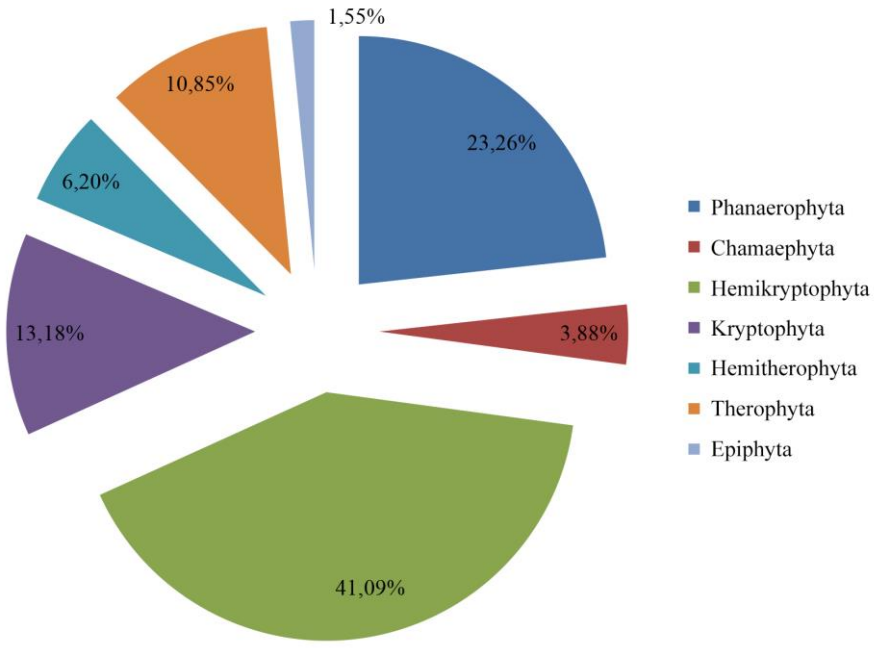


**Fig. 7.** The status of medicinal plant species from the studied area with reference to the biological group aspect

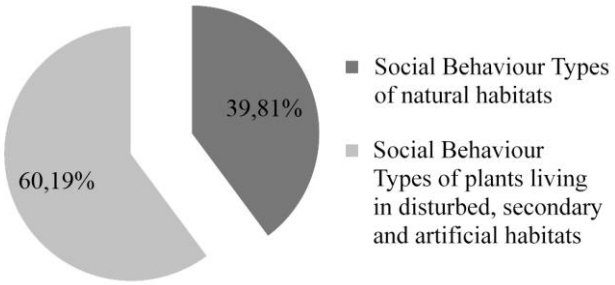


**Fig. 8.** The status of medicinal plant species from the studied area with reference to the social behavior types

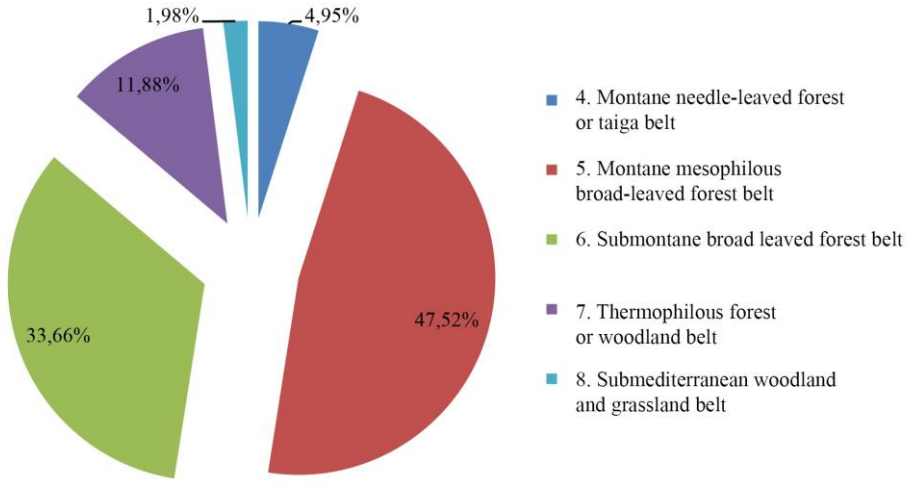


**Fig. 9.** The status of medicinal plant species from the studied area with reference to the terms of relative „temperature figures"

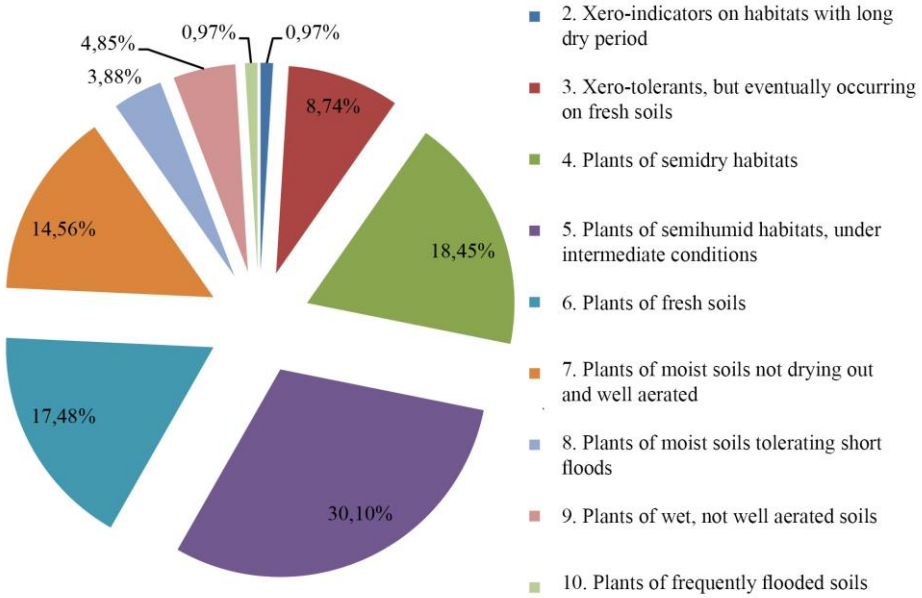


**Fig. 10.** The status of medicinal plant species from the studied area with reference to the terms of relative „moisture figures"

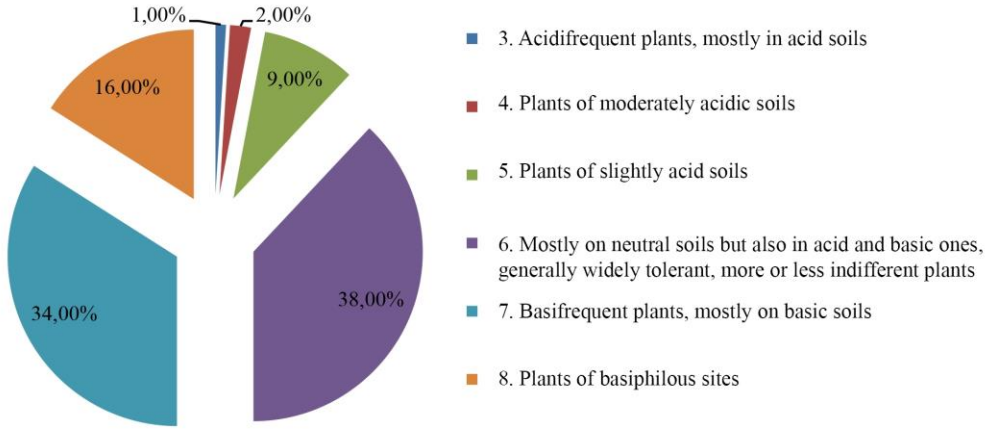


**Fig. 11.** The status of medicinal plant species from the studied area with reference to the terms of relative soil „reaction figures"

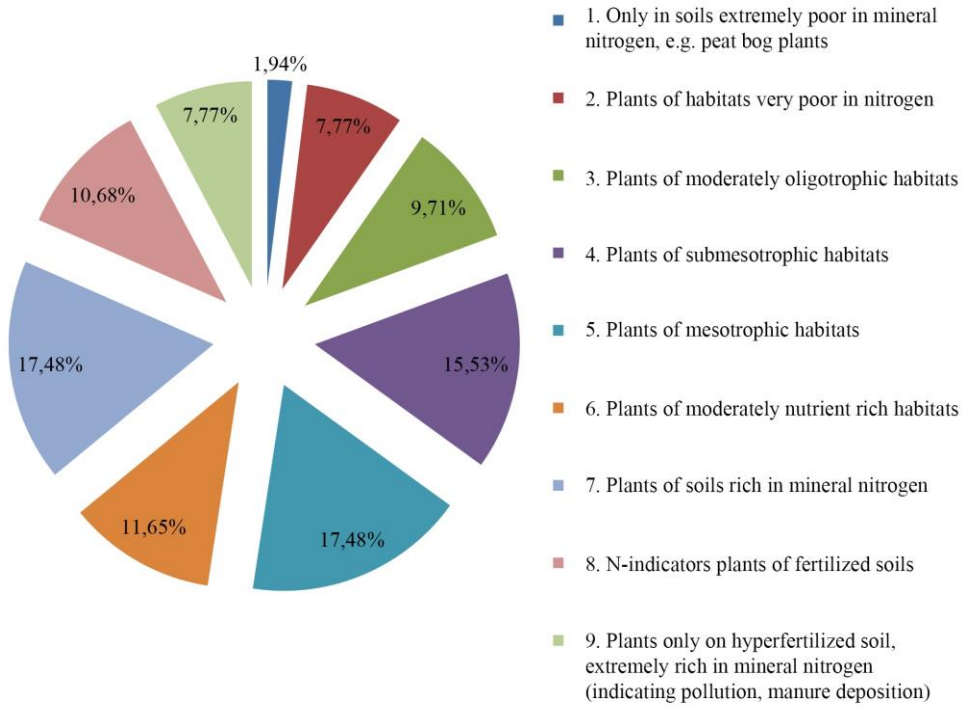


**Fig. 12.** The status of medicinal plant species from the studied area with reference to the terms of relative „nitrogen figures"

In terms of relative „nitrogen figures" (N value/index), the majority is plants of mesotrophic habitats (18 species – 17.48%), and plants of soils rich in mineral nitrogen (18 species – 17.48%) (**Fig. 12**).

The classification of the (semi)spontaneous medicinal plants in the area according to the relative „light figures" (L value/index) shows that in case of deforestation the number of medicinal taxa would increase spectacularly. Hence in the underbrush of dense forests there are only a few or no species of medicinal plants at all. The so-called half-light plants that live in mostly full light but are also shadow tolerants are in majority in the area's flora (40 species – 39.60%) (**Fig. 13**).

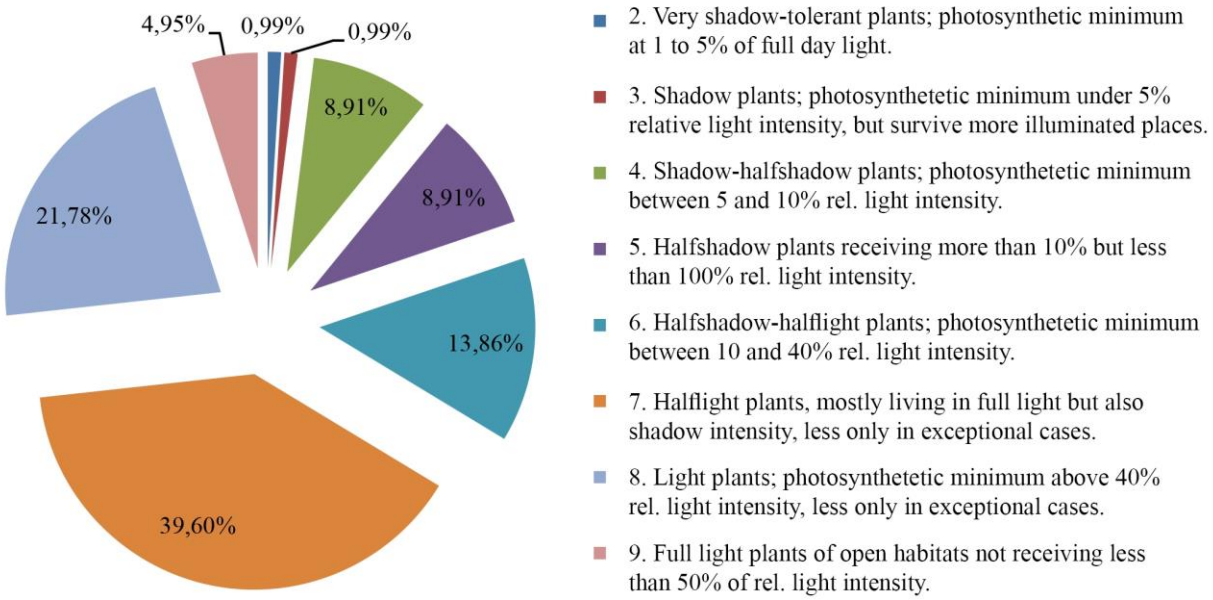


**Fig. 13.** The status of medicinal plant species from the studied area with reference to the terms of relative „light figures"

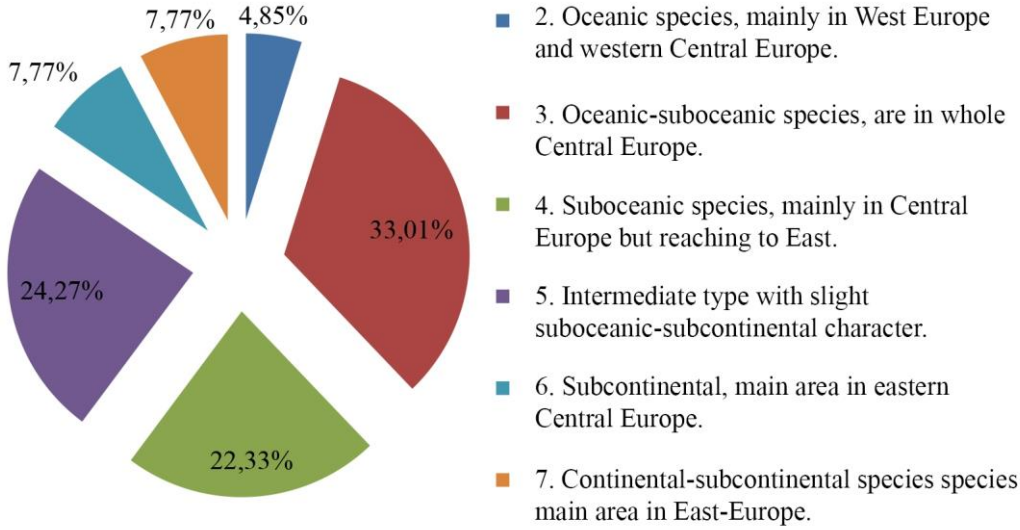


**Fig. 14.** The status of medicinal plant species from the studied area with reference to the terms of „continentality figures"

In terms of tolerance towards climate change or climate extremes („continentality figures" – C value/index), the majority of the species resulted to be oceanic–suboceanic species, which can be found in whole Central Europe (34 species – 33.01%) (**Fig. 14**).

The resistance of the (semi)spontaneous medicinal plants to salt is limited. According to the salt resistance index („salt figures" – S value/index) 90 species (88.24%) are halophob, not occuring in salty or alkalic soils (**Fig. 15**).

