PhD Thesis

Ethnobotanical evaluation of Covasna County; Histological, phytochemical, microbiological and cytotoxic analysis of *Aristolochia clematitis* L.

Dr. Sámuel Gergely Bartha

Doctoral School of Pharmacological and Pharmaceutical Sciences

Program for isolation and analysis of bioactive compounds

Head of doctoral school: Dr. Erika Pintér full professor

Head of the program: Prof. Dr. József Deli full professor

Supervisor:

Dr. Nóra Papp

Dr. Mónika Kerényi

University of Pécs

Faculty of Pharmacy

Department of Pharmacognosy

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1. Introduction

Ethnopharmacobotany focuses on the connection between people and (medicinal) plants, and the research on people's knowledge related to the application of plants. According to the definition by Gunda (1971), "*Ethnobotany is the common research area of ethnography and botany that examines the role of plants in human culture, the ways of their application together with the related beliefs and customs.*" Today it is a task of high priority all over the world to record and to document people's knowledge about plants, medicinal plants passed on from generation to generation. The young generations are less interested in the knowledge from their parents and grandparents, which is another aspect that highlights the consequence of the collections.

During the past 30 years, the field research and collections related to ethnomedicine had upsurged again; according to a survey conducted earlier, between 1992 and 2014, there were 182 studies related to the topic had been published.The publication of the results has been going on from the area of several countries even in recent times; between 2011 and 2014, the number of publications was 25% higher than during the previous years. These data underlies the actuality of our choice of topic.

In Transylvania, the first pieces of art, books on herbs with relevance from an ethnobotanical point of view have been published in the 16th century (Lencsés 1570; Melius 1578). During the past 60-70 years research activities revived e.g. in the Gheorgheni Basin, the Ghimeș region, along the Homorod rivers, within the area of Țara Călatei and Satu Mare, and also in the Úz Valley. The settlements subject to the research covered by the thesis are located in Covasna County of diverse flora; its neighbour counties are Brașov and Harghita.

The common birthwort (*Aristolochia clematitis* L., Aristolochiaceae) selected based on earlier field works occurs in the Caucasus, Western-Asia and Europe at floodplain forests, along the roads and orchards. The leaf to be harvested in full flower contains e.g. resin, bitter substance, aristolochic acid (I, II), minerals, and polyphenols. In ethnomedicine, its leaves are used to treat eczema, chancrous wounds (mainly for animals), cracked heel, phlebitis and ulceration in Transylvania, but there are records on its use for wound care from Kosovo and Serbia as well. Based on pharmacological data collected earlier the aristolochic acid stimulates the operation of the phagocytes, therefore the immunity against certain pyogenic bacteria increases, however, it can cause the inflammation of the mucous membrane, or might cause diverticulitis or nephritis, reduces the blood pressure, and in the case of severe poisoning cardiac paralysis might

occur as well. Today we have no information on the internal application of the species considering its aristolochic acid content.

2. Aims

The most important objectives of the thesis can be classified around two main topics: planning the ethnopharmacobotanical survey in the confined regions of Transylvania, and the analysis of common birthwort (*A. clematitis*) selected based on earlier collected data in the region, as follows:

- field research on people's knowledge about medicinal plants in Transylvania, including 17 settlements of Covasna County, where even today we can find elements of the traditional knowledge, and so far there has been no similar survey made regarding the local folk data; the research performed included primarily the identification and documenting the mentioned plant species used in the local healing methods, administration / the way of their application, preparation, and the related knowledge;
- evaluation and comparison of the recorded data with results from scientific databases (Google Scholar, PubMed, Research Gate, ScienceDirect);
- histological, phytochemical, microbiological, and cytotoxic study and evaluation of the common birthwort based on the available methods and opportunities, as there are relatively limited technical data available about this species in literature sources to the present.

3. Methods

3.1. Methods of ethnobotanical collection

During the field research between 2010 and 2018, we performed ethnopharmacobotanical collection in 17 settlements of Covasna County, these were the following: Brăduț, Biborţeni, Bodos, Filia, Racoşul de Sus, Băţanii Mici, Aita Medie, Herculian, Micloșoara, Băţanii Mari, Tălişoara, AitaSeacă, Dobоșeni,Ozunca-Băi, Ormeniș, Vârghiş, and Valea Zălanului. During selecting the study areathe following aspects were taken into consideration:the informants should be Hungarian-speaking people; whether there have been similar surveys performed earlier in the region based on data from literature; is there permanent medical/veterinary/dentist service available on the field.

In these 17 settlements, we interviewed altogether 188 data providers (of ages between 11 and 98 years, mainly from the age group above 60 years), during discussions and semi-structured interviews we directed the discussions by using determined issues, according to disease groups. The selection of data providers has been performed by using the system of recommendations ("snowball sampling technique").The discussions were recorded by dictaphone (Olympus WS-110 and VN-7700; altogether 168 hours), meanwhile, we made notes and created photos of the informants (about 2700 pieces), the mentioned plant species, their habitats and the preparations (Canon Ixy Digital, Panasonic Lumix DMC-FZ8). Some of the plant species were documented on the field as *in vivo* plants, others were documented in dried form, as stored at home by the informant. We performed the identification of the questionable species by using plant identification handbooks.

During the interviews, we have recorded the habitat of the mentioned plant, its local name, used part, preparation type, and the method of preparation for veterinary and medical purposes as well.Furthermore, beliefs and local customs were also recordedrelated to the diseases, plants and their application, and the plants mentioned for other purposes (e.g.dyeing plants and others used as food or fodder).

3.2. Histological methods

Histological analyses of common birthwort were performed at the Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs. The parts subject to the analysis were the root, leaf, petiole, stem, flower and fruit. The samples were collected in Romania, on the confines of Augustin, Romania in 2017, from plant vegetation located along the road.

Samples were fixed in 1:1:1 mixture of 96% ethanol, glycerine and distilled water. After dehydration infiltration and blocks were created, then glued the blocks on the basic part, and cut 10 μm thick sections with rotation microtome (Anglia Scientific). The sections were dried, stained with toluidine blue and have been covered. The sections were documented in the Institute of Biology, Faculty of Sciences, University of Pécs, using a Nikon Eclipse 80i microscope and Spot Basic v4.0 program.

3.3. Phytochemical analyses – HPLC

We performed the phytochemical analysis of the stem, the root, the leaf and the fruit of common birthwort with HPLC (high-pressure liquid chromatography) at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Semmelweis University. The sample phases made of methanol, hexane, chloroform, ethyl acetate, butanol, and water were solved in 1 mL of methanol.

The analysis of aristolochic acid I (AAI) and aristolochic acid II (AAII) was based on the Sorenson and Sullivan method validated earlier, applied minor modifications. HPLC analysis was performed using the following equipment: Agilent 1260 Infinity LC system12B binary gradient pump, G1367E auto-sampler, G1315C diode-array detector, Agilent Technologies, Waldbronn, Germany), for which Kinetex C18 column was applied (100 mm×4.6 mm, 2.6 μm; Phenomenex, Los Angeles, California, USA) at (20°C). The flow rate of the gradient elution program was 0.7 mL/minute, where eluent A was 0.1% (v/v) formic acid and eluent B was acetonitrile: 0 minute: 20% (v/v) B, 25 minutes: 70% (v/v) B, 30 minutes: 100% (v/v) B, 31 minutes: 20% (v/v) B, 40 minutes: 20% (v/v) B. The UV measuring was made at 390 nm. The calibration curve was prepared using six concentrations between 1 and 500 μg/mL. The calibration curves were created with regression analysis according to the least-squares best-fit line and constant weighting. For both isomers, linear correlation was identified according to the following equation: $y = 41.164x-0.4646$ ($r^2 = 0.9998$) and $y = 40.3225x-0.4516$ ($r^2 = 0.9997$) for AA I and for AA II (x = the concentration of the components in μg/mL, y = the peak area of the components).

3.4. Study of cytotoxicity

The cytotoxicity tests were performed at the Department of Medical Microbiology and Immunology, Medical School, University of Pécs. First, water extracts of the leaf, the root and the fruit of common birthwort were tested on cell culture grown on 96-well culture plates: the growth medium (RPMI 1640 Lonsa) was removed from the cultures, then 5 μL, 2.5 μL and 1.25 μL of each extract were added into 4 wells on the cell culture using the pipette, then 200 μL of growth medium (RPMI 1640 Lonsa) was measured. After incubation and the removal of the mixture of extract and growth medium, DPBS (salt solution with diphosphate buffer) was used for washing three times, then 200 μL 2% DPBS solution with formalin was used for each well for fixation. Then, the cell culture has been stained using 0.13% crystal violet, 0.5% ethanol and 2% PBS solution with formalin. After staining we rinsed twice using DPBS, then dissolved the remaining fixated cells with 10% SDS (sodium dodecyl sulfate) and PBS of 50%

ethanol.The measurement was performed at 595 nm useda BMG LABTECH spectrophotometer. The tested cell lines were the following: Vero – monkey kidney cell line, Int-407 – human small intestine cell line, HeLa – human cervical cancer cell line, and HACAT – human epidermis cell line.

3.5. Microbiological study

The antimicrobial studieswerecarried out at the Department of Medical Microbiology and Immunology, Medical School, University of Pécs. In the case of the Mueller-Hinton growth medium,the microdilution technique was performed on sterile 96-well culture plates (Sarstedt). After drying and grinding the plant parts (leaf, stem, root, fruit; 3 g each) methanol was added, then placed in a shaking thermostat (New Brunswick) (24 hours, 37 °C, 150 rpm). After filtering and boiling off (Buchi vacuum evaporator), we received 6 extracts altogether for each plant part: methanol, hexane, chloroform, ethyl acetate, butanol, and water extracts (see the detailed description in the thesis). The boiled-off extracts were solved in dimethyl sulfoxide (DMSO).