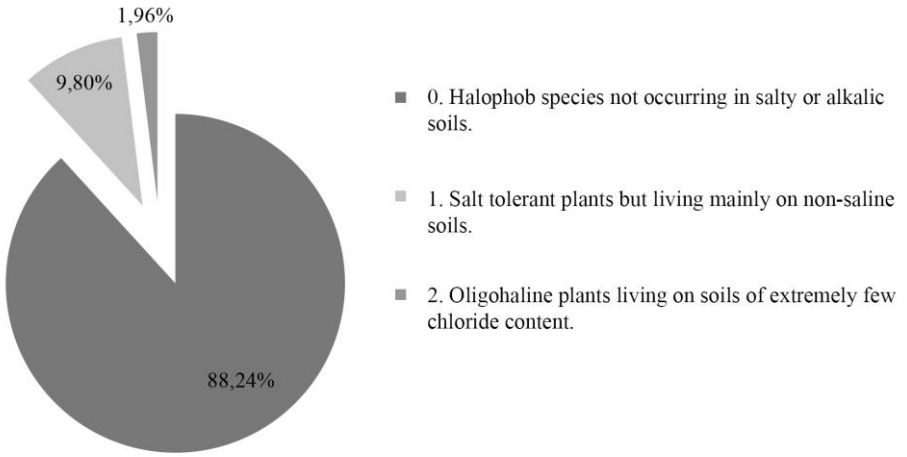


**Fig. 15.** The status of medicinal plant species from the studied area with reference to the terms of „salt figures"

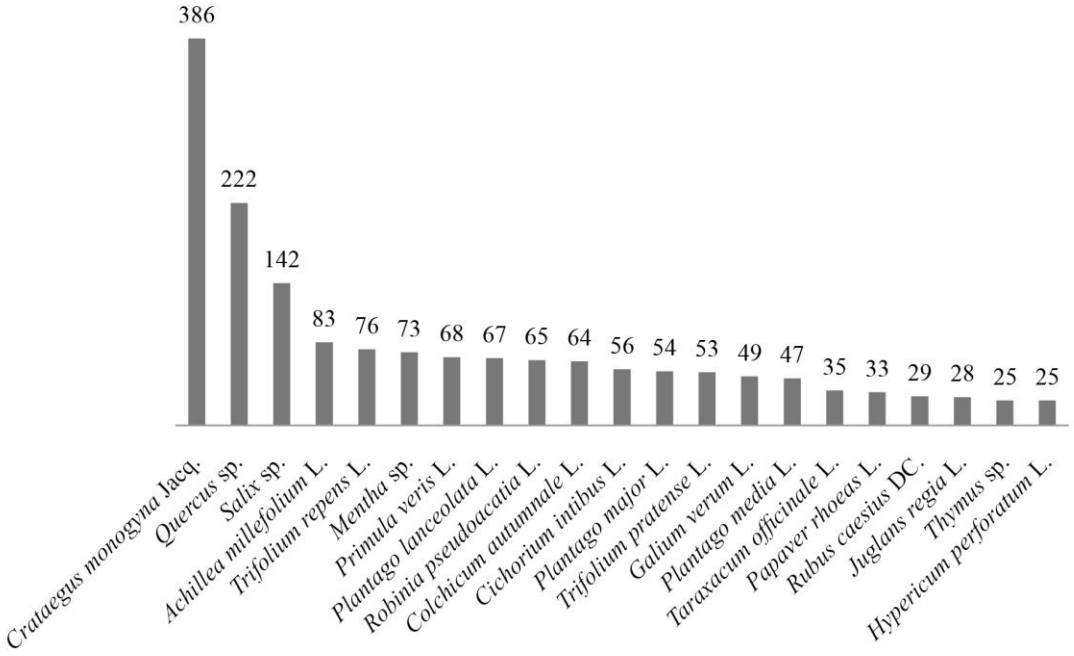


**Fig. 16.** The size of the area (ha) of the twenty most widespread taxa in the studied territory

The number of the documented factors that change the habitat in the area between Târnava Mică și Niraj rivers is almost 50 (natural disasters – flood, earthfall etc.; environmental pollution; constructions). These factors regarding the medicinal flora are positive, negative, but also positive and negative at the same time, as the examples above illustrate it (erosion, deforestation etc.). In most cases, the factors that endanger the habitat in terms of medicinal flora are only changing it. With reference to the abundance-dominance index, the most common 20 taxa are the most commonly known, most often used, and most often traded nowadays (**Fig. 16** and **Fig. 17**). Therefore, there is huge potential in the production and trade of drugs or drug preparations.

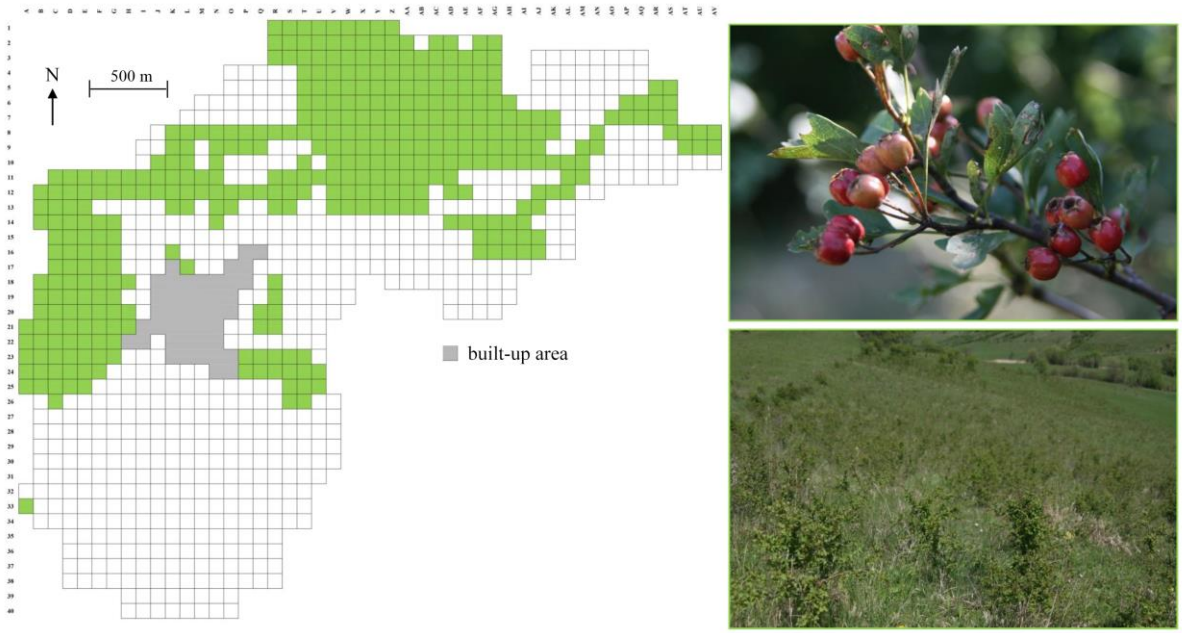


**Fig. 17.** The most widespread species: the spreading of *Crataegus monogyna* Jacq. in the studied area (original photo)

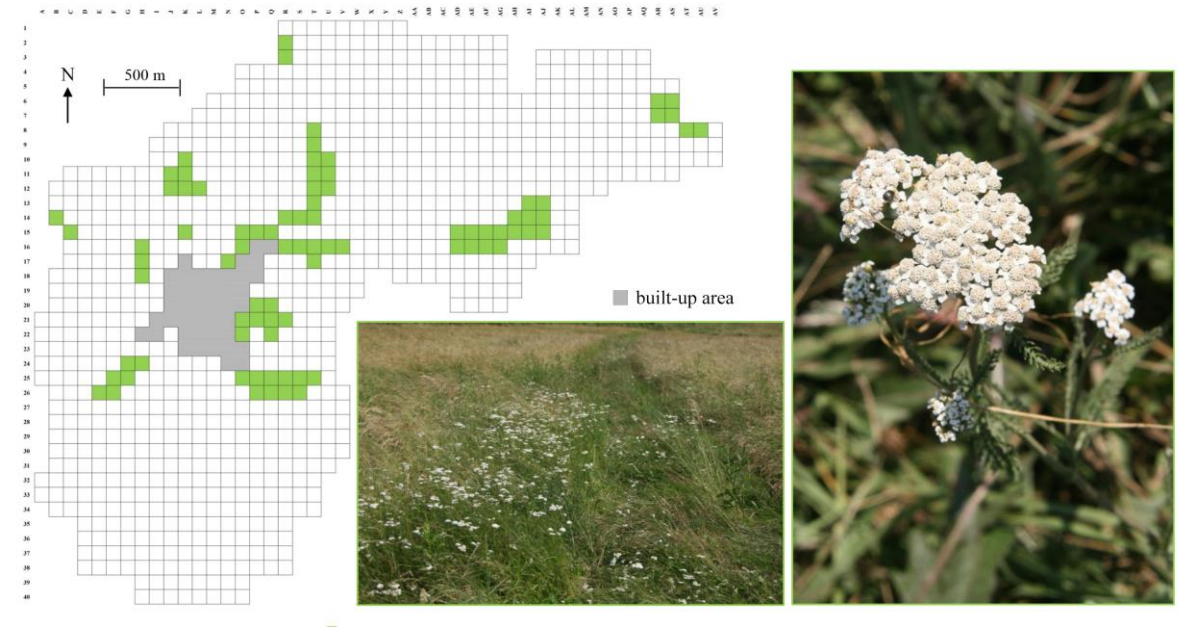


**Fig. 18.** Example 1 – the spreading of *Achillea millefolium* L. in the studied area (original photo)

The 69 maps show the spreading (area) of the documented medicinal plants (**Fig. 18**). They also reveal which species are tolerant towards human activities and which are trying to avoid the habitats disturbed by human activities. In case of tree species, the maps revealed areas of dense, homogeneous forests. In case of *Salix* and *Populus* genus, the maps show areas of temporal and permanent watercourses or water areas (**Fig. 19**).

In case of invasive-adventitious species – the group of parcels that could be potential risk factors. In the case of *Viscum album* L. – where are groups of sick and/or old trees (**Fig. 20**) etc.

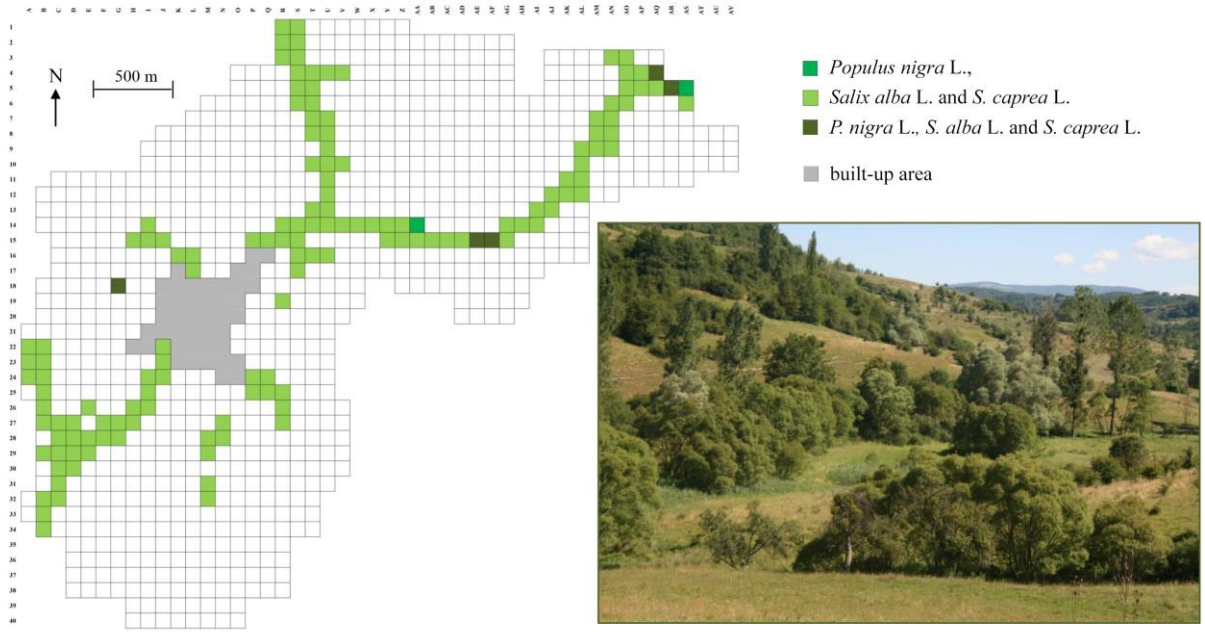


**Fig. 19.** The spreading of medicinal plants belonging to the *Salicaceae* family in the studied area (original photo)

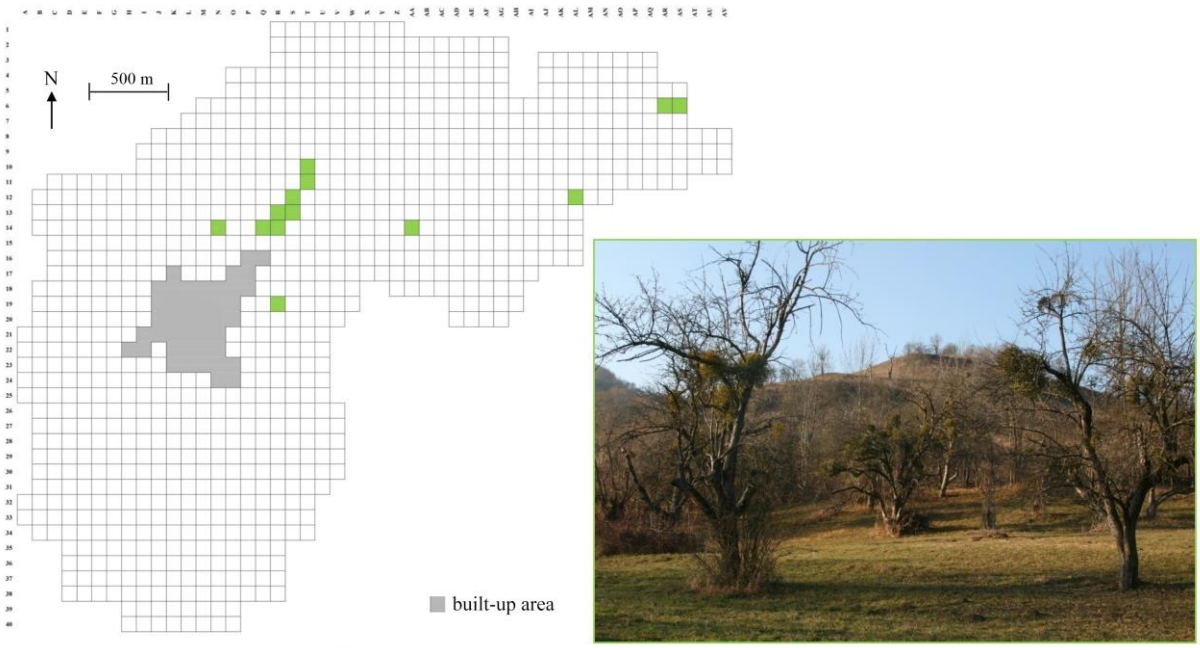


**Fig. 20.** The spreading of *Viscum album* L. in the studied area (original photo)

The mapping of ineffective or poisonous facsimile (26 species) highlights those parts of the area where there is a possibility to mistake the medicinal plant with the phenotypically similar but unusable species (**Fig. 21**). The 101 documented medicinal plants offer 195 drugs and drug preparations. This number constitutes 58.03% of the total number of the drugs and drug preparations from the (semi)spontaneous medicinal flora potential of the Carpathian Basin.

The number of the obtained drugs from the 101 plants is almost four times higher (79.49%) than the number of drug preparations. These results shows us that in the studied area, not only the harvest of the medicinal plants, but also the processing and marketing of three quarters of the species as a finished product can be accomplished relatively easily with cheap technologies and machinery, since these plants require only primary processing (drying, conditioning) and rudimentary storage. The 155 drugs contain the aerial part of the plants (herba) in highest percentage (32.26%) (**Fig. 22**).

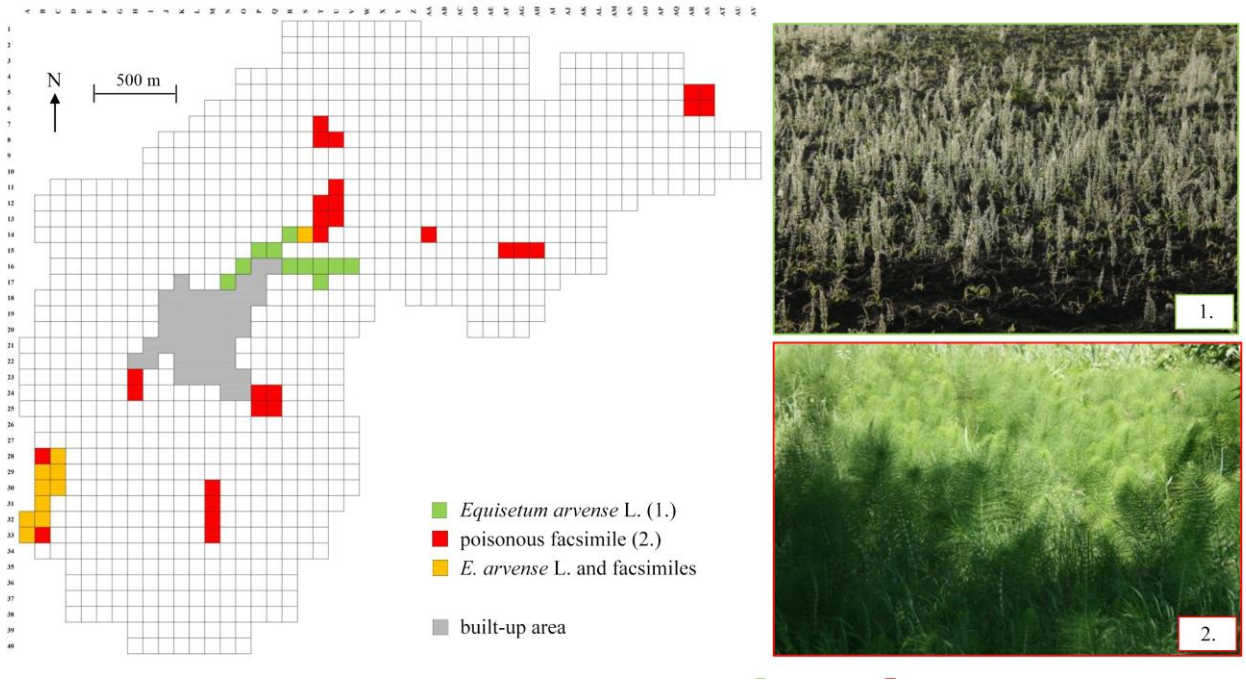


**Fig. 21.** Example 2 – the spreading of medicinal plants belonging to the *Equisetum* genus and its facsimiles in the studied area (original photo)

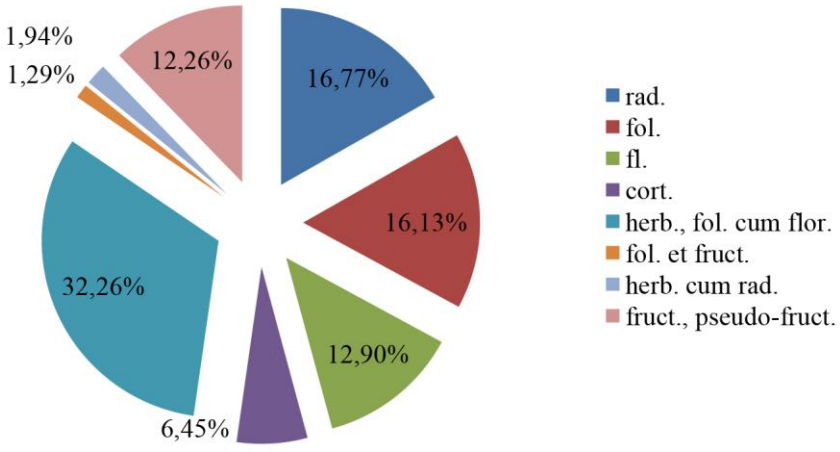


**Fig. 22.** Morphological grouping of drugs provided by the medicinal plants and the percentage distribution of groups (abbreviations: rad.–radix; fol.–folium; fl.–flos; cort.–cortex; herb., fol. cum flor.–herba, folium cum flore; fol. et fruct.–folium et fructus; herb. cum rad.–herba cum radicae; fruct., pseudo-fruct.-fructus, pseudofructus)

The 40 drug preparations mostly (11) and in highest percentage (27.50%) contain extracts (extractum) obtained by various method (**Fig. 23**).