Then 100-100 µl of Mueller-Hinton growth medium were added into the wells of the 96-well culture plates, then made a twofold dilution per row from the extracts (three parallel dilutions were prepared from the same extract). Finally, the plates were inoculated with bacteria culture according to 10^5 cfu (colony forming unit)/mL. The same bacterium was inoculated on each plate. After incubation, the reproduction of the bacteria strains was checked by turbidity test (no turbidity = inhibitor effect). Based on the above the minimum inhibitor concentration (MIC) of DMSO (dimethyl sulfoxide) was determined for the bacteria strains tested (µg/mL), these were the following:*Staphylococcus aureus* ATCC 23923, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 700698, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853,and clinical isolates of the broad spectrum*Escherichia coli* and *Klebsiella pneumoniae* (ESBL) producing beta-lactamase, multiresistant *Pseudomonas aeruginosa* (MDR), *Salmonella* Typhimurium(its abbreviated scientific name: *Salmonella enterica* subsp. *enterica* (Le Minor and Popoff) serovar. Typhimurium ATCC 14028), and *Acinetobacter baumannii* in the case of MDR.

4. Results and discussion

4.1.Etnopharmacobotanical data in Covasna County

During our field survey, we worked on 17 appointed research points and recorded 135 plant species which were classified into 52 plant families. The highest ratio is represented by Rosaceae/roses (16 species), Asteraceae/aster (14 species) and Lamiaceae/mint (11 species) families. Altogether 1-11 different local names/species were documented. There are 121 medicinal plants among 135 species, including 73 wild, 43 cultivated and 5 exotic plant species. Of the 121 species, all the 121 taxons were mentioned in human medicine, and 32 taxa were for veterinary purposes. There are overlaps among 55 plants for consumption, 12 fodder plants and 7 plants for colouring aims. Some of the species were mentioned frequently at the selected settlements (e.g. *Achillea millefolium, Petroselinum crispum, Plantago lanceolata, Solanum tuberosum*), while others were mentioned only by 1-2 persons (e.g. *Artemisia vulgaris, Leonurus cardiaca*).

Among the mentioned plants part the root,flowering shoot, flower, fruit, bark and tuber can be listed; the most frequently mentioned was the flowering shoot (44%). Among 12 preparation types the most frequently was the decoction (83%). In addition, creams, ointments, baths, washes, foments and tinctures were also mentioned. At the studied settlements 13 disease groupswere recorded, among them, the application of plants was most frequently mentioned for problems of the digestive system (51%), while less frequently for diseases related to vision and hearing (7%).

The collected elements of knowledge were compared to the data of the 8thHungarian Pharmacopoeia (focusing on the used drug part and the application), and it included 40 drugs from the species mentioned at the settlements. Comparing it to the content of the 10th Romanian Pharmacopoeia, overlaps of 20 drugs were observed.

The following sample article shows the detailed introductions of each species included in the thesis, and similar subtitles are used for the evaluation and the classification of the collected data for all species (after the names and the data the abbreviations of the settlements are mentioned in brackets – see in the thesis, while in the case of the drug, if it is relevant, the inscriptions of the8th Hungarian Pharmacopoeia and the 10th Romanian Pharmacopoeiaare shown; the local names and the quotations of the informants are printed in italics). Sample section of an ethnobotanically mentioned species of the thesis:

***Artemisia absinthium* L. / wormwood (Asteraceae/aster family)**

Local name: *üröm*(BA, BD, NB, SZA), *pelin* (MI), fehér üröm (BF, BD, EF, FR, KA, KB, NB, OT, SZA)

Drug: Absinthii herba (8th Ph. Hg., 10th F. R.)

Habitat: *"It cannot be purchased everywhere. At the mine road, it is possible. It was brought from there. The wormwood has always been brought from the mine road at Vargyas.*" (BF)

Morphology: *"It has white flowers. It is high, weedy*." (BF)

Preparation:decoction

Data collection: anthelmintic (BD), (FR), for *fever* of cattle (= hepatitis) (BF, BD, EF, FR, KB, KA, MH, NB, OT, SZA), liver diseases (BD, MI, SZA), icterus (BF), hepatogastritis (BF), (BD), (FR), nausea (BD, OT), gall problems (BD, MI), blast of cattle (EF), with blue-berry againstthe blast of sheep (EF), pneumonia (FR), as wine with wormwood it is aperitive (MI), against diarrhoea in veterinary treatment (KA).

"For the cow wormwood is good against hepatogastritis. Its decoction can be filled into its mouth. Then it will turn the inflammation. It is also good for humans against diarrhoea. Just it is very-very bitter." (BF)

"The wormwood is also good for humans when he has stomach and liver inflammation. (...) *Only a small glass of wormwood tea should be added into the cup, as small as the one used for brandy*." (BD)

"One of my brothers says (...), *that he cures himself with this when he has nausea."* (BD)

"If it badly hurts here. He keeps drinking wormwood. Every morning before breakfast, for three-four days. He keeps asking me, why don't you drink wormwood for your gall? It is good for all problems." (BD)

In addition to the data of medicinal plants, 14 materials from animals and 30 other materials were mentioned in the local practices, which are available in the annex of the thesis.

4.2. Result of the study of common birthwort

4.2.1. Ethnopharmacobotanical data

A. clematitis was called "*farkasalmalapi*" in 13 settlements in the study area. The parts used as drugs are the flowering shoot and the leaf; its decoction is used as foment for inflammations and purulent wounds (in veterinary and human medicine as well); in dried the plant is applied against wheatworm, for clothes against moths and worms. Details from the interviews:

"*When the horse has wounds, which are sensitive, the name is "farkasalmalapi". Prepare tea from it, and rinse the wound with this. No other medicine will do so much good for the horse*." (Biborţeni) *"They brewed the leaves of the birthwort, warmed it, covered with it, and it stops the inflammation.*" (Bodos)

4.2.2. Histological results

Among the studied parts of common birthwort, under the rhizodermis of the root, there are several rows of ground tissues that form the primary cortex. The epidermis cells of the stem are isodiametric, under those 4-7 rows of angular collenchyma cells, 3 rows of sclerenchyma cells, then storage ground cells were found. The structure of the leaf is heterogeneous and dorsiventral; the epidermis cells are flattened, there are flattened isodiametric cells on the abaxial side and at the midrib as well. The epidermis cells of the petiole are isodiametric on both sides, under those a few rows of angular collenchyma cells and ground cells were observed. The epidermis cells of the yellow tepals of the flower are elongated and cylindrical shaped. Ground tissues and vascular bundles are located in the mesophyll. Under the flattened epidermis cells of the globe-shaped capsule fruit, there are 3 rows of angular collenchyma cells. Towards the inner part of the fruit, isodiametric cells of the ground tissue were documented.

4.2.3. Phytochemical results – HPLC

In all tested fractions of *A. clematitis*, the presence of aristolochic acid I (AAI) and aristolochic acid II (AAII) were detected (Figure 1-4). The highest AAI component content was found in the ethyl acetate phase of the root (1347.9 µg), the stem (160.4 µg), the leaf (278.4 µg), and the chloroform phase of the fruit (821 µg). The highest AAII component content was present in the ethyl acetate phase of the root (953.6 µg) and the stem (37.1 µg), and in the chloroform phase of the leaf (44.2 µg), moreover in the ethyl acetate phase of the fruit (436.5 µg).

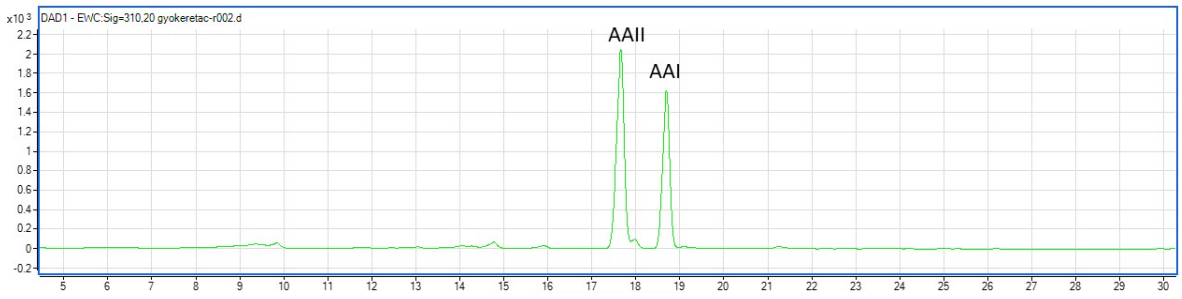

Figure 1. AAI and AAII content in the root extract of *A. clematitis* used ethyl acetate

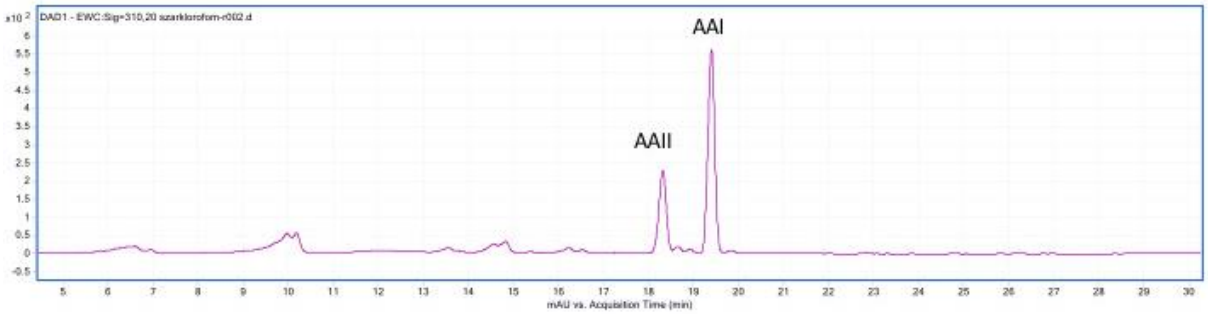

Figure 2. AAI and AAII content in the stem extract of *A. clematitis* used chloroform