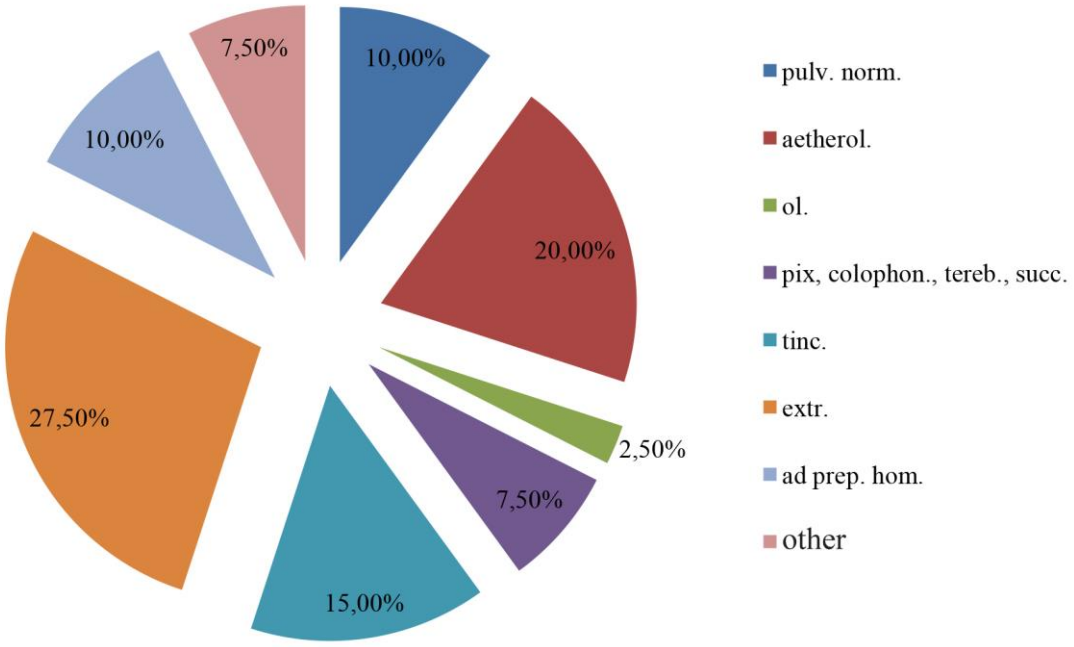


**Fig. 23.** Type grouping of drug preparations provided by the medicinal plants and the percentage distribution of groups (abbreviations: pulv. norm.–pulvis normatus; aetherol.–aetheroleum; ol.–oleum; pix, colophon., tereb., succ.–pix, colophonium, terebinthia, succus; tinc.–tinctura; extr.–extractum; ad prep. hom.–ad preparations homeopathicas)

## Conclusions

The botanical inventory and the pharmaco-botanical mapping of the area is wished to be continued until 2020, based on the 6-year plan. The second year of mapping was started on 4th March 2017. The second year's partial results also indicate the presence of new medicinal plant species in the studied area. The processing of statistical data and the preparation of specimens for the herbarium is currently ongoing.

It is believed that this study based on the researches carried out will effectively contribute to the rational and sustainable valorization of the potential of the (semi)spontaneous vegetation of the studied area in order to create alternative rural development opportunities in the territory that provide a better living for the local society.

## Conflict of Interest

The author 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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**ORIGINAL PAPER**

# THE STUDY OF ESSENTIALS OILS OBTAINED FROM *THYMUS PANNONICUS* L. - MICROBIOLOGICAL ASPECTS

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**Abstract:** Essentials oils have been used over time in the food and cosmetics industry, but also in the medical and pharmaceutical industry. Environmental factors such as temperature, radiation and photoperiod play an extremely important role in the quantity and quality of volatile oils. It is also known that the vegetation stage can play an important role in the chemical composition of volatile oils. The purpose of this paper is to establish the antibacterial and antifungal activity of volatile oils of *Thymus pannonicus*, taking into account the ontogenetic stage in which the plants were collected, highlighting the compounds of therapeutic importance. To test the antimicrobial activity of essential oils two methods of work were used: Kirby-Bauer disc diffusion method and microplate method. The essential oils studies were tested on *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans*. It was find that all essential oils tested have antimicrobial activity at all stages of development tested. The maximum antimicrobial activity has been recorded for the oils extracted from individuals collected at the anthesis stage.

**Keywords:** volatile oils, composition, antimicrobial activity, thyme, ontogenetic stage.

## 1. Introduction

The species of the *Thymus* genus exhibit tonic, carminative, digestive, antitussive, expectorant properties (Mojab et al., 2008), which is why volatile oils have been extensively studied and tested on various microorganisms. Thus, essentials oil of *Thymus vulgaris* exhibits antifungal properties, being tested on *Aspergillus, Candida, Penicillium, Mucor, Cladosporium, Trichoderma, Chaetomium* (Segvic Klaric et al., 2006; Giordani et al., 2004; Faleiro et al., 2003). At present, there are numerous studies on the antibacterial activity of volatile oils belonging to the genus *Thymus* (Kowal and Kuprinska, 1979; Marino, 1999; Nelson, 1997; Pina-Vaz et al., 2004; Smithpalmer et al., 1998). Timol and carvacrol seem to play an important role in this. These phenolic compounds bind to the amino- and hydro-amino groups of proteins in the bacterial membrane, altering their permeability, thereby leading to battery death (Juven et al., 1994). According to studies conducted by Pina-Vaz C. et al. (2004), essential oil from *Thymus vulgaris*, *Thymus zygioides* ssp. *zygioides* and

*Thymus mastichine*, can be used for medicinal purposes. The antibacterial activity of the main components of volatile oil (carvacrol, timol, p-cimen and 1,8 cineol) and the possible interactions between these components were studied. Oils from *Thymus vulgaris* and *Thymus zygioides* have shown similar antibacterial activity, and higher than that of *Thymus mastichina*. Also, volatile oil of *Thymus vulgaris* was also tested on *E. coli* (Marino, 1999), demonstrating that *E. coli* cells are destroyed at a relatively low concentration of oil. Possible antimicrobial activities of volatile oil of *Thymus* have been investigated by Faleiro et al. (2003). The authors analyze the chemical composition and test the antimicrobial activity of oils obtained from *Thymus mastichina* ssp. *mastichina*, *Thymus camphoratus* and *Thymus lotocephalus*, species harvested in different areas of Portugal. The antimicrobial activity of these oils was tested on *Candida albicans, Escherichia coli, Listeria monocytogenes, Proteus mirabilis, Salmonella* spp. and *Staphylococcus aureus*. The studied *Thymus* species demonstrated antimicrobial activity, but the tested microorganisms exhibited different sensitivities. Also, this antimicrobial activity is due to several components of volatile oils. The antimicrobial properties of the *Thymus pubescens* and *Thymus serpyllum*, species harvested before and during flowering, have been studied by Rasooli and Mirmoreafa (2002). Also volatile oil extracted from *Thymus revolutus*, a species growing on the territory of Turkey, presents important antibacterial and antifungal activities.

## 2. Materials and Methods

### 2.1 Plant material

The plant material is represented by *Thymus pannonicus* ssp. *auctus* All. and *Thymus pannonicus* ssp. *pannonicus* (Lyka) Soo. collected from Dealul Șorogari, jud. Iași, in three phenophases of development: vegetative, anthesis and fructification.

Species identification was performed by Dr. Ioan Sârbu from Botanic Garden "Anastasie Fătu", Iași and by prof. Dr. Nicolae Ștefan, taxonomist at Faculty of Biology, University "Al. I. Cuza" University of Iași.

The identification of taxa has been done using the following papers: Flora Europaea, vol. 3 and Flora ilustrată a României - Pteridophyta et Spermatophyta (Ciocârlan, 2009). The collected material was registered and stored in „Alexandru Ioan Cuza" University's Herbarium from Iași (*Thymus pannonicus* ssp. *auctus – no. 182354, Thymus pannonicus* ssp. *pannonicus – no. 182353)*.

### 2.2 Isolation of essential oils

100 g dried plant were subjected to hydro-distillation, 3 hours, using a NeoClevenger apparatus, according to the method recommended by the European Pharmacopeia (1997). The obtained essential oils were stored at +4°C until analysis.

### 2.3 Antimicrobial activity methods

To test the antimicrobial activity of essential oils two methods of work were used: Kirby-Bauer disc diffusion method and microplate method. The essential oils studies were tested on *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans*.

#### *2.3.1 Kirby-Bauer diffusion method*

The sensitivity of microorganisms to the volatile oils under study was tested "in vitro" using optimal and standardized cultivation conditions (culture medium, inoculation, incubation etc.). Diffusion method Kirby-Bauer adopted by CLSI (Clinical Laboratory Standards International, 2009) in the US is the usual method, widely used in laboratories to

test a relatively small number of microbial strains with rapid growth. By submitting steel cylinders containing 50µl quantities of samples tested (volatile oil to be tested in various concentrations: 10%, 1% and 0.1%, oil was dissolved in 10% DMSO), on the surface of a solid medium inoculated with a microbial culture, active antimicrobial substance will diffuse into the environment, with a steady decline of the concentration gradient from the edge of the cylinder toward the periphery. After the incubation time two separate zones will appear: one in which microbial growth is inhibited by concentrations of the antimicrobial substance, and a zone where the concentration is too low to inhibit the growth.

The culture medium used is Mueller Hinton medium (for bacteria) and Sabouraud medium (for yeast), distributed in Petri dishes in a uniform layer thickness of 4 mm, a pH of 7.2 to 7.4 (for bacteria ) and pH 6.5 (for yeast) measured before pouring into plates. These medium have nutritional value which allows optimum development of a wide variety of germs and contains no inhibitors of bacterial substances.

*2.3.2 Minimum inhibitory concentration*

The second method used is microplate method (Sarker et al., 2007). We used 96-well microplate, each containing 80 ml culture medium, 10 µl of diluted bacterial culture, 100 µl essential oil to be tested in different concentrations (10%, 1% and 0.1%; oil was dissolved in 10% DMSO) and 10 µl resazurine, resulting in a total volume of 200 µl per well. Microplates were incubated at 37°C for 24 hours. Of course, each plate contained wells and representing control (represented by DMSO). The colour changes were then evaluated visually. Thus, growth and development of microorganisms was indicated by changing colour from dark blue to purple. MIC (minimum inhibitory concentration) is the lowest concentration at which the colour changes.

**2.4 Statistical analysis**

For both working methods, positive controls (kanamycin for *Staphyloccocus aureus* and *Escherichia coli* and Nystatin for *Candida albicans*) and negative controls (distilled sterile water) were used. Also, all the determination was performed in triplicates and the results presented in tables representing an average.

## 3. Results and discussions

**3.1 Testing antibacterial activity of essentials oils used on *Staphylococcus aureus***

The results of testing the antibacterial activity of volatile oils from the two subspecies of Thymus pannonicus are shown in **Table 1.** As can be seen, all the oils tested show antibacterial activity, differing only in MIC (minimum inhibitory concentration). The largest inhibition zone (17 mm) was recorded for the volatile oil of *Thymus pannonicus* ssp. *pannonicus* - vegetative stage. The main compounds for this oil are germacren D, farnesol and trans nerolidol. Germacren D is a known compound due to its antimicrobial properties. Farnesol is generally known as a pesticide and pheromone (Boz et al., 2016) In the literature, there are studies on the antibacterial activity of volatile oils of *Thymus pannonicus* (anthesis stage) (Maksimović, 2008), but not on the activity of volatile oils from the two subspecies of the genus.

**3.2 Testing the antibacterial activity of essentials oils analyzed for *Escherichia coli***

Data on antibacterial activity of volatile oils derived from *Thymus pannonicus* ssp. *auctus* and *Thymus pannonicus* ssp. *pannonicus* collected in various phenophases are shown in **Table 2.** As can be seen, the minimal inhibitory concentration (MIC) for the tested concentrations is 0.1% with the

exception of volatile oils collected from plants in the fructification stage, where the MIC is 1%. The largest inhibition zone (11 mm) was found in the *auctus* ssp, at the anthesis stage and at a concentration of 10%. The main compounds of this oil are germacren D, farnesol, trans nerolidol and terpinyl acetate (Boz et al., 2016).

### 3.3 Testing the antifungal activity of essentials oils analyzed for *Candida albicans*

Data on the antifungal activity of volatile oils from *Thymus pannonicus* ssp. auctus and *Thymus pannonicus* ssp. *pannonicus* collected in various phenophases are shown in **Table 3.** As can be seen, the MIC for the tested concentrations is 0.1% with the exception of volatile oils collected from plants in the fructification stage, where the MIC is 1%. The largest inhibition zone (10 mm) is observed for the volatile oil extracted from the *Thymus pannonicus* ssp. auctus (anthesis stage) at a concentration of 10%. The main compounds of this oil are germacren D, farnesol, α terpinyl acetate and trans-nerolidol (Boz et al., 2016).

The results obtained show that all the oils tested show antimicrobial activity, differing only in MIC. Generally, the oils obtained from plants in the vegetative stage and the anthesis stage has a higher activity. From the available literature, we have not identified studies on the chemical composition and antimicrobial activity of volatile oils from the 2 subspecies of *Thymus pannonicus*. There are, however, studies on volatile oil of *Thymus pannonicus*. Thus, Maksimovic and colleagues identified a total of 33 chemical compounds in the volatile oil of *Thymus pannonicus*, collected from Serbia, in 2008, the main ones being geranial (41.42%) and neral (29.61%). These compounds have not been identified in volatile oil from species collected in Romania (Boz et al., 2009).