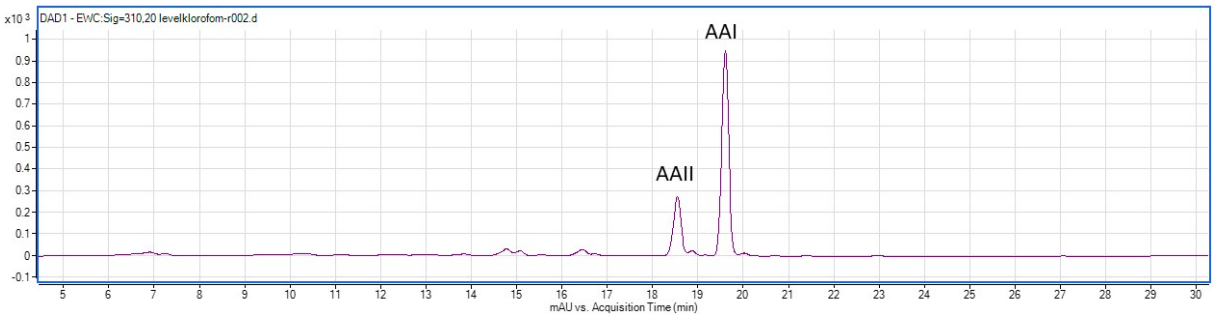

Figure 3. AAI and AAII content in the leaf extract of *A. clematitis* used chloroform

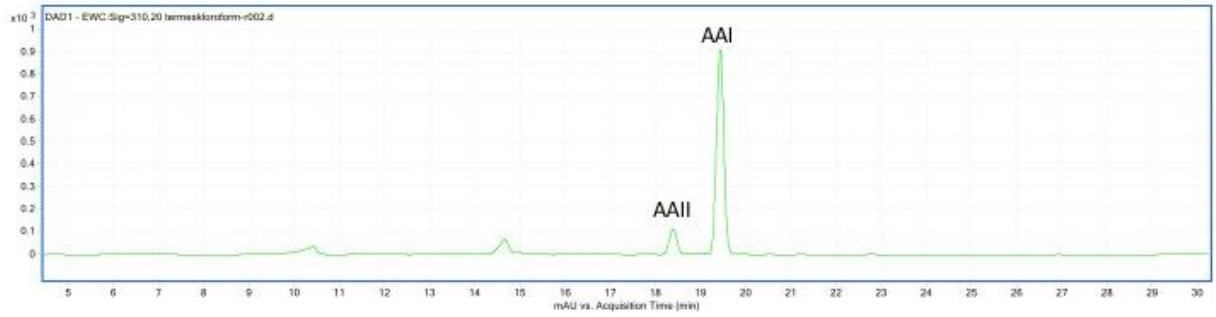

Figure 4. AAI and AAII content in the fruit extract of *A. clematitis* used chloroform

4.2.4. Results of cytotoxicity tests

In the case of HACAT cell line, the highest value was observed in the fruit extract of the plant (1886 µg/mL, 29.63%). The leaf was found to be cytotoxicin 18.05% (438,1 µg/mL), while the water extract of the root in 23.52% (462 µg/mL).

During the analysis of the small intestine cell line, the stem had 84.32% (105,4 μg/mL), the leaf 85.45% toxicity (114,6 μg/mL), while the root extract showed the highest value of cytotoxicity, being 88.46% (120 μg/mL).

In the case of HeLa cell line, the extracts of the parts of the plant subject to the test ended up with low cytotoxicity values. The stem showed 7.7% (105,4 μg/mL), the leaf 13.76% (114,6 μg/mL), while the extract of the root 17.64% inhibition (9.72 mg/mL).

Analysing Vero cell line, the water extract of the leaf reached the highest value of 71.72% (114,6 μg/mL). In the case of the stem, the cytotoxicity value was 62.66% (105,4 μg/mL), while the fruit had a toxicity value of 53.9% (488,9 μg/mL).

4.2.5. Microbiological results

All the studied 6 extracts of the root of the species inhibited the MRSA ATCC 700698 strain: the inhibition effect of ethyl acetate and butanol extracts was measured in 1000 μg/mL, while that of the methanol, hexane, chloroform and water extracts in 2000 μg/mL concentration.

Among the extracts of the stem, the butanol phase proved to be successful against *Staphylococcus aureus* ATCC 23923 (1000 μg/mL), MRSA ATCC 700698 (1000 μg/mL), *Klebsiella pneumoniae* ATCC 13883 (2000 μg/mL), *Pseudomonas aeruginosa* ATCC 27853 (2000 μg/mL), *P. aeruginosa* MDR (2000 μg/mL), and *Acinetobacter baumannii* MDR (1000 μg/mL).

The extracts of the leaf with methanol, hexane, and ethyl acetate had an inhibition effect against *Staphylococcus aureus* ATCC 23923 (2000 μg/mL) and MRSA ATCC 700698 (2000 μg/mL). In the case of *Klebsiella pneumoniae* ATCC 13883 the methanol phase of the leaf (2000 μg/mL) proved to be inhibitory, while the phase with methanol and ethyl acetate (2000-2000 μg/mL) against *Pseudomonas aeruginosa* ATCC 27853.

The extract of the fruit with methanol and butanol (2000 μg/mL), those with hexane and chloroform (500 μg/mL), and that with ethyl acetate (125 μg/mL) presented inhibitory effect against *Staphylococcus aureus* ATCC 23923. In the case of MRSA ATCC 700698 the same extracts showed inhibition (with methanol and butanol: 2000 μg/mL, with hexane and chloroform: 500 μg/mL, with ethyl acetate: 62,5 μg/mL). Against *Klebsiella pneumoniae* ATCC 13883 that of ethyl acetate (2000 μg/mL), while for *Pseudomonas aeruginosa* ATCC

27853 that of hexane (2000 (μg/mL) and ethyl acetate (1000 μg/mL) proved to be effective as inhibitory samples.

5. New observations

- In the 17 settlements selected in Covasna County of Transylvania, ethnopharmacobotanical collection was performed in the region as first, during which data of 121medicinal plants/135mentioned specieswere recorded used in the local human medicine and veterinary practices.
- Among the recorded species, 40 drugs were listed in the 8th Hungarian Pharmacopoeia and 20 drugs in the 10th Romanian Pharmacopoeia.
- Compared the collected data of people's knowledge about medicinal plants with the results of additional Transylvanian and international field works, the conducted survey was confirmed as successful fieldwork, which underlies the richness of the elements of traditional knowledge in the region, and the necessity of collections and documentation and its role in saving values.
- During data documentation and evaluationof our fieldwork, they were compared with those of official sources, resulted in additional species for further analyses; as an example, *Aristolochia clematitis* (common birthwort) analysed in the thesis can be mentioned.
- Based on the collected ethnobotanical records, the decoction of the leaf and the shoot of *A. clematitis* were mentioned in 13 settlements as a foment primarily for treating wounds and inflammations in humans and veterinary practices as well.
- In a histological aspect, the main histological features of the aerial and underground parts of the species were characterised.
- In the HPLC analysis, all fractions of the studied plant parts included aristolochic acid I (AAI) and aristolochic acid II (AAII).
- Among the results of the cytotoxicity tests, the HACAT cell line showed the highest value observed in the fruit extract of the common birthwort, for the small intestine cell line the extracts of the stem, the leaf and the root, while for the Vero cell line the water extract of the leaf. In the case of the HeLa cell line, the extracts showed low cytotoxicity values.

- In the antimicrobial tests, all the 6 extracts of the root inhibited the reproduction of the MRSA ATCC 700698; while the butanol phase of the stem proved to be successful against *Staphylococcus aureus* ATCC 23923, MRSA ATCC 700698, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* MDR, and *Acinetobacter baumannii* MDR; the extracts of the leaf with methanol, hexane and ethyl acetate demonstrated inhibitory effect against *Staphylococcus aureus* ATCC 23923 and MRSA ATCC 700698, while extract of the fruit with methanol, butanol, hexane, chloroform and ethyl acetate presented inhibition against *S. aureus* ATCC 23923 as well.
- The current results related to common birthwort contribute to the knowledge available about the species, which requires further (e.g. pharmacological) studies in the future.

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7. List of own publications

Publications related to the present PhD thesis

Papp N, **Bartha S**, Boris Gy, Balogh L. Traditional use of medicinal plants for respiratory diseases in Transylvania. *Natural Product Communications* 2011; 6(140): 1459-1460. **[IF:1.242]**

Nóra P, **Bartha SG**, Balogh L. Jelenkori etnobotanikai értékű adatok egy erdélyi (nagybaconi) falusi herbáriumból (Recent data of ethnobotanical value from a herbarium in Nagybacon, Transylvania). *Botanikai Közlemények* 2013; 100(1-2): 177-199.

Bartha SG, Quave CL, Balogh L, Papp N. Ethnoveterinary practices of Covasna County, Transylvania, Romania. *Journal of Ethnobiology and Ethnomedicine* 2015; 11:35 **[IF: 2.414]**

Papp N, Tóth M, Dénes T, Gyergyák K, Filep R, **Bartha SG**, Csepregi R, Balázs VL, Farkas Á. Ethnomedicinal treatment of gastrointestinal disorders in Transylvania, Romania. *Acta Ethnographica Hungarica* 2017; 62(1): 207-220.