**Table 1.** Testing of antibacterial activity of volatile oils of *Thymus pannonicus* ssp. *auctus* and *Thymus pannonicus* spp. *pannonicus*, collected in various phenophases, on *Staphylococcus aureus*

| **Sample** | **Oil concentration %** | ***Staphyloccocus aureus*** | |
|---|---|---|---|
| | | **Diffusimetric method Inhibition zone - mm** | **Microplates method** + positive reaction, - negative reaction |
| *Thymus pannonicus* ssp. *auctus* – vegetative stage | 10 | 12 | +++ |
| | 1 | 8 | +++ |
| | 0.1 | 6 | +++ |
| *Thymus pannonicus* ssp. *auctus* – anthesis stage | 10 | 10 | +++ |
| | 1 | 6 | +++ |
| | 0.1 | 0 | ++- |
| *Thymus pannonicus* ssp. *auctus* – fruit stage | 10 | 8 | +++ |
| | 1 | 0 | ++- |
| | 0.1 | 0 | --- |
| *Thymus pannonicus* ssp. *pannonicus* - vegetative stage | 10 | 17 | +++ |
| | 1 | 10 | +++ |
| | 0.1 | 6 | +++ |
| *Thymus pannonicus* ssp. *pannonicus*– anthesis stage | 10 | 12 | +++ |
| | 1 | 6 | +++ |
| | 0.1 | 5 | +++ |
| *Thymus pannonicus* ssp. *pannonicus*– fruit stage | 10 | 12 | +++ |
| | 1 | 5 | +++ |
| | 0.1 | 0 | --- |

**Table 2.** Antibacterial activity testing of volatile oils of *Thymus pannonicus* ssp. *auctus* and *Thymus pannonicus* ssp. *pannonicus* collected in various phenophases on *Escherichia coli*

| **Sample** | **Oil concentration %** | ***Escherichia coli*** | |
|---|---|---|---|
| | | **Diffusimetric method Inhibition zone** - mm | **Microplates method** + positive reaction, - negative reaction |
| *Thymus pannonicus* ssp. *auctus* – vegetative stage | 10 | 10 | +++ |
| | 1 | 7 | +++ |
| | 0.1 | 6 | +++ |
| *Thymus pannonicus* ssp. *auctus* – anthesis stage | 10 | 11 | +++ |
| | 1 | 7 | +++ |
| | 0.1 | 5 | +++ |
| *Thymus pannonicus* ssp. *auctus* – fruit stage | 10 | 9 | +++ |
| | 1 | 5 | +++ |
| | 0.1 | 0 | --- |
| *Thymus pannonicus* ssp. *pannonicus* - vegetative stage | 10 | 10 | +++ |
| | 1 | 8 | +++ |
| | 0.1 | 6 | +++ |
| *Thymus pannonicus* ssp. *pannonicus*– anthesis stage | 10 | 9 | +++ |
| | 1 | 6 | +++ |
| | 0.1 | 5 | +++ |
| *Thymus pannonicus* ssp. *pannonicus*– fruit stage | 10 | 10 | +++ |
| | 1 | 6 | +++ |
| | 0.1 | 0 | --- |

**Table 3.** Antifungal activity testing of volatile oils of *Thymus pannonicus* ssp. *auctus* and *Thymus pannonicus* ssp. *pannonicus* collected in various phenophases on *Candida albicans*

| **Sample** | **Oil concentration %** | ***Candida albicans*** | |
|---|---|---|---|
| | | **Diffusimetric method Inhibition zone** - mm | **Microplates method** + positive reaction. - negative reaction |
| *Thymus pannonicus* ssp. *auctus* – vegetative stage | 10 | 8 | +++ |
| | 1 | 7 | +++ |
| | 0.1 | 6 | +++ |
| *Thymus pannonicus* ssp. *auctus* – anthesis stage | 10 | 10 | +++ |
| | 1 | 7 | +++ |
| | 0.1 | 5 | +++ |
| *Thymus pannonicus* ssp. *auctus* – fruit stage | 10 | 9 | +++ |
| | 1 | 5 | +++ |
| | 0.1 | 0 | --- |
| *Thymus pannonicus* ssp. *pannonicus* - vegetative stage | 10 | 8 | +++ |
| | 1 | 8 | +++ |
| | 0.1 | 6 | +++ |
| *Thymus pannonicus* ssp. *pannonicus*– anthesis stage | 10 | 7 | +++ |
| | 1 | 6 | +++ |
| | 0.1 | 5 | +++ |
| *Thymus pannonicus* ssp. *pannonicus*– fruit stage | 10 | 7 | +++ |
| | 1 | 6 | +++ |
| | 0.1 | 0 | --- |

Other scientists identified large amounts of thymol (25-41%) and p-cimen (17-38%) in the volatile oil of *Thymus pannonicus* (Pluhar et al., 2007). The antimicrobial activity of the oil was evaluated using agar disc diffusion and broth microdilution method against *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli*, two strains of *Klebsiella pneumoniae* and two strains *of Candida albicans*. The essential oil exhibited antimicrobial activity to varying degrees against all tested strains (Maksimović et al., 2008).

## Conclusions

In conclusion, we can say that all essential oils tested have antimicrobial activity at all stages of development tested, differing only in MIC. In most of the variants tested, intensification of antimicrobial activity occurs with an increase in oil concentration of up to 10%. Considering the plant development phase, we find that for the most part the maximum antimicrobial activity has been recorded for the oils extracted from individuals collected at the anthesis stage, also knowing that the flow of volatile compounds is intensified in order to ensure attraction of pollinators.

## 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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**ORIGINAL PAPER**

# FLORA FROM FĂRĂGĂU AREA (MUREŞ COUNTY) AS POTENTIAL SOURCE OF MEDICINAL PLANTS

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**Abstract** The aim of this study was to identify a potential source of medicinal plant from Transylvanian Plain. Also, the paper provides information about the hayfields floral richness, a great scientific value for Romania and Europe. The study of the flora was carried out in several stages: 2005-2008, 2013, 2017-2018. In the studied area, 397 taxa were identified, distributed in 82 families with therapeutic potential, represented by 164 medical taxa, 37 of them being in the European Pharmacopoeia 8.5. The study reveals that most plants contain: volatile oils (13.41%), tannins (12.19%), flavonoids (9.75%), mucilages (8.53%) etc. This plants can be used in the treatment of various human disorders: disorders of the digestive system, respiratory system, skin disorders, muscular and skeletal systems, genitourinary system, in gynaecological disorders, cardiovascular, and central nervous sistem disorders. In the study plants protected by law at European and national level were identified: *Echium maculatum*, *Cephalaria radiata*, *Crambe tataria*, *Narcissus poeticus* ssp. *radiiflorus*, *Salvia nutans*, *Iris aphylla*, *Orchis morio*, *Orchis tridentata*, *Adonis vernalis*, *Dictamnus albus*, *Hammarbya paludosa* etc.

**Keywords:** Fărăgău, medicinal plants, human disease, Mureş County

## 1. Introduction

Fărăgău is one of the most attractive places in the Transylvanian Plain, a region widespread, with hills, valleys dug in the creeks, marshes and sarmatic sands, with eroded flanks and muddy streams, landslides that have sometimes battered the rivers, forming lakes. In Fărăgău, floristic and geobotanic investigations were carried out in order to declare the protected area ”Lacurile Fărăgău - Glodeni” (ROSCI0100, Longitude 24.580755, Latitude 46.678636 and 244.8000 ha area) (**Fig. 1**). This protected area is considered by specialists to be the last natural lake in Transylvania (Cernei, 1995). The area has a conservative interest due to its good representation both in terms of quality and quantity of habitat and flora species protected by national law and U.E Directives. In Flora României, vol. I-XIII, in Fărăgău only 17 species were cited (Săvulescu, 1952-1976). Here it is recorded for the first time in literature, *Trifolium ambiguum* Bieb. We mention that since its reporting in Flora R.P.R. vol. V (Săvulescu, 1957), there is no data on its presence. The main objective of this study was the inventory of vascular spontaneous flora, especially the medicinal one, and its complex

analysis. The paper presents the results of the researches carried out during 2005-2018.

**Fig. 1.** Lake Fărăgău

## 2. Materials and Methods

The inventory of vascular flora was based on information from botanical literature and field research. In general, the taxonomic concept of "Flora Europaea" (Tutin, 1991; Tutin et al., 1964-1980) was respected. The botanical nomenclature used complies with the rules of the "International Code of Botanical Nomenclature" (Code de Melbourne 2012) and the book of Sârbu I. et al. 2013. In the floral inventory, the adopted classification system was updated according to the most recent publications (Cristea, 2014; Oroian, 2000; Sârbu et al., 2013). Within each family, the genera have been put in alphabetical order, as well as the species.

The medicinal plants were grouped according to the dominant active principles for which they are used in traditional medicine, respectively in phytotherapy, adopting the grouping of plants after Eşianu and Laczkó (2016) as well as the most recent specialized publications (Istudor, 1998, 2001, 2005; Stănescu et al., 2002, 2004).

The identification and classification of the protected plants were made on the basis of the specialty literature (Bilz et al., 2011; Boșcaiu et al., 1994; Mihăilescu et al., 2015; Oltean et al., 1994). All figures from this paper represent original photos.

## 3. Results and discussions

### 3.1 List of taxa identified

The floristic inventory includes 397 taxa, distributed in 82 families. The most representative families are: Asteraceae (52 taxa), Fabaceae (34 taxa), Lamiaceae (35 taxa), Poaceae (25 taxa), Scrophulariaceae (16 taxa), Apiaceae (17 taxa), Ranunculaceae (14 taxa).

The following taxa are identified: Equisetaceae: *Equisetum arvense*, *Equisetum maximum*; Dryopteridaceae: *Dryopteris filix-mas*, *Thelypteris palustris*; Pinaceae: *Picea abies*; Aristolochiaceae: *Asarum europaeum*, *Aristolochia clematitis*; Ranunculaceae: *Aconitum anthora*, *Adonis aestivalis*, *Adonis vernalis*, *Anemone nemorosa* L. subsp. *nemorosa*, *Anemone ranunculoides*, *Caltha palustris* L. subsp. *laeta*, *Consolida regalis*, *Helleborus purpurascens*, *Ranunculus acris*, *Ranunculus ficaria* subsp. *ficaria*, *Ranunculus polyanthemos*, *Ranunculus repens*, *Ranunculus sceleratus*, *Thalictrum minus*; P*apaveraceae*: *Chelidonium majus*, *Papaver rhoeas*; Caryophyllaceae: *Cerastium holosteoides*, *Dianthus armeria*, *Dianthus carthusianorum*, *Silene italica* subsp. *nemoralis*, *Silene latifolia*, *Silene otites*, *Silene vulgaris*, *Stellaria graminea*, *Stellaria holostea;* Chenopodiaceae: *Chenopodium album*, *Chenopodium hybridum;* Amaranthaceae: *Amaranthus retroflexus*; Polygonaceae: *Fallopia convolvulus*, *Reynoutria sachalinensis*, *Polygonum amphibium*, *Polygonum aviculare*, *Rumex acetosa*, *Rumex acetosella*, *Rumex crispus*, *Rumex sanguineus*; Betulaceae: *Carpinus betulus*, *Corylus avellana*; Moraceae: *Morus alba* (subspontan); Cannabaceae: *Humulus lupulus*; Urticaceae: *Urtica dioica*; Fagaceae: *Quercus robur*; Juglandaceae: *Juglans regia*;

Crassulaceae: *Sedum maximum*; Parnassiaceae: *Parnassia palustris*; Rosaceae: *Agrimonia eupatoria*, *Crataegus monogyna*, *Filipendula ulmaria*, *Filipendula vulgaris*, *Fragaria vesca*, *Fragaria viridis*, *Geum urbanum*, *Malus sylvestris*, *Potentilla anserina*, *Potentilla argentea*, *Potentilla arenaria*, *Potentilla recta*, *Prunus avium*, *Prunus spinosa*, *Prunus tenella*, *Pyrus pyraster*, *Rosa canina*, *Rosa gallica*, *Rubus caesius*, *Sanguisorba minor*; Fabaceae: **Amorpha fruticosa*, *Anthyllis vulneraria*, *Astragalus austriacus*, *Astragalus glycyphyllos*, *Astragalus monspenssulanus*, *Chamaecytisus albus*, *Chamaecytisus hirsutus*, *Cytisus leucotrichus*, *Chamaespartium sagittale*, *Coronilla varia*, *Dorycnium pentaphyllum* subsp. *herbaceum*, *Genista tinctoria* subsp. *tinctoria*, *Lathyrus palustris*, *Lathyrus tuberosus*, *Lotus corniculatus*, *Medicago falcata*, *Medicago lupulina*, *Medicago minima*, *Medicago sativa*, *Melilotus albus*, *Melilotus officinalis*, *Onobrychis viciifolia*, *Ononis arvensis*, *Robinia pseudacacia*, *Tetragonolobus maritimus* subsp. *siliquosum*, *Trifolium ambiguum*, *Trifolium arvense* subsp. *arvense*, *Trifolium campestre*, *Trifolium hybridum*, *Trifolium montanum*, *Trifolium pratense*, *Trifolium repens*, *Trifolium pannonicum*, *Vicia cracca*, *Vicia sepium*; Onagraceae: *Epilobium hirsutum*, *Epilobium palustre*, *Oenothera biennis*; Lythraceae: *Lythrum salicaria*; Haloragaceae: *Myriophyllum spicatum*; Aceraceae: *Acer campestre*, *Acer tataricum*; Rutaceae: *Dictamnus albus*; Oxalidaceae: *Oxalis corniculata*; Linaceae: *Linum catharticum*, *Linum flavum*, *Linum hirsutum*, *Linum perenne*; Geraniaceae: *Geranium palustre*, *Geranium pratense*; Polygalaceae: *Polygala comosa*, *Polygala major*; Celastraceae: *Euonymus europaeus*; Rhamnaceae: *Frangula alnus*; Euphorbiaceae: *Euphorbia cyparissias*; Elaeagnaceae: *Hippophaë rhamnoides*; Araliaceae: *Hedera helix*; Apiaceae: *Bupleurum falcatum*, *Carum carvi*, *Conium maculatum*, *Daucus carota* subsp. carota, *Eryngium campestre*, *Eryngium planum*, *Falcaria vulgaris*, *Ferulago sylvatica*, *Heracleum sphondylium* subsp. *sphondylium*, *Laser trilobum*, *Oenanthe aquatica*, *Oenanthe silaifolia*, *Pastinaca sativa* subsp. *urens*, *Peucedanum cervaria*, *Peucedanum oreoselinum*, *Pimpinella saxifraga*, *Sanicula europaea*, *Sium latifolium*; Hypericaceae: *Hypericum perforatum*; Cistaceae: *Helianthemum nummularium* subsp. *nummularium*; Brassicaceae: *Armoracia rusticana*, *Brassica elongata*, *Capsella bursa-pastoris*, *Crambe tataria*, *Erophila verna*, *Erysimum odoratum*, *Lepidium campestre*, *Raphanus raphanistrum*, *Rorippa amphibia*, *Rorippa pyrenaica*, *Sinapis arvensis*; Salicaceae: *Populus nigra*, *Populus tremula*, *Salix alba*, *Salix caprea*, *Salix cinerea*; Tiliaceae: *Tilia cordata*; Malvaceae: *Hibiscus trionum*, *Lavathera thuringiaca*, *Malva sylvestris*; Cornaceae: *Cornus sanguinea*; Santalaceae: *Thesium linophyllon*; Primulaceae: *Anagallis arvensis*, *Anagallis foemina*, *Lysimachia nummularia*, *Lysimachia vulgaris*, *Primula veris*; Gentianaceae: *Centaurium erythraea* (**Fig. 2**), *Gentiana cruciata* (**Fig. 3**); Apocynaceae: *Vinca herbacea*, *Vinca minor*; Asclepiadaceae: *Vincetoxicum hirundinaria*; Rubiaceae: *Asperula cynanchica*, *Cruciata glabra*, *Cruciata laevipes*, *Galium album*, *Galium aparine*, *Galium mollugo*, *Galium odoratum*, *Galium rivale*, *Galium rubioides*, *Galium uliginosum*, *Galium verum*; Oleaceae: *Ligustrum vulgare*; Caprifoliaceae: *Sambucus ebulus*, *Sambucus nigra*, *Viburnum opulus*; Valerianaceae: *Valeriana officinalis* subsp. *officinalis*; Dipsacaceae: *Cephalaria radiata*, *Dispacus fullonum*, *Dipsacus laciniatus*, *Knautia arvensis*, *Scabiosa ochroleuca*; Convolvulaceae: *Calystegia sepium*, *Convolvulus arvensis*; Cuscutaceae: *Cuscuta epithymum*, *Cuscuta europaea*; Solanaceae:

*Datura stramonium*, *Physalis alkekengi* (**Fig. 4**), *Solanum dulcamara*, *Solanum nigrum*; Boraginaceae: *Anchusa officinalis*, *Cerinthe minor* subsp. *minor*, *Echium russicum*, *Echium vulgare*, *Myosotis scorpioides*, *Nonea pulla*, *Pulmonaria officinalis* subsp. *officinalis*, *Symphytum officinale*; Scrophulariaceae: *Digitalis grandiflora*, *Euphrasia rostkoviana*, *Euphrasia stricta*, *Linaria vulgaris*, *Melampyrum arvense*, *Melampyrum barbatum*, *Melampyrum bihariense*, *Melampyrum cristatum*, *Odontites verna* subsp. *verna*, *Rhinanthus rumelicus*, *Verbascum chaixii* subsp. *austriacum*, *Verbascum lychnitis*, *Veronica beccabunga*, *Veronica chamaedrys*, *Veronica orchidea*, *Veronica teucrium*; Orobanchaceae: *Orobanche alba*, *Orobanche lutea*; Plantaginaceae: *Plantago lanceolata*, *Plantago major*, *Plantago media*; Verbenaceae: *Verbena officinalis*; Lamiaceae: *Acinos arvensis*, *Ajuga chamaepitys*, *Ajuga genevensis*, *Ajuga laxmani*, *Ajuga reptans*, *Ajuga salicifolia*, *Clinopodium vulgare*, *Galeopsis speciosa*, *Galeopsis x tetrahit*, *Lamium album*, *Lamium purpureum*, *Leonurus cardiaca*, *Lycopus europaeus*, *Mentha arvensis*, *Mentha longifolia*, *Mentha* x *verticillata*, *Nepeta nuda* (*Nepeta pannonica*), *Origanum vulgare*, *Phlomis tuberosum* (**Fig. 5**), *Prunella grandiflora* (**Fig. 6**), *Prunella laciniata*, *Prunella vulgaris* (**Fig. 7**), *Salvia austriaca*, *Salvia nemorosa*, *Salvia nutans*, *Salvia pratensis*, *Salvia transsilvanica*, *Salvia verticillata*, *Scutellaria galericulata*, *Stachys germanica*, *Stachys officinalis*, *Stachys recta*, *Teucrium chamaedrys*, *Thymus glabrescens*, *Thymus pannonicus*, *Thymus pulegioides*; Campanulaceae: *Asyneuma canescens*, *Campanula bononiensis*, *Campanula glomerata*, *Campanula persicifolia*, *Campanula rapunculoides*, *Campanula sibirica*; Asteraceae: *Achillea millefolium*, *Achillea setacea*, *Anthemis tinctoria*, *Arctium lappa*, *Artemisia absinthium*, *Artemisia campestris* subsp. *campestris*, *Artemisia pontica*, *Artemisia vulgaris*, *Aster linosyris*, *Bellis perennis*, *Bidens cernua*, *Bidens tripartita*, *Carduus acanthoides*, *Carlina vulgaris*, *Centaurea apiculata* subsp. *spinulosa*, *Centaurea biebersteinii*, *Centaurea cyanus*, *Centaurea rhenana*, *Cichorium intybus*, *Cirsium arvense*, *Cirsium canum*, *Conyza canadensis*, *Crepis biennis*, *Echinops sphaerocephalus*, *Erigeron acris*, *Eupatorium cannabinum*, *Galinsoga parviflora*, *Pilosella officinarum*, *Hieracium bauhini*, *Hieracium x sulphureum*, *Hypochoeris maculata*, *Hypochoeris radicata*, *Inula britannica*, *Inula ensifolia*, *Inula hirta*, *Jurinea mollis*, *Leontodon hispidus* subsp. *hispidus*, *Leucanthemum vulgare*, *Matricaria perforata*, *Matricaria recutita*, *Mycelis muralis*, *Scorzonera purpurea*, *Senecio jacobaea*, *Senecio paludosus*, *Sonchus arvensis*, *Sonchus palustris*, *Tanacetum vulgare*, *Taraxacum officinale*, *Tragopogon pratensis* subsp. *orientalis*, *Tussilago farfara*, *Xanthium strumarium*; Butomaceae: *Butomus umbellatus*; Alismataceae: *Alisma plantago-aquatica*; Juncaginaceae: *Triglochin palustre*; Najadaceae: *Najas marina*; Potamogetonaceae: *Potamogeton natans*; Alliaceae: *Allium albidum* subsp. *albidum*, *Allium paniculatum*, *Allium scorodoprasum* subsp. *scorodoprasum*; Amaryllidaceae: *Galanthus nivalis*, *Narcissus poeticus* ssp. *radiiflorus*; Iridaceae: *Iris aphylla*, *Iris pseudacorus*, *Iris ruthenica*; Liliaceae: *Anthericum ramosum*, *Asparagus officinalis*, *Convallaria majalis*, *Erythronium dens-canis*, *Muscari comosum*, *Muscari tenuiflorum*, *Ornithogalum pyramidale*, *Polygonatum latifolium*, *Polygonatum multiflorum*, *Polygonatum odoratum*, *Veratrum nigrum* (**Fig. 8**); Orchidaceae: *Epipactis palustris*; *Hammarbya paludosa*, *Orchis morio*, *Orchis tridentata* ssp. *tridentata*; Juncaceae: *Juncus conglomeratus*, *Juncus effusus*, *Juncus tenuis*, *Luzula campestris*; Cyperaceae: *Carex*

*caryophyllea*, *Carex paniculata*, *Carex vulpina*, *Cyperus flavescent*, *Scirpus sylvaticus*; Typhaceae: *Typha angustifolia*, *Typha latifolia*; Poaceae: *Agrostis capillaris*, *Agrostis stolonifera*, *Alopecurus aequalis*, *Alopecurus pratensis*, *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Brachypodium pinnatum*, *Briza media*, *Bromus erectus* subsp. *erectus*, *Calamagrostis epigejos*, *Chrysopogon gryllus*, *Cynosurus cristatus*, *Dactylis glomerata* subsp. *glomerata*, *Deschampsia caespitosa*, *Echinochloa crus-galli*, *Festuca rupicola* subsp. *rupicola* f. *hirsuta*, *Festuca valesiaca*, *Glyceria maxima*, *Holcus lanatus*, *Lolium perenne*, *Phragmites australis*, *Poa palustris*, *Phleum phleoides*, *Setaria viridis*, *Stipa tirsa* (*S. stenophylla*), *Trisetum flavescens*, Lemnaceae: *Lemna minor*.

### 3.2 Medicinal plants identified in Fărăgău area and their uses in human disease

Of the 397 taxa identified 164 species are medicinal. Thus, it was observed that most plants contain: volatile oils (13.41%), tannins (12.19%), flavonoids (9.75%), mucilages (8.53%), coumarins (7.92%), saponins (7.31%), alkaloids (6.70%), iridoids (5.48%), phenolic glycosides (4.87%), organic acids, vitamins and provitamins 2.43%), anthryquinone derivatives (1.21%), cardiotonic glycosides (3.65%), bitter principles (2.43%), bitter-aromatic principles (4.26%), homoglycans, senevoline glycosides, depside, fatty oils, allantoin, resins, sulfurized compounds and floroglucines (0.60% each). Thus, we mention medicinal herbs that contain these principles: homoglicans: *Arctium lappa* (radix); mucilage: *Hibiscus trionum* (herba), *Lavathera thuringiaca* (radix), *Malva sylvestris* (flos et folium), *Orchis morio*, *O. tridentata* (tuber), *Plantago sp.* (folium), *Tussilago farfara* (folium), *Verbascum* sp. (flos); **senevolic glycosides**: *Raphanus raphanistrum* (radix); **phenolic glycosides**: *Filipendula ulmaria* (flos), *Populus* sp. (gemma), *Pyrus pyraster* (folium), *Salix* sp. (cortex), *Viburnum opulus* (cortex); **anthraquinone derivatives**: *Frangula alnus* (cortex), *Rumex crispus* (rhizoma); **naphtodianthrones**: *Hypericum perforatum* (herba); **cardiac glycosides**: *Convallaria majalis* (herba), *Digitalis grandiflora* (folium), *Erysimum odoratum* (herba), *Euonymus europaea* (cortex), *Helleborus purpurascens* (rhizoma et radix), *Leonurus cardiaca* (herba); **saponins**: *Anagallis* sp. (herba), *Bellis perennis* (flos), *Eryngium* sp. (herba), *Equisetum arvense* (herba), *Hedera helix* (herba), *Ononis arvensis* (radix), *Polygala* sp. (herba), *Primula veris* (rhizoma cum radicibus), *Ranunculus ficaria* (radix); **flavonoids**: *Bidens* sp. (herba), *Capsella bursa-pastoris* (herba), *Crataegus monogyna* (folium, fructus et flos), *Eupatorium cannabinum* (rhizoma et radix), *Filipendula ulmaria* (herba), *Linaria vulgaris* (herba), *Morus alba* (folium), *Pilosella officinarum* (herba), *Polygonum aviculare* (herba), *Prunus avium* (stipites), *Robinia pseudacacia* (flos), *Sambucus nigra* (flos), *Veronica* sp. (herba), *Vincetoxicum hirundinaria* (radix); **anthocyanins**: *Centaurea cyanus* (flos), *Consolida regalis* (flos), *Papaver rhoeas* (flos), *Rosa gallica* (flos); **coumarins**: *Cruciata* sp. (herba), *Galium* sp. (herba), *Heracleum sphondylium* (radix, folium et fructus), *Medicago* sp. (herba), *Melilotus officinalis* (flos et herba), *Pastinaca sativa* (radix), *Pimpinella saxifraga* (radix); **tannins**: *Agrimonia eupatoria* (herba), *Anthyllis vulneraria* (flos), *Cornus sanguinea* (cortex), *Corylus avellana* (folium), *Epilobium hirsutum* (herba), *Erigeron acris* (summitates), *Fragaria vesca* (folium), *Geum urbanum* (rhizoma), *Juglans regia* (folium), *Lysimachia* sp. (herba), *Lythrum salicaria* (herba), *Polygonum aviculare* (herba), *Potentilla anserina* (herba), *P. argentea*, *P. recta* (rhizoma), *Prunus spinosa* (flos, fructus),

*Quercus robur* (cortex), *Salix* sp. (cortex); **depsides**: *Cichorium intybus* (herba et radix); **fatty oils**: *Oenothera biennis* (semen); **essential oils**: *Achillea millefolium* (flos), *Asarum europaeum* (rhizoma), *Carum carvi* (fructus), *Iris* sp. (rhizoma), *Matricaria recutita* (flos), *Mentha* sp. (folium), *Nepeta nuda* (summitates), *Origanum vulgare* (herba), *Peucedanum oreoselinum* (rhizoma), *Pimpinella saxifraga* (radix), *Thymus* sp. (herba), *Tilia cordata* (flos), *Valeriana officinalis* (radix), *Xanthium strumarium* (herba); **allantoin**: *Symphytum officinale* (radix); **resins**: *Humulus lupulus* (strobuli); **glycoresins**: *Calystegia sepium* (herba), *Convolvulus arvensis* (herba); **sulfur compounds**: *Armoracia rusticana* (radix); **iridoids**: *Ajuga* sp. (herba), *Euphrasia* sp. (herba), *Lamium album* (herba), *Sambucus ebulus* (radix, flos, fructus), *Stachys* sp. (herba), *Verbena officinalis* (herba); **alkaloids**: *Aconitum anthora* (tuber), *Chelidonium majus* (herba), *Conium maculatum* (fructus), *Datura stramonium* (folium), *Echium vulgare* (herba), *Galanthus nivalis* (bulbus), *Genista tinctoria* (herba), *Solanum dulcamara* (stipes), *Thalictrum minus* (herba), *Vinca* sp. (herba); **bitter compounds**: *Centaurium erythraea* (herba), *Euphorbia cyparisias* (herba), *Gentiana cruciata* (radix), *Taraxacum officinale* (radix et herba); **bitter-aromatic compounds**: *Artemisia* sp. (herba), *Teucrium chamaedrys* (herba), *Tanacetum corymbosum* (flos), *T. vulgare* (herba); **floroglucine**: *Dryopteris filix-mas* (rhizoma); **organic acids, vitamins and provitamins**: *Daucus carota* (radix), *Hippophae rhamnoides* (fructus), *Physalis alkekengi* (fructus), *Rosa canina* (fructus), *Urtica dioica* (fructus).

Of the 164 medicinal species included in the floral inventory, 37 species supply plant products included in the Romanian Pharmacopoeia and European Pharmacopoeia, the 8$^{th}$ edition: *Achillea millefolium*, *Oenothera biennis*, *Agrimonia eupatoria*, *Origanum vulgare*, *Artemisia absinthium*, *Papaver rhoeas*, *Carum carvi*, *Plantago lanceolata*, *Centaurium erythraea*, *Polygonum multiflorum*, *Chelidonium majus*, *Polygonum aviculare*, *Crataegus monogyna*, *Primula veris*, *Datura stramonium*, *Prunella vulgaris*, *Equisetum arvense*, *Quercus robur*, *Filipendula ulmaria*, *Robinia psudacacia*, *Frangula alnus*, *Rosa canina*, *Hedera helix*, *Salix* sp., *Humulus lupulus*, *Sambucus nigra*, *Hypericum perforatum*, *Taraxacum officinale*, *Leonurus cardiaca*, *Tilia cordata*, *Lythrum salicaria*, *Valeriana officinalis*, *Malva sylvestris*, *Verbena officinalis*, *Matricaria recutita*, *Urtica dioica*, *Melilotus officinalis*.

Even if only partial, the data on Romania's medicinal flora clearly reveals two fundamental features of it, namely: a great taxonomic diversity and an exceptional therapeutic potential. Flora from Fărăgău area 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 (27 taxa), respiratory system (15 taxa), skin disorders (9 taxa), muscular and skeletal systems (11 taxa), genitourinary system (9 taxa), in gynaecological disorders (4 taxa), cardiovascular (3 taxa), CNS disorders (4 taxa) (**Table 1**). The paper highlights the importance of herbs that can be used as remedies for human diseases. 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.

### 3.3 Protected plants in Fărăgău area

The special interest manifested today in the world for natural medicine, where phytotherapy occupies a privileged place, can sometimes have negative repercussions for the

**Table 1.** Medicinal plant used in various disorders

| **Phytotherapy for human disease** | **Disorders of various systems** | **Taxa** |
|---|---|---|
| **Phytotherapy for digestive system disorders** | Phytotherapy of mouth<br>▪ gingivitis<br>▪ stomatitis<br>▪ periodontitis<br>▪ dental abscesses<br>▪ tonsillitis | *Achillea millefolium, Agrimonia eupatoria, Centaurium erythraea, Geum urbanum, Lysimachia nummularia, Lythrum salicaria, Matricaria chamomilla, Potentilla sp., Quercus robur, Thymus* sp. |
| | Hyperacid gastritis and ulcer disease | *Equisetum arvense, Hypericum perforatum, Medicago sativa, Melilotus officinalis, Symphytum officinale* |
| | Gastric hypoacidity - dyspepsia, anorexia | *Artemisia vulgaris, Centaurium erythrea, Gentiana cruciata* |
| | Acute and chronic liver disease | *Achillea millefolium, Hypericum perforatum, Taraxacum officinalis;* |
| | Functional disorders of the gallbladder and biliary tract | *Achillea millefolium, Agrimonia eupatoria, Cichorium intybus, Eupatorium cannabinum, Hypericum perforatum, Mentha longifolia, Pastinaca sativa, Taraxacum officinale* |
| | Phytotherapy in constipation | *Cichorium intybus, Convolvulus arvense, Rumex* sp. |
| | Phytotherapy in diarrhea | *Agrimonia eupatoria, Geum urbanum, Lythrum salicaria, Potentilla anserina, Quercus robur, Rosa canina* |
| | Vomiting - nausea | *Mentha longifolia* |
| | Abdominal colic | *Achillea millefolium, Matricaria chamomilla, Mentha longifolia, Potentilla anserine, Salix* sp. |
| | Flatulence (bloating) | *Carum carvi, Mentha longifolia* |
| | Helminthiasis - anthelmintic plant | *Achillea millefolium, Dryopteris filix-mas, Gentiana cruciata, Rosa canina, Tanacetum vulgare, Thymus* sp. |
| **Phytotherapy for cardiovascular system disorders** | Hearth failure | *Digitalis grandiflora* |
| | Cardiac neurosis | *Convallaria majalis, Crataegus monogyna* |
| | Angina pectoris | *Crataegus monogyna* |
| **Phytotherapy for respiratory system disorders** | Immuno-stimulatory plant | *Achillea millefolium, Equisetum arvense, Hypericum perforatum, Rosa canina* |
| | Central and peripheral antitussives | *Datura stramonium, Plantago* sp., *Thymus* sp., *Tussilago farfara, Verbascum lychnitis* |
| | Expectorant | *Eryngium planum, Hedera helix, Primula veris, Picea abies* |
| | Asthma | *Ajuga reptans, Datura stramonium, Origanum vulgare, Thymus* sp. |
| **Phytotherapy for genitourinary system disorders** | Diuretic / acvaretice | *Equisetum arvense, Hibiscus trionum, Lamium album, Ononis arvensis, Polygonum aviculare, Prunus avium, Taraxacum officinale, 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* |
| | Eczema | *Achillea millefolium, Taraxacum officinale* |
| | Dermatomycosis | *Achillea millefolium, Populus* sp, *Thymus* sp. |
| | Alopecia (hair loss) | *Urtica dioica* |
| | Wounds | *Equisetum arvense, Hypericum perforatum, Rosa canina, Populus* sp., *Plantago* sp., *Symphytum officinale* |
| | Light burns | *Hypericum sp., Populus* sp. |
| | Bruises | *Achillea millefolium, Symphytum officinale* |
| **Phytotherapy for locomotory system disorders** | Plant products with anti-inflammatory / analgesic anti-rheumatic and hyperemic action | *Filipendula ulmaria, Helleborus purpurascens, Hypericum perforatum, Rosa canina, Medicago sativa, Mentha longifolia, Picea abies, Salix alba, Taraxacum officinale, Urtica dioica* |
| **Phytotherapy for CNS system disorders** | Sleep disturbances; Nervousness. depression | *Humulus lupulus, Hypericum* sp., *Valeriana officinalis, Viburnum opulus* |

conservation of some plant species in the spontaneous flora. This can happen in cases where the species vegetates in a restricted area and the natural regeneration capacity is low. As a result of this study, we bring novel information about floristic richness of hayfields, an invaluable scientific value for the Romania and Europe. In the follow we present some plants protected by law at European and national level: *Echium maculatum* (**Fig. 9**), *Cephalaria radiata* (**Fig. 10**), *Crambe tataria* (**Fig. 11**), *Narcissus poeticus* ssp. *radiiflorus* (**Fig. 12**), *Salvia nutans* (**Fig. 13**), *Iris aphylla*, *Orchis morio* (**Fig. 14**), *Orchis tridentata*, *Adonis vernalis* (**Fig. 15**), *Dictamnus albus* (**Fig. 16**), *Hammarbya paludosa* etc.