Bartha SG, Tóth G, Horváth P, Kiss E, Papp N, Kerényi M. Analysis of aristolochic acids and evaluation of antibacterial activity of *Aristolochia clematitis* L. *Biologia Futura* 2019; 70: 323-329. **[IF: 0.585]**

The total impact factor of the publications used as a basis for the thesis: 4.241

Publications and posters related to the PhD thesis

Papp N, Birkás-Frendl K, Vántus V, Csepregi K, Bencsik T, Vojkovics É, Vincz D, Bóna V, Farkas I, **Bartha S**, Gajdos L, Fancsali I, Grynaeus T, Csedő K. Etnobotanikai kutatások a Pécsi Tudományegyetemen (Ethnobotanical researches at the University of Pécs). XX. Tudományos Ülésszak, Kézdivásárhely, April 22-24, 2010. *Orvostudományi Értesítő* 83(1): 41. (poster)

Bartha SG, Balogh L, Papp N. Népi növényismeret Nagybaconban és környékén (Ethnobotanical data in Băţanii Mari and its neighbourhood). *Magyar Biológiai Társaság Pécsi Csoport 236. szakülése,* Pécs, March 10, 2011 (poster)

Papp N, **Bartha S**, Boris GY, Balogh L. Traditional use of medicinal plants for respiratory diseases in Transylvania. *CIPAM 2011: The International Congress on Aromatic and Medicinal Plants* (April 13-15, 2011, Cagliari, Italy). Abstract Book: p. 334. (poster)

Bartha S, Balogh L, Papp N. Népi gyógynövényismereti adatok Nagybaconban és környékén (Ethnopharmacobotanical data in Băţanii Mari and its neighbourhood). *XII. Magyar Gyógynövény Konferencia,* Szeged, May 5-7, 2011. *Gyógyszerészet Supplementum*: p. 23-24. (poster)

Bartha SG, Balogh L, Papp N. Traditional ethnobotanical data in Erdővidék (Romania). *Second Eastern European Ethnobiology Workshop,* Királyrét, October 13-16, 2011. Abstract Book: p. 4. (oral presentation)

Papp N, Boris GY, **Bartha S,** Horváth D, Birkás-Frendl K. (Medicinal herbs of ethnomedicine in Transylvania today). A népi orvoslás gyógynövényei napjainkban Erdélyben. Debrecen, *Current flora and vegetation research in the Carpathian-Basin 9th*, Gödöllő, February 24-26, 2012, Issued in memory of Tuba Zoltán, 2012; *Kitaibelia*17(1): 49. (oral presentation)

Bartha SG, Papp N,Balogh L. Traditional ethnoveterinary data in Széklerland (Romania). *The Third Eastern European Ethnobiology Workshop,*K ików, Poland, October 9-13, 2013. Abstract Book: p. 4 (oral presentation)

Bartha SG, Balogh L, Papp N. Ethnoveterinary medicine in the county Covasna, Romania. *X. Szentágothai János Transzdiszciplináris Konferencia és Hallgatói Verseny,* Pécs, November 4-5, 2013. Abstract Book: p. 53. (poster)

Balogh L, **Bartha SG**, Papp N. Ethnobotanical and floristic values of a herbarium of a Transylvanian village (Egy erdélyi falusi herbárium etnobotanikai és florisztikai értékei). *Current flora and vegetation research in the Carpathian-Basin 10th* (*X. Aktuáli sflóra- és vegetációkutatás a Kárpát-medencében),* Sopron, March 7-9th, 2014. Abstract Book: pp. 106-107. (poster)

Budán F, **Bartha SG**, Andreidesz K, Bufa S, Dénes T, Varga E, Papp N. Légúti megbetegedések kezelése – népgyógyászati és tudományos megközelítés (Ethnomedicinal and scientific approach of the treatment of respiratory diseases*). Fiatal Higiénikusok Fóruma X.,* Pécs, May 14-16, 2014. *Egészségtudomány* 58(2): 85. (poster)

Papp N, Dénes T, Kaszás A, **Bartha SG**, Varga E, Boros B. Study of some medicinal plants used in the Transylvanian ethnobotany. *6th ICEB Congress,* November 17-21, *2*014. Cordoba, Spain. Abstract Book: p. 277-278 (oral presentation)

Sali N, Csepregi R, Tóth M, Dénes T, KaszásA, **Bartha S**, Kőszegi T, Nóra Papp. Antioxidant activity of plants used in the Transylvanian ethnomedicine. *7th International Student Medical*

Congress in Košice. June 24-26, 2015. Kassa, Szlovákia. Folia Medica Cassoviensia, Tomus 70, No. 1, Supl. 1, p. 244. ID: 170. (poster)

Papp N, TóthM, Dénes T, **Bartha S**, Varga E, Gyergyák K. Studii etnobotanice si fitochimice ale drogurilor recoltate din Transilvania. *Simposium de Fitoterapia − Actualitati in fitoterapie,* Cséfa, July 4, 2015 (oral presentation)

Papp N, Csepregi R, Dénes T, Tóth M, **Bartha SG**, Gyergyák K, Czégényi D. Relevance of Transylvanian plants in the European ethnomedicine.*9th Conference on Medicinal and Aromatic Plants of Southeast European Countries 9th CMAPSEEC,* Plovdiv, Bulgaria, May 26-29, 2016 Abstract Book: SL 3. (oral presentation)

Bartha SG, Papp N, Kopcsányi M, Mágó M, Kerényi M. A farkasalma (*Aristolochia clematitis* L.) antimikrobás hatásának vizsgálata (Study of the antimicrobial effect of birthwort (*Aristolochia clematitis* L.). *Fiatal Gyógynövénykutatók Fóruma,* Budakalász, May 12, 2017, Abstract: A4, p. 9. doi: 10.14232/fgykf.2017.a4 (oral presentation)

Papp N, Dénes T, Tóth M, **Bartha SG,** Gyergyák K, Filep R, Czégényi D. Aspects of Transylvanian ethnomedicine – overview. *Ceská a slovenská farmacie* 2017; 66: 292. (oral presentation)

Kerényi M, **Bartha S**, Dénes T, Tóth M, Papp N. Erdélyi gyógynövények antimikrobás hatásának vizsgálata (Study of the antimicrobial effect of Transylvanian medicinal herbs). *Erdélyi népigyógyászat – hagyományoktól az alkalmazásig Konferencia,* Pécs, April 5-6, 2019.Abstract: p. 22. (oral presentation)

Other publications (out of scope of the PhD thesis)

Dénes T, **Bartha SG**, Kerényi M, Varga E, Balázs VL, Csepregi R, Papp N. Histological and antimicrobial study of *Ononis arvensis* L. *Acta Biologica Hungarica*2017; 68(3): 320-332. **[IF: 0.506]** (resigned)

Papp N, Sali N, Csepregi R, Tóth M, Gyergyák K, Dénes T, **Bartha SG**, Varga E, Kaszás A, Kőszegi T. Antioxidant potential of some plants used in folk medicine in Romania. *Farmacia,* 2019; 67(2): 323-330. **[IF: 1.527]** (resigned)

Varga E, Becsek E, **Bartha SG**, Stranczinger Sz, Mihalovits F, PappN. Determination of polyphenols and *in vitro* antimicrobial and antioxidant activity of *Calluna vulgaris* (L.) Hull. *Biologia Futura* 2021; https://doi.org/10.1007/s42977-020-00059-9 **[IF= 0.821]**

Other poster and oral presentations (out of scope of the PhD thesis)

Csepregi R, Kocsis M, **Bartha SG**, Gyergyák K, Papp N. Az *Anthyllis vulneraria, Galium mollugo* és *Veronica beccabunga* elterjedése és etnobotanikai adataiErdélyben (Ethnobotanical data and distribution of *Anthyllis vulneraria, Galium mollugo* and *Veronica beccabunga* in Transylvania). *IX. Aktuális flóra- és vegetációkutatás a Kárpát-medencében*, February 12-14, 2016, Budapest. Abstract Book: 140-142. (poster)

Dénes T, Kerényi M, **Bartha SG**, Varga E, Papp N. Ethnobotanical and microbiological study of *Ononis arvensis* L.*9th Conference on Medicinal and Aromatic Plants of Southeast European Countries, 9th CMAPSEEC,* Plovdiv, Bulgaria,May 26-29, 2016. Abstract Book: p 23. (poster)

Tóth MM, **Bartha SG**, Papp N, Kerényi M. Ethnomedicinal and antimicrobial potential of *Lilium candidum* L. *9th Conference on Medicinal and Aromatic Plants of Southeast European Countries, 9th CMAPSEEC,* Plovdiv, Bulgaria, May 26-29, 2016. Abstract Book: p. 73. (poster)

Varga E, Becsek E, **Bartha SG**, Papp N. Phytochemical and antimicrobial study of the aerial part of *Calluna vulgaris* (L.) Hull. *9th Conference on Medicinal and Aromatic Plants of Southeast European Countries, 9th CMAPSEEC,* Plovdiv, Bulgaria, May 26-29, 2016.Abstract Book: p. 161. (poster)

Papp N, Balázs VL, **Bartha SG,** Bencsik T, Dénes T, Filep R, Gyergyák K, Joós-Békésiné Kallenberger H, Patay É, Tóth M, Farkas Á. Gyógynövények hisztológiai értékelése – oktatás és kutatás a pécsi Farmakognóziai Intézetben (Histological evaluation of medicinal plants – teaching and research at the Department of Pharmacognosy, Pécs). *XV. Magyar Növényanatómiai Szimpózium,* Budapest, September 7, 2017. Program and summaries: p. 12. (oral presentation)

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