## Conclusions

The work highlights the importance of medicinal plants in the Fărăgău area, which can be used as remedies for human diseases. The florist inventory includes 397 plant species, distributed in 82 families. The most represented are the families: Asteraceae (52 taxa), Fabaceae (34 taxa), Lamiaceae (35 taxa), Poaceae (25 taxa), Scrophulariaceae (16 taxa), Apiaceae (17 taxa), Ranunculaceae (14 taxa) etc. The 164 species of medicinal plants were grouped according to the dominant active principles: volatile oils (13.41%), tannins (12.19%), flavonoids (9.75%), mucilages (8.53%), coumarins (7.92%), saponins (7.31%), alkaloids (6.70%), and iridoids (5.48%). Of the 164 medicinal species, 37 species are included in the Romanian Pharmacopoeia and the European Pharmacopoeia 2008. The identified medicinal plants can be used in the treatment of various human diseases. Also, a significant number of plants are protected by law at national and European level.

## 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.

**Fig. 2.** *Centaurium erythraea*

**Fig. 3.** *Gentiana cruciata*

**Fig. 4.** *Physalis alkekengi*

**Fig. 5.** *Phlomis tuberosa*

**Fig. 6.** *Prunella grandiflora*

**Fig. 7.** *Prunella vulgaris*


**Fig. 8.** *Veratrum nigrum:* a – general aspect, b – flowers, c - fruits

**Fig. 9.** *Echium maculatum*

**Fig. 10.** *Cephalaria radiata*

**Fig. 11**. *Crambe tataria*

**Fig. 12.** *Narcissus poeticus* ssp. *radiiflorus*

**Fig. 13.** *Salvia nutans*

**Fig. 14.** *Orchis morio*

**Fig. 15.** *Dictamnus albus*

**Fig. 16.** *Adonis vernalis*

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**ORIGINAL PAPER**

# THE NYÁRÁDY ERAZMUS GYULA'S HERBARIUM IN THE DEBRECEN UNIVERSITY'S PLANT COLLECTION

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**Abstract:** After the publication of the book entitled "Recollection of Gyula E. Nyárády" (2016), the interest of his inheritance increased significantly. His left behind herbarium was estimated to have 55,000 sheets (1988), while till 2016 its number increased up to 85,000. The herbarial investigations are taking place over the Romania's borders too, such as in the plant collection of the Debrecen University. With this occasion, we have studied the part collections of Rezső Soó (40,000 specimens), the Zoltán Siroki's (20,000 specimens), together with the kryptogame (3,000 bryophytes) ones. In the Debrecen University plant collection we found 166 plant species collected and determined by E. Gy. Nyárády, among them 154 are superior plant and 12 are moss. In the Soó collection 112 plants arose from E. Gy. Nyárády, 69% of them are from Slovakia, 29% from Romania and 1% from Poland. The Slovakian collections took place in the 1905-1916 period, the most of them (34 species) are from 1910, being collected in Késmárk and Tatra's region, where E. Gy. Nyárády was secondary school teacher. The Romanian collections took place in the 1905-1942 period, the 33 species mainly arise from the high mountains (especially Rodna Mountains), as well as from the Transylvanian Plain. We have found three endemic species among them: *Festuca carpathica* Dietr., *Koeleria transsilvanica* Schur (syn. *Koeleria macracantha* ssp. *transsilvanica* (Schur) A. Nyár., and *Thymus pulcherrimus* Schur. In the Siroki collection there are 42 plant sheets, originated from Slovakia, from 1908-1913 period. The four Romanian plants came from the Rodna Mountains. The Kryptogam Herbarium contains 12 Romanian moss species, collected between 1925 and 1929, most of them being from Székelyudvarhely (Odorheiu Secuiesc). The genus *Carex* occurs most frequently in the Nyárády-collection, due to his increased interest to sedges, forming 39% of the studied species. In accordance with the labels, in his collecting trips he was occasionally accompanied by Béla Husz (1911, Szepes) and Ádám Boros (1929, Korond).

**Keywords:** herbarium, collection of Rezső Soó, collection of Zoltán Siroki, Romania, Slovakia, history of botany.

## 1. Introduction

After the publication of the book entitled "Recollection of Gyula E. Nyárády" (Bartók et al., 2016), the interest on the work and inheritance of Nyárády increased significantly. The book presents in details the plant collections of Nyárády held in the Romanian universities and museums. Based on these data the number of herbarium sheets collected by Nyárády is estimated to more than 85,000.

In this study, our aim was to conduct researches at the universities and museums beyond the borders of Romania, primarily in

Hungary, and find new materials and plant collections of Gyula E. Nyárády. At the beginning of our research we found 154 Nyárády sheets in the Móra Ferenc Museum from Szeged (disposable on the Internet), which arrived there in 1909 via exchange. These collections originate from the first decade of the 1900s. Today, the materials of the Museum and University of Szeged are owned by the Hungarian Natural History Museum, Budapest (Bartók et al., 2016). In October 2016, with the Domus Hungarica scholarship, a research was conducted in the Herbarium Carpato-Pannonicum from the Natural History Museum. This collection is not digitized, but the sheets are organized by taxonomic criteria. There is no separate collection for Gyula E. Nyárády. From the 630,000 herbarium pages found here, about 12,000 were checked, among which 98 plant taxa were collected and prepared by Nyárády. These data were digitized and the herbarium sheets were photographed. Our goal is to finish this research with a new Domus scholarship.

In the second part of the Domus scholarship (4-18 December, 2016), the research was continued with the botanical collections from the University of Debrecen. The Faculty of Humanities from this University was founded relatively late (in 1929), so their herbarium consists of a small number of sheets (under 100,000). The herbarium is composed of two parts: the vascular plant collection and the cryptogam collection. The vascular plant collection consists of 3 part collections: Rezső Soó Herbarium (RSH ~ 40,000 sheets), Zoltán Siroki Herbarium (ZSH ~ 20,000 sheets) and Árpád Degen Herbarium (ÁDH ~ 30,000 sheets); the cryptogam collection contains 3000 capsules with mosses and 2500 with lichens.

The RSH and the ZSH is digitized from 2013. Attila Takács, collaborator of the Department of Botany (Debrecen University), renewed the herbarium sheets, placed them in a systematic order, recorded the data in a database, and photographed the sheets. All these have been the subject of his doctoral thesis (Takács, 2016).

The aims of this study were: the selection of herbarium sheets collected by Gyula E. Nyárády held at the University of Debrecen; the inclusion of the data in an electronic database; the completion of the sheets with different important collection attributes; the preparation of the photo collection about the specimens found.

## 2. Materials and Methods

The data about each specimen were recorded in a table: scientific name, exact place of collection (country, county, locality, mountains etc.), collection time (year, month, day), reviewer name, and review time (if applicable). Pictures were taken about all specimens found.

## 3. Results and discussions

In the herbarium of the University of Debrecen, 166 plants collected by Gyula E. Nyárády were found, of which 154 are vascular plants and 12 moss species (kept in capsules).

### 3.1 The Herbarium of Rezső Soó (RSH)

The RSH contains 112 plant sheets collected by Nyárády. The plants were collected in: Slovakia (69%, 79 sp.), Romania (29%, 33 sp.) and Poland (1%).

The material from Slovakia was collected by Nyárády between 1905 and 1919 (**Fig. 1**) when he was a secondary school teacher at Késmárk. Collection places, their meticulous description, and phenological observations of plants are found in the book “Recollection of Gyula E. Nyárády” (Chapter II/16 "Gyula E. Nyárády Self-description, life and work" p. 193-212, in Bartók et al., 2016). In his manuscripts Nyárády describes in details his

fieldtrips in Késmárk and its surroundings, the excursions in the Tatras, and the interesting plant taxa found during the fieldtrips.

The number of plants collected increases with the number of years spent in Késmárk and also with the length of the fieldtrips. While the median number of collected specimens did not exceed 5 herbarium sheets between 1905 and 1909, in 1911 their number reached 34. In the summer of 1911, he stopped being teacher in Késmárk (**Fig. 2**) and in autumn of the same year, he became teacher of the Roman Catholic Gymnasium from Marosvásárhely (Târgu Mureș), where he activated until 1922. According to herbarium data, he returned only one time in Slovakia, in 1916, when he collected *Carex ornithopoda* W. near Bratislava. Nyárády made a statistic on his fieldtrips from the period 1905-1911 (Bartók et al., 2016, Chapter II/16, p. 204). According to this, he attended 106 trips in Késmárk and its surroundings, 59 in the High Tatras and the Low Tatras, 7 in the Western Tatras, 4 in Lőcse Mountains, 18 in Transylvania, and 1 in Bánát (Banat). Thus, in 7 years, he attended 225 fieldtrips. Would a botanist be able to do this nowadays, keeping in mind the road conditions and travel opportunities at that time?

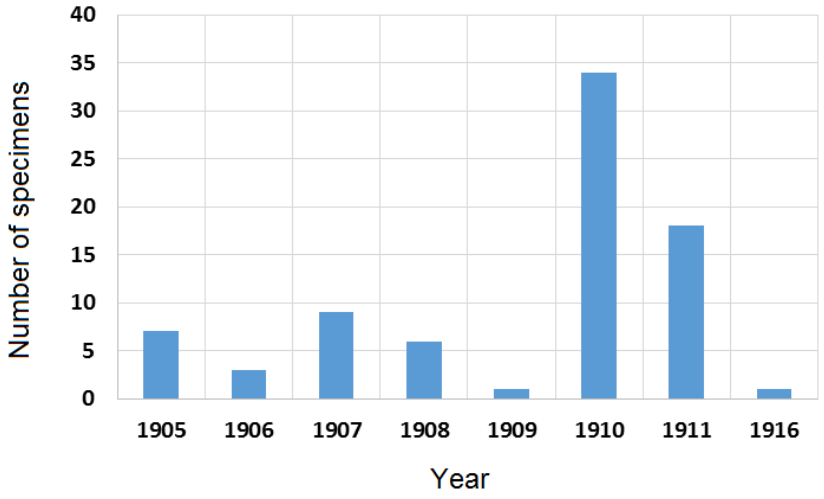


**Fig. 1.** The number of yearly collected taxa by Nyárády in Slovakia (data from the Rezső Soó Herbarium)

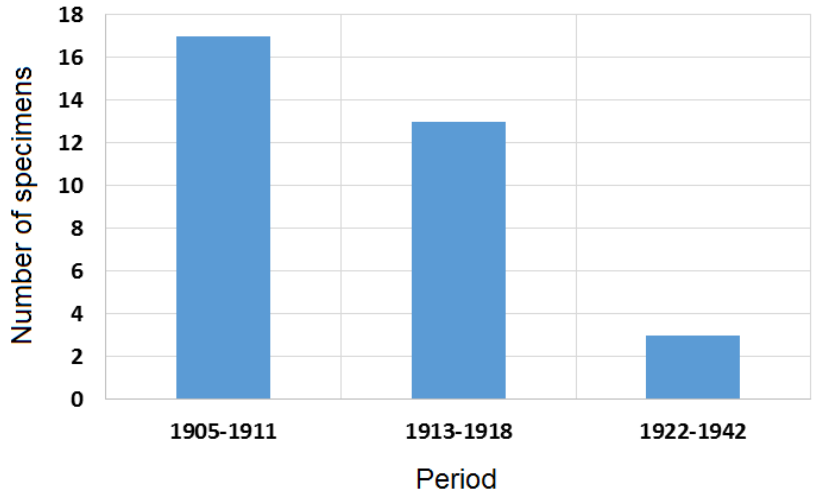


**Fig. 2.** Number of taxa collected by Nyárády during his teacher reasoning period (data from the Rezső Soó Herbarium)

According to the RSH, Nyárády collected a total of 33 plant taxa in Romania (Transylvania), between 1905 and 1942. Most plants (17 sp.) were collected in the period when he was teaching in Késmárk (1905-1911), spending the summer holydays at home, in Transylvania. From the time when he was teacher in Marosvásárhely (Târgu Mureş) (1912-1918) there are 13 herbarium sheets in the Soó-collection, while from the period 1922-1942, only 3 species were found (**Fig. 3**).

The data presented in **Fig. 1-3** do not reflect the real work of Nyárády as a botanist or the volume of his collections. It is known that between 1922 and 1942, Nyárády was the curator of University herbarium at Cluj, and it was his duty to enrich the herbarium material. During these years he traveled not only in Transylvania, but all around Romania, creating a remarkable herbarium collection. However, the RSH is almost completely lack of collections from these years. Why was not enriched this herbarium during these years? What sort of selection was made in case of those sheets which were included in the herbarium from that period? These remain unanswered questions. The majority of specimens from the RSH are collected from high mountain area: Radnai-(Rodnei), Görgény (Gurghiu), Kelemen (Călimani), Fogarasi (Făgăraş). A smaller part was collected from the Transylvanian Plain. Three endemic species were found. Their accepted scientific names and conservation status were checked in Flora Europaea, List of Plant, certain internationally accepted technical books etc., and also in the latest Romanian botanical works (Ciocârlan 2009, Sârbu et al., 2013). First the name of the species found in the RSH is mentioned, followed by the valid synonym utilized today:

- *Festuca carpathica* F. Dietr. (Ciocârlan 2009; Sârbu et al. 2013); syn. *Leucopea carpatica* (F. Dietr.) H. Scholz (Flora Europea)-scientific name not used by Sârbu and Ciocârlan; Carpathian endemic present in Romania, Poland, Slovakia, Ukraine;
- *Koeleria transsilvanica* Shur.; syn. *Koeleria macrantha* ssp. *transsilvanica* (Schur) A. Nyár. (Ciocârlan, 2009; Sârbu et al. 2013; Flora Europaea), Endemic in Romania-R (Rare);
- *Thymus pulcherrimus* Schur. (Ciocârlan 2009; Sârbu et al., 2013; Flora Europaea). Carpathian endemic present in Romania, Poland, Czech Republic, Ukraine-R (Rare).

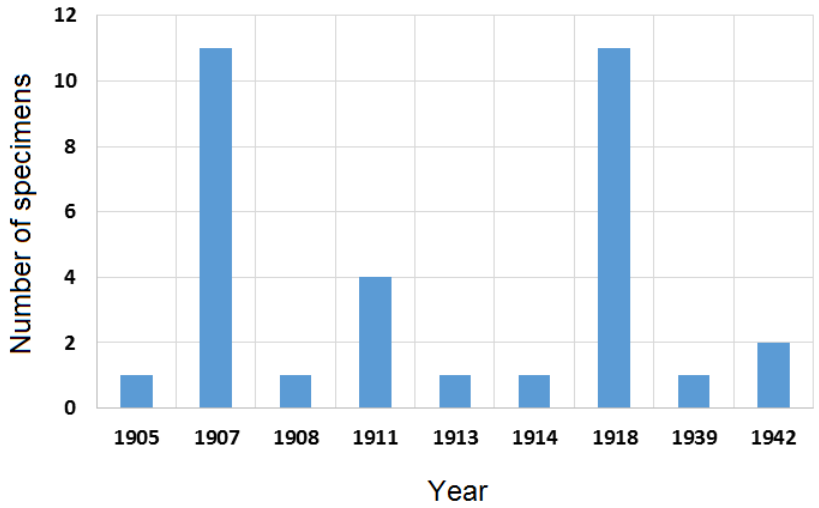


**Fig. 3.** The number of yearly collected specimens by Nyárády in Transylvania (data from the Rezső Soó Herbarium)

### 3.2 The Herbarium of Zoltán Siróki (ZSH)

Zoltán Siróki (1906-1987), ornithologist-botanist, agricultural engineer, is well-known in Debrecen, since his last workplace was at the Agricultural Academy of Debrecen, Department of Biology, where he was an associate professor. Zoltán Siróki is not known in Transylvania, so a few words about his activity will be noted. When he retired, he became preoccupied to study the Department's plant collection. With his successor, György Mándy, he accepted the entire herbarium of the former Seed Testing Station of Budapest, which was formed under Árpád Degen's board. It is likely that the species collected by Nyárády found in the ZSH are from the Degen-collection. These are kept by the Herbarium of the University of Debrecen. In the ZSH 42 species collected and preserved by Nyárády were found. From Slovakia were collected up to 90% (38 species) of the species, in the period 1908-1913. The herbarium sheets dating from 1910 are in higher number (24). The remaining sheets (11) are from 1911 (**Fig. 4**). There are only 4 sheets from Romania: *Carex pyreneica* Whlbg. (the same species from two collection points, dating from 22 July, 1909) from the Zănoaga Lake, Retezat Mountain; *Ranunculus crenatus* W. et K. from the Rodna Mountains, near Ünökő (Ineu), dating from 8 July, 1918; *Daphne mezereum* L. from Ratosnya (Răstolița), Mureș Valley, dating from 7 June, 1914. When the number of plant species is observed as a function of collecting time (**Fig. 5**), the month of June is the most favorable for both collections (RSH-36 and SZH-15 collected specimens) (**Fig. 6**).

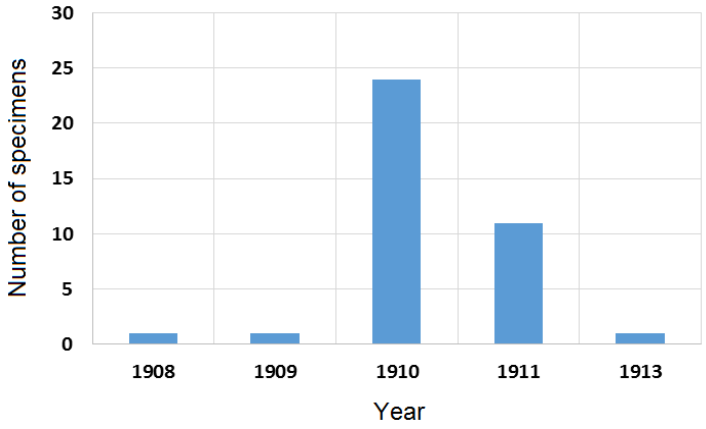


**Fig. 4.** The number of yearly collected specimens in Slovakia (data from the Zoltán Siroki Herbarium)

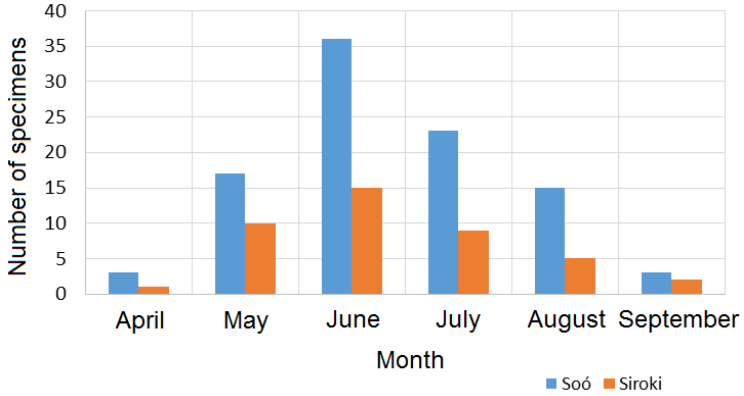


**Fig. 5.** Number of specimens in function of collecting time (months)

It should be also mentioned that in the ZSH 29 plants belonging to the *Carex* genus were found (collected in different locations), while in the RSH 31 species. There are a total of 60 sheets with *Carex* species in the herbarium from Debrecen, which represents 39% of the entire collection. Nyárády's special interest for the sedge species had resulted in the discovery of *Carex chordorrhiza* in 1910, in the area of Késmárk, a new species identified in the Carpathians. The discovery was reported in 1911, in the periodical Magyar Botanikai Lapok (Nyárády, 1911; Bartók et al., 2016). His interest for sedges remained, so later in 1962 he published an article on *Caricetum humilis* phytoconosis, from Transylvania and Moldova.

### 3.3 The cryptogam collection

Of the 3000 moss species from the cryptogam collection only 12 were collected by Gyula E. Nyárády, between 1925 and 1929. These species were all collected from Romania (Transylvania):

- 8 species near Székelyudvarhely (Odorheiu Secuiesc, Harghita County) from different humid areas (Tolvajos rivulet, Festőmalom, Lucs, Oroszhegy);
- two species from the area around Gyergyóalfalu (Joseni, Harghita County).

These collections can be related with the foundation of the Department of Natural Sciences of the Székely National Museum from Sepsiszentgyörgy (Sfântu Gheorghe). The basis of the newly formed department consisted of vascular plants and cryptogam species collected, determined and donated to the Museum by Gyula E. Nyárády. The results of his botanical researches from Szeklerland have appeared in several works in 1929, for example: in the Memorial Book of the Sepsiszentgyörgyi Museum, Csíki Lapok, Ifjú Erdély, Pásztortűz (Bartók et al., 2016).

### 3.4 The Herbarium of Árpád Degen (ÁDH)

This part of the collection is not digitalized, so we had great hopes that we will find plant sheets collected by Gyula E. Nyárády. It is known (Bartók et al, 2016) that Degen, during a study trip around Késmárk, has discovered in Nyárády a young and novice teacher. The friendship and collegiality between the two botanists lasted until the death of Degen. Nyárády remembers Degen as his mentor, who helped greatly to define plants with dubious status. So it is impossible that there would be no exchange or gift making between them with the collected plants.

Unfortunately, we did not find any herbarium sheets from Nyárády in ÁDH, although we have reviewed many plant genera (*Poa*, *Nardus*, *Bromus*, *Lolium*, *Campanula*, *Solidago*, *Phyteuma*, *Bellis*) and families (Asteraceae, Rosaceae, Orchidracea).

The Degen Herbarium, which we have reviewed, is from the second half of the 1800's, so it is made up of more than 150 years old plant sheets. The collectors were not Hungarians. We mention some of them: Richter (1876), Kugler (1882), Schultz (1861), Border (1879) etc. At that time Nyárády was not even born.

## Conclusions

It passed more than 50 years since the death of the great botanist Gyula E. Nyárády, although his work, his spread herbarium is still not fully known. The size of his herbarium from Romania is known, only accidental discoveries could increase the number of these herbarium sheets. We are currently searching for his herbarium sheets beyond the borders of Romania. Thus the purpose of our study was to explore the herbarium of the University of Debrecen. A total of 166 plants were found in the Rezső Soó Herbarium (RSH), Siroki Zoltán

Herbarium (ZSH), and in the cryptogam collection: 154 vascular plants and 12 moss species. There are 112 plants in the RSH, 42 in the ZSH. Both herbariums are dominated by the specimens collected in Slovakia (77 and 38, respectively), and the month of June was the preferred collection time. In the list of plants collected from Romania, three endemic species of Transylvania were found. Nyárády was very interested in the *Carex* genus, so in his collection 39% of the vascular plants are different sedge species.

## 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.

## Acknowledgments

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**Fig. 6.** Herbarium sheets of Gyula E. Nyárády from the University of Debrecen: **A**-*Athyrium alpestre* (Tatras, 4 February, 1907); **B**-*Carex atrata* (Bélai Mountains, 29 June, 1910); **C**-*Ranunculus dentatus* (Călimani Mountains, 20 May, 1918); **D**-*Aster alpinus* (Szádelői Valley, 3 June, 1911).

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## SUPPLEMENTARY MATERIALS

**Supplementary material 1**

**Table 2.** Plants used in the traditional medicine and/or phytotherapy from some representative plant communities in the Niraj Valley

| | **Taxa in alphabetical order/ Sozological categories** [a] | **Dominant active principles** | **Drugs / Presence in the Pharmacopoeia** [b] | **Harvesting period** |
|---|---|---|---|---|
| 1. | *Acer campestre* ssp. *campestre* | Tannins | Cortex, folium | V-IX |
| 2. | *Acer platanoides* | Tannins | Cortex | II-IV |
| 3. | *Achillea millefolium* ssp. *millefolium* | Essential oils | Millefolii flos, Millefolii herba **Eur. Ph., Rom. Ph.** | VI-IX |
| 4. | *Achillea millefolium* var. *collina* | Essential oils | Millefolii flos, Millefolii herba | VI-IX |
| 5. | *Achillea ptarmica* **RL, Oltean et al.** | Essential oils | Flos | - |
| 6. | *Achillea setacea* | Essential oils | Flos | VI-IX |
| 7. | *Adonis aestivalis* | Cardiotonic glycosides | Adonidis herba | IV-VI |
| 8. | *Agrimonia eupatoria* ssp. *eupatoria* | Tannins | Agrimoniae herba **Eur. Ph.** | VI-VIII |
| 9. | *Ajuga reptans* | Iridoids | Ajugae herba, folium, flos | IV-VI |
| 10. | *Alliaria petiolata* | Compuși sulfurați | Alliariae herba | IV-VI |
| 11. | *Alnus glutinosa* | Tannins | Alni cortex, Alni folium, Alni gemma | III-IV, IV-V |
| 12. | *Anagallis arvensis* | Saponins | Anagallidis herba | V-IX |
| 13. | *Anthriscus sylvestris* | Flavonoids | Herba, fructus | V-VII |
| 14. | *Anthyllis vulneraria* ssp. *vulneraria* | Tannins | Anthyllis herba, flos | V-VIII |
| 15. | *Arctium lappa* | Homoglycans | Bardanae radix | II-III, X-XI |
| 16. | *Artemisia vulgaris* | Bitter-aromatic principles | Artemisiae vulgaris herba | VI-VIII |
| 17. | *Arum maculatum* | Saponins | Rhizoma | III-IV, IX-XI |
| 18. | *Asarum europaeum* | Essential oils | Asari rhizoma | IV-V, VIII-IX |
| 19. | *Asparagus officinalis* | Proteins, lipids, carbon hydrates | Asparagi rhizoma et radix | I-XII |
| 20. | *Atropa bella-donna* | Alkaloids | Belladonnae folium et radix **Eur. Ph., Rom. Ph.** | V-VII, IX-XI |
| 21. | *Ballota nigra* | Bitter principles | Ballotae nigrae herba **Eur. Ph.** | VIII |

| | | | | |
|---|---|---|---|---|
| 22. | *Brassica nigra* | Senevolic glycosides | Sinapis nigrae semen **Rom. Ph.** | VII-VIII |
| 23. | *Bupleurum falcatum* ssp. *falcatum* | Saponins | Radix **Eur. Ph.** | IX-XI |
| 24. | *Calluna vulgaris* | Phenolic glycosides | Callunae herba | VI-IX |
| 25. | *Calystegia sepium* | Glicorezine | Herba | VI-IX |
| 26. | *Capsella bursa-pastoris* | Flavonoids | Bursae pastoris herba | V-VIII |
| 27. | *Cardamine bulbifera* | Bitter principles | Rhizoma | IX-XI |
| 28. | *Cardamine pratensis* | Organic acids, vitamins and provitamins | Folium | IV-VII |
| 29. | *Carlina acaulis* ssp. *acaulis* | Flavonoids | Carlinae radix | VIII-IX |
| 30. | *Centaurea jacea* ssp. *jacea* | Anthocyanins | Flos | VI-IX |
| 31. | *Centaurium erythraea* ssp. *erythraea* | Bitter principles | Centaurii herba **Eur. Ph.** | VI-VII |
| 32. | *Chelidonium majus* | Alkaloids | Cheledonii herba, Chelidonii radix **Eur. Ph., Rom. Ph.** | IV-V, III-IV |
| 33. | *Cichorium intybus* | Depside | Cichorii radix et herba **Eur. Ph.** | II-III, X-XI, VI-VIII |
| 34. | *Clematis vitalba* | Alkaloids | Folium | VI-IX |
| 35. | *Colchicum autumnale* | Alkaloids | Colchici bulbus et semen **Rom. Ph.** | IX-X, VI |
| 36. | *Conium maculatum* | Alkaloids | Conii fructus et herba | VI-VII |
| 37. | *Convallaria majalis* | Cardiotonic glycosides | Convallariae herba | V-VI |
| 38. | *Convolvulus arvensis* | Glycoresins | Convolvuli herba | IV-IX |
| 39. | *Cornus sanguinea* | Tannins | Cortex | III-IV |
| 40. | *Coronilla varia* | Alkaloids | Herba, Semen | VI-IX |
| 41. | *Corydalis cava* | Alkaloids | Tuber | V-XI |
| 42. | *Corylus avellana* | Tannins | Coryli folium | VI-VIII |
| 43. | *Crataegus monogyna* | Flavonoids | Crataegi folium cum flos, Crataegi fructus **Eur. Ph., Rom. Ph.** | V-VI, VII-VIII |
| 44. | *Cruciata laevipes* | Coumarins | Herba | IV-V |
| 45. | *Daucus carota* ssp. *carota* | Organic acids, vitamins and provitamins | Radix | IX-XI |
| 46. | *Digitalis grandiflora* | Cardiotonic glycosides | Folium | V-VI |
| 47. | *Dryopteris filix-mas* | Floroglucine | Filicis maris rhizoma | IX-XI, III-IV |
| 48. | *Echium vulgare* | Alkaloids | Echii herba | VI-VIII |

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|---|---|---|---|---|
| 49. | *Elymus repens* ssp. *repens* | Homoglycans | Graminis rhizoma **Eur. Ph.** | III-X |
| 50. | *Epilobium hirsutum* | Tannins | Epilobi hirsuti herba | VI-VIII |
| 51. | *Epilobium montanum* | Tannins | Epilobi montani herba | VI-IX |
| 52. | *Equisetum arvense* | Saponins | Equiseti herba **Eur. Ph., Rom. Ph.** | V-VII |
| 53. | *Erigeron acris* ssp. *acris* | Tannins | Summitates | V-X |
| 54. | *Erigeron annuus* ssp. *annuus* | Tannins | Summitates | VII-VIII |
| 55. | *Eryngium campestre* | Saponins | Eryngii campestris rhizoma | III-IV, X-XI |
| 56. | *Eryngium planum* | Saponins | Eryngii plani herba | VII-VIII |
| 57. | *Euonymus europaeus* | Cardiotonic glycosides | Euonymi cortex | IX-XI |
| 58. | *Euonymus verrucosus* | Cardiotonic glycosides | Cortex | IX-XI |
| 59. | *Eupatorium cannabinum* | Flavonoids | Eupatorii cannabini rhizoma, radix et folium | VII-VIII |
| 60. | *Euphorbia cyparissias* | Resins | Radix | IX-XI |
| 61. | *Euphorbia salicifolia* | Organic acids, vitamins and provitamins | Flos | V-VI |
| 62. | *Euphrasia rostkoviana* ssp. *rostkoviana* | Iridoids | Euphrasiae herba | VI-IX |
| 63. | *Fagus sylvatica* | Tannins | Creosotum | IV-V |
| 64. | *Filipendula ulmaria* | Phenolic glycosides | Ulmariae herba **Eur. Ph.** | VI-VIII |
| 65. | *Filipendula vulgaris* | Flavonoids | Filipendulae flos, Filipendulae herba cum radicibus, Filipendulae radix | V-VII, VIII-IX |
| 66. | *Fragaria vesca* | Tannins | Fragariae folium | V-IX |
| 67. | *Fragaria viridis* ssp. *viridis* | Tannins | Folium | V-IX |
| 68. | *Frangula alnus* | Anthraquinone derivatives | Frangulae cortex **Eur. Ph., Rom. Ph.** | II-IV |
| 69. | *Fraxinus excelsior* | Coumarins | Fraxini folium **Eur. Ph.** | VI-IX |
| 70. | *Galeobdolon luteum* | Iridoids | Herba | IV-V |
| 71. | *Galium aparine* | Coumarins | Galii herba | V-IX |
| 72. | *Galium mollugo* | Coumarins | Herba | VI-IX |
| 73. | *Galium odoratum* | Coumarins | Asperulae herba | IV-VI |
| 74. | *Galium verum* | Coumarins | Galii veri herba | V-IX |
| 75. | *Genista sagittalis* | Alkaloids | Herba | VI-VII |
| 76. | *Genista tinctoria* ssp. *tinctoria* | Alkaloids | Genistae tinctoriae herba | VI-VIII |
| 77. | *Geranium phaeum* | Tannins | Herba | V-VI |
| 78. | *Geranium robertianum* | Tannins | Geranii robertiani herba | VI-VIII |

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|---|---|---|---|---|
| 79. | *Geum urbanum* | Tannins | Gei rhizoma | III-IV, IX-X |
| 80. | *Glechoma hederacea* | Bitter principles | Hederae terestris herba | IV-VI |
| 81. | *Hedera helix* | Saponins | Hederae helicis folium **Eur. Ph.** | I-XII |
| 82. | *Helianthemum nummularium* ssp. *nummularium* | Tannins | Folium | V-VII |
| 83. | *Helleborus purpurascens* | Cardiotonic glycosides | Hellebori radix et rhizoma | III-IV, VIII-X |
| 84. | *Heracleum sphondylium* | Coumarins | Heraclei sphondylii radix, Heraclei sphondylii herba | VI-IX, IX-XI |
| 85. | *Humulus lupulus* | Resins | Lupuli strobuli, Lupuli glandulae **Eur. Ph.** | VIII-IX |
| 86. | *Hypericum perforatum* | Naftodiantrone | Hyperici herba **Eur. Ph., Rom. Ph.** | VI-VIII |
| 87. | *Iris pseudacorus* | Essential oils | Iridis pseudacori rhizoma | VIII-IX |
| 88. | *Iris sibirica* **RL, Dihoru and Dihoru** | Essential oils | Rhizoma | - |
| 89. | *Juglans regia* | Tannins | Juglandis folium, Juglandis pericarpium, Juglandis cortex | VI-VII, IX |
| 90. | *Lamium album* | Iridoids | Lamii albi flos, Lamii albi herba | IV-VIII |
| 91. | *Lamium purpureum* | Tannins | Lamii purpureumi herba | III-IX |
| 92. | *Leucojum vernum* | Alkaloids | Bulbus | IV-XI |
| 93. | *Linaria vulgaris* | Flavonoids | Linariae herba | VI-IX |
| 94. | *Linum austriacum* | Mucilages | Semen | VI-VIII |
| 95. | *Linum catharticum* | Mucilages | Semen | VI-VIII |
| 96. | *Linum flavum* | Mucilages | Semen | VI-VIII |
| 97. | *Linum hirsutum* | Mucilages | Semen | VI-VIII |
| 98. | *Lotus corniculatus* | Anthocyanins | Flos | V-IX |
| 99. | *Lysimachia nummularia* | Tannins | Lysimachiae herba | V-VII |
| 100. | *Lysimachia vulgaris* | Tannins | Herba | VI-VII |
| 101. | *Lythrum salicaria* | Tannins | Salicariae herba **Eur. Ph.** | VI-VIII |
| 102. | *Malus sylvestris* | Tannins | Malusi sylvestrisi fructus **Eur. Ph.** | IX-X |
| 103. | *Malva sylvestris* ssp. *sylvestris* | Mucilages | Malvae flos et folium | VI-VII |
| 104. | *Medicago falcata* | Coumarins | Herba | V-X |
| 105. | *Melilotus officinalis* | Coumarins | Meliloti herba, flos **Eur. Ph.** | VI-VIII |
| 106. | *Mentha aquatica* | Essential | Menthae aquaticae | VI-VIII |

| | | | | |
|---|---|---|---|---|
| | | oils | folium | |
| 107. | *Mentha arvensis* spp. *arvensis* | Essential oils | Folium | VI-VIII |
| 108. | *Mentha longifolia* ssp. *longifolia* | Essential oils | Folium | VI-VIII |
| 109. | *Mentha pulegium* | Essential oils | Menthae pulegii herba | VI-VIII |
| 110. | *Mercurialis perennis* | Saponins | Herba | IV-VI |
| 111. | *Mycelis muralis* | Iridoids | Myceli herba | VI-VIII |
| 112. | *Narcissus poeticus* ssp. *radiiflorus* **BC** | Alkaloids | Bulbus | - |
| 113. | *Nepeta nuda* ssp. *nuda* | Essential oils | Semen | VII-VIII |
| 114. | *Oenanthe aquatica* | Essential oils | Fructus | VII-VIII |
| 115. | *Ononis arvensis* ssp. *arvensis* | Saponins | Radix | III-IV, X-XI |
| 116. | *Orchis laxiflora* ssp. *elegans* **RL, Oltean et al.** | Mucilages | Tuber | - |
| 117. | *Orchis militaris* **RL, Oltean et al.** | Mucilages | Tuber | - |
| 118. | *Orchis morio* **RL, Oltean et al.** | Mucilages | Salep tuber | - |
| 119. | *Origanum vulgare* ssp. *vulgare* | Essential oils | Origani herba **Eur. Ph.** | VI-VIII |
| 120. | *Papaver rhoeas* | Anthocyanins | Rhoeados flos **Eur. Ph.** | VI-VII |
| 121. | *Pastinaca sativa* ssp. *sativa* | Coumarins | Pastinaci radix | IX-XI |
| 122. | *Pastinaca sativa* ssp. *sylvestris* | Coumarins | Radix | IX-XI |
| 123. | *Petasites hybridus* | Essential oils | Petasitidis rhizoma | III-IV, IX-X |
| 124. | *Peucedanum oreoselinum* | Essential oils | Rhizoma | IX-XI |
| 125. | *Physalis alkekengi* | Organic acids, vitamins and provitamins | Alkekengi fructus | VIII-IX |
| 126. | *Pimpinella saxifraga* ssp. *saxifraga* | Essential oils, Coumarins | Pimpinellae radix | IV-X |
| 127. | *Plantago lanceolata* | Mucilages | Plantaginis folium **Eur. Ph.** | V-X |
| 128. | *Plantago major* | Mucilages | Folium | V-X |
| 129. | *Plantago media* | Mucilages | Folium | V-X |
| 130. | *Polygala comosa* | Saponins | Herba | IV-VI |
| 131. | *Polygala major* | Saponins | Herba | IV-VI |
| 132. | *Polygala vulgaris* | Saponins | Polygalae radix | IX-XI |
| 133. | *Polygonatum odoratum* | Cardiotonic | Polygonati rhizoma | IX-X |

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| | | glycosides | et radix | |
| 134. | *Polygonum bistorta* | Tannins | Bistortae rhizoma **Eur. Ph.** | III-IV, IX-X |
| 135. | *Polygonum hydropiper* | Flavonoids | Polygoni hydropiperis herba | VII-IX |
| 136. | *Populus alba* | Phenolic glycosides | Gemma | II-III |
| 137. | *Populus nigra* | Phenolic glycosides | Populi gemma | II-III |
| 138. | *Populus tremula* | Phenolic glycosides | Populi cortex | II-IV |
| 139. | *Potentilla argentea* ssp. *argentea* | Tannins | Rhizoma | IX-X |
| 140. | *Potentilla erecta* | Tannins | Tormentillae rhizoma **Eur. Ph.** | IX-X |
| 141. | *Potentilla recta* ssp. *recta* | Tannins | Rhizoma | IX-X |
| 142. | *Potentilla reptans* | Tannins | Rhizoma | IX-X |
| 143. | *Primula veris* ssp. *veris* | Saponins | Primulae rhizoma cum radicibus **Eur. Ph., Rom. Ph.** | IV-V, IX-X |
| 144. | *Prunella vulgaris* | Tannins | Prunellae herba | VI-VIII |
| 145. | *Prunus avium* | Flavonoids | Cerasorum stipites | VI-VI |
| 146. | *Prunus spinosa* | Tannins | Pruni spinosae flos et fructus | IV-V, X-XI |
| 147. | *Prunus tenella* **RL, Oltean et al.** | Cyanogenic glycosides | Folium, semen | - |
| 148. | *Pulicaria dysenterica* | Flavonoids | Herba | VII-IX |
| 149. | *Pulmonaria officinalis* | Saponins | Pulmonariae folium | III-IV |
| 150. | *Pyrus pyraster* | Phenolic glycosides | Folium | IV-V |
| 151. | *Quercus petraea* | Tannins | Cortex | III-IV |
| 152. | *Quercus robur* | Tannins | Quercus cortex **Eur. Ph.** | III-IV |
| 153. | *Ranunculus ficaria* | Saponins | Ficariae radix, Ficariae folium | III, IX-X |
| 154. | *Robinia pseudoacacia* | Flavonoids | Acaciae flos | V-VI |
| 155. | *Rosa canina* | Organic acids, vitamins and provitamins | Cynosbati fructus **Eur. Ph.** | IX-X |
| 156. | *Rosa gallica* | Anthocyanins | Rosae petalum | V-VI |
| 157. | *Rubus caesius* | Organic acids, vitamins and provitamins | Folium | V-IX |
| 158. | *Rubus idaeus* | Organic acids, vitamins and provitamins | Rubi ideae folium | V-VII |
| 159. | *Rumex acetosa* | Anthraquinone derivatives | Rhizoma | III-V, IX-X |
| 160. | *Rumex acetosella* | Anthraquinone | Rhizoma | III-V, |

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| | ssp. *acetosella* | derivatives | | IX-X |
| 161. | *Rumex crispus* | Anthraquinone derivatives | Rhizoma | III-V, IX-X |
| 162. | *Salix alba* | Tannins, Phenolic glycosides | Salicis cortex **Eur. Ph.** | II-VI |
| 163. | *Salix caprea* | Tannins, Phenolic glycosides | Cortex | II-VI |
| 164. | *Salix cinerea* | Tannins, Phenolic glycosides | Cortex | II-VI |
| 165. | *Salix fragilis* | Tannins, Phenolic glycosides | Cortex | II-VI |
| 166. | *Salix purpurea* ssp. *lambertiana* | Tannins | Cortex | II-VI |
| 167. | *Sambucus nigra* | Flavonoids | Sambuci flos **Eur. Ph.** | VI-VII |
| 168. | *Sanguisorba officinalis* | Tannins | Sanguisorbae herba **Eur. Ph.** | V-VIII |
| 169. | *Sanicula europaea* | Saponins | Saniculae europaeae rhizoma, herba | V-VII, IX-XI |
| 170. | *Saponaria officinalis* | Saponins | Saponariae rubrae radix | VIII-IX |
| 171. | *Scabiosa ochroleuca* | Flavonoids | Herba | VI-VIII |
| 172. | *Scrophularia nodosa* | Iridoids | Herba | VI-VIII |
| 173. | *Scrophularia scopolii* | Iridoids | Herba | V-VIII |
| 174. | *Senecio jacobea* | Flavonoids | Herba | VII-VIII |
| 175. | *Solanum dulcamara* | Alkaloids | Dulcamarae stipes | VI-VII, IX-X |
| 176. | *Solidago virgaurea* ssp. *virgaurea* | Saponins | Solidaginis summitates **Eur. Ph.** | VII-IX |
| 177. | *Stachys germanica* | Iridoids | Herba | VI-VIII |
| 178. | *Stachys officinalis* | Iridoids | Stachysi herba | VI-VIII |
| 179. | *Stellaria media* | Saponins | Herba | III-X |
| 180. | *Symphytum officinale* | Allantoin | Symphyti radix et folium | III-IV, X |
| 181. | *Tanacetum corymbosum* | Bitter-aromatic principles | Flos | VI-VIII |
| 182. | *Tanacetum vulgare* | Bitter-aromatic principles | Tanaceti flos | VII-IX |
| 183. | *Taraxacum officinale* | Bitter principles | Taraxaci radix, Taraxaci herba **Eur. Ph.** | X-XI, III-IX |
| 184. | *Teucrium chamaedrys* | Bitter-aromatic principles | Chamaedryos herba | VI-IX |
| 185. | *Thalictrum minus* | Alkaloids | Herba | V-VIII |
| 186. | *Thymus glabrescens* | Essential oils | Herba | V-VIII |

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| 187. | *Thymus pannonicus* ssp. *pannonicus* | Essential oils | Herba | V-VIII |
| 188. | *Thymus pulegioides* ssp. *pulegioides* | Essential oils | Herba | V-VIII |
| 189. | *Tilia cordata* | Essential oils | Tiliae flos **Eur. Ph., Rom. Ph.** | VI-VII |
| 190. | *Trifolium campestre* | Flavonoids | Herba | V-IX |
| 191. | *Trifolium pratense* | Flavonoids | Trifolii rubi flos | V-IX |
| 192. | *Trifolium repens* | Flavonoids | Trifolii albi herba | V-X |
| 193. | *Tussilago farfara* | Mucilages | Farfarae folium, Farfarae flos | IV-VI, II-IV |
| 194. | *Urtica dioica* | Organic acids, vitamins and provitamins | Urticae folium et radix **Eur. Ph.** | V-IX, III-V, IX-X |
| 195. | *Valeriana officinalis* | Essential oils | Valerianae rhizoma et radix **Eur. Ph., Rom. Ph.** | X-XI |
| 196. | *Veratrum album* | Alkaloids | Veratri rhizoma | IX-X |
| 197. | *Verbascum nigrum* | Mucilages | Flos | VI-VIII |
| 198. | *Verbascum phoeniceum* | Mucilages | Flos | V-VII |
| 199. | *Veronica chamaedrys* ssp. *chamaedrys* | Flavonoids | Herba | IV-VIII |
| 200. | *Veronica officinalis* | Flavonoids | Veronicae herba | VI-VIII |
| 201. | *Veronica orchidea* | Flavonoids | Herba | VI-IX |
| 202. | *Veronica spicata* ssp. *spicata* | Flavonoids | Herba | VI-IX |
| 203. | *Veronica teucrium* ssp. *teucrium* | Flavonoids | Herba | V-VII |
| 204. | *Viburnum opulus* | Phenolic glycosides | Viburni cortex | III-IV, IX-X |
| 205. | *Vinca minor* | Alkaloids | Vincae minoris herba **Rom. Ph.** | V-VI |
| 206. | *Vincetoxicum hirundinaria* ssp. *hirundinaria* | Flavonoids | Radix | V-VIII |
| 207. | *Viola odorata* | Saponins | Violae odoratae radix | II-III, IX-X |
| 208. | *Viola tricolor* | Saponins | Violae tricoloris herba **Eur. Ph.** | V-VIII |

Note: a - Sozological categories: **BC** - Species included in the Bern Convention; **RL Oltean et al.** - Species on the Red List of Superior Plants from Romania: Oltean M, Negrean G, Popescu A, Roman N, Dihoru GH, Sanda V, Mihăilescu S (1994) Lista roşie a plantelor superioare din România-Studii, sinteze, documentaţii de ecologie, Ed. Acad. Română, Inst. de Biol., București; **RL Dihoru and Dihoru** - Species on the Red List: Dihoru G, Dihoru A (1994) Plante rare, periclitate și endemice în Flora României-Lista Roșie. Acta Bot Horti bucurest 1993-1994:173–197; b - Pharmacopoeia: **Eur. Ph.** - European Pharmacopoeia Online 9.3 http://online6.edqm.eu/ep903/. Accessed 16 June 2018; **Rom. Ph.** - Farmacopeea Română (1993) Ed. a X-a, Ed. Medicală, București.

**Supplementary material 2**

**Table 1.** The number of the medicinal plant taxa for each family from the Carpathian Basins medicinal flora and in the studied area

| | **Family in alphabetical order** | **Number of the medicinal plant taxa from the Carpathian Basin** | **Number of the medicinal plant taxa in the studied area** |
|---|---|---|---|
| 1. | Acoraceae | 1 | 0 |
| 2. | Adoxaceae | 4 | 3 |
| 3. | Anacardiaceae | 1 | 0 |
| 4. | Amaryllidaceae | 1 | 0 |
| 5. | Apiaceae | 7 | 2 |
| 6. | Apocynaceae | 1 | 1 |
| 7. | Araliaceae | 1 | 1 |
| 8. | Aristolochiaceae | 1 | 1 |
| 9. | Asparagaceae | 3 | 1 |
| 10. | Asteraceae | 29 | 13 |
| 11. | Berberidaceae | 1 | 0 |
| 12. | Betulaceae | 3 | 2 |
| 13. | Boraginaceae | 5 | 3 |
| 14. | Brassicaceae | 4 | 1 |
| 15. | Cannabaceae | 2 | 2 |
| 16. | Caprifoliaceae | 1 | 1 |
| 17. | Caryophyllaceae | 5 | 1 |
| 18. | Colchicaceae | 1 | 1 |
| 19. | Convolvulaceae | 2 | 2 |
| 20. | Crassulaceae | 1 | 0 |
| 21. | Cupressaceae | 1 | 0 |
| 22. | Droseraceae | 1 | 0 |
| 23. | Dryopteridaceae | 1 | 1 |
| 24. | Eleagnaceae | 1 | 1 |
| 25. | Ephedraceae | 1 | 0 |
| 26. | Ericaceae | 4 | 0 |
| 27. | Equisetaceae | 1 | 1 |
| 28. | Fabaceae | 9 | 7 |
| 29. | Fagaceae | 4 | 3 |
| 30. | Gentianaceae | 7 | 1 |
| 31. | Geraniaceae | 2 | 1 |
| 32. | Grossulaceae | 1 | 0 |
| 33. | Hypericaceae | 4 | 1 |
| 34. | Juglandaceae | 1 | 1 |
| 35. | Lamiaceae | 26 | 7 |
| 36. | Lycopodiaceae | 2 | 0 |

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| 37. | Lythraceae | 1 | 1 |
| 38. | Malvaceae | 10 | 2 |
| 39. | Melanthiaceae | 1 | 0 |
| 40. | Menyanthaceae | 1 | 1 |
| 41. | Oleaceae | 3 | 1 |
| 42. | Onagraceae | 4 | 0 |
| 43. | Orobanchaceae | 1 | 0 |
| 44. | Papaveraceae | 8 | 2 |
| 45. | Pinaceae | 3 | 1 |
| 46. | Plantaginaceae | 8 | 4 |
| 47. | Poaceae | 2 | 2 |
| 48. | Polygonaceae | 9 | 2 |
| 49. | Polypodiaceae | 1 | 0 |
| 50. | Primulaceae | 2 | 1 |
| 51. | Ranunculaceae | 9 | 4 |
| 52. | Rhamnaceae | 1 | 1 |
| 53. | Rosaceae | 49 | 8 |
| 54. | Rubiaceae | 3 | 3 |
| 55. | Rutaceae | 1 | 0 |
| 56. | Salicaceae | 24 | 3 |
| 57. | Santalaceae | 1 | 1 |
| 58. | Scrophulariaceae | 4 | 0 |
| 59. | Solanaceae | 5 | 2 |
| 60. | Taxaceae | 1 | 0 |
| 61. | Urticaceae | 2 | 1 |
| 62. | Verbenaceae | 1 | 0 |
| 63. | Violaceae | 4 | 2 |
| 64. | Zygophyllaceae | 1 | 0 |