Ethnobotanical studies in Transylvania / PD OTKA 108534 (2013-2017)

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1. Introduction, aims

Traditional health systems involve the relationship between people and nature including the special use of medicinal plants, animals, human materials, minerals, or combinations thereof. Ethnomedicinal practices play an important role in the everyday life of people in Transylvania, currently part of Romania nowadays.

Based on earlier reports published mostly from the 1960s in many regions of the country, the aims of my project were the followings:

1. to collect ethnomedicinal data in the Homoród-valley in Székely Land namely in Meresti, Martinis, Petreni and Sanpaul between 2013-2016
2. to collect ethnomedicinal data in Lueta completed the survey started in 2008
3. evaluation and comparison of the recorded plants' data with those obtained from scientific references and databases
4. to select plants for laboratory analyses including the study of the earlier reported smooth bedstraw (*Galium mollugo* L.), hoary plantain (*Plantago media* L.) and fuchsia species (*Fuchsia* sp.) used in the local therapy in the region.

2. Material and methods

2.1. Data collection and documentation

The performed ethnobotanical field work corresponds to the planned methods (semi-structured interviews, documentation of local name, drug, use, collection and storage place of plants by notes, photos, herbaria and dictaphone), highlighted the origine of the records (inherited or read/heard). Study areas:

1st schedule (autumn of 2013, summer of 2014): Martinis, Meresti, Lueta
2nd schedule (summer of 2015): Petreni, Sanpaul, Martinis, Meresti, Lueta
3rd schedule (spring and summer of 2016): Petreni, Sanpaul, Martinis, Meresti, Lueta
+ extra 4th schedule (summer of 2017): Martinis, Lueta

2.2. Data evaluation

Documented data of each settlement were classified into tables according to the identified scientific and local name and use. The recorded interviews were literally noted from words to words used the phrases and verbs of local dialect of voice recordings.

Thereafter, data were compared with the published results of scientific reports and references (e.g. 8th Ph. Hg. VIII, 6th Ph. Eur., 10th Ph. Ro., Escop Monograph, and scientific databases - Pubmed, Science Direct, Scopus), which focused on the similarities and the differences, as well. Based on this evaluation, plants were selected for further analyses for new projects in the future.

The earlier documented data of *Galium mollugo*, *Plantago media* and *Fuchsia* sp. (local use: leaves for wounds) were completed with those collected in field and databases to plan and optimize the analyses.

2.3. Laboratory analyses

1st schedule: Stationeries, chemicalizes and solutions were provided for the planned laboratory analyses.

2nd schedule: Based on previosuly screened scientific papers, the extractions of the selected 3 plants were prepared from the whole herb of *G. mollugo* and the leaf of *P. media*

(samples collected at fields) and *Fuchsia* sp. (from Botanical Garden, University of Pécs). The antioxidant potential of the samples were studied by 1,1-diphenyl-2-picryl-hydrazyl (DPPH), enhanced chemiluminescence (ECL), ferric reducing ability of plasma (FRAP), oxygen radical absorbance capacity (ORAC), and Trolox equivalent antioxidant capacity (TEAC) assays.

3rd schedule: The measurement of the antioxidant potential of the samples were repeated and tested on cell cultures. The cytoprotective and anti-inflammatory effect of the extracts were investigated and repeated by chemiluminescence and fluorescence spectroscopy methods. As complementary assays, the histological structure of the used plant parts of these and other herbs were investigated on preparations made by rotation microtome, the polyphenol content by TLC and HPLC, and the antimicrobial potential by disc diffusion method against bacteria and fungi strains.

3. Results

Results of all subsections of the project were published in peer-reviewed journals in English and Hungarian, as well as in posters and oral presentations at national and international conferences (52 items, see in References).

3.1. Field work

The performed semi-structured interviews and field trips involved data on the local knowledge and use of medicinal plants, vegetables, ornamental and fodder plants, connected beliefs, used animals and minerals in all studied villages. In general, first field trip involves the majority of the used pants in each village, which were completed with minor elements by further trips.

Based on the geographical position of Martinis, Petreni and Sanpaul, as neighbouring villages along the Nagy-Homoród river, the local name and use of the mentioned plants particularly overlap, but they also differ from data collected in Lueta and Meresti located along the Kis-Homoród river.

In Meresti, 90 medicinal plants/all 117 mentioned species were listed by 48 informants between 2014-2015, which data were confirmed by introduction of plant samples in living or dried form for the correct botanical identification in 2016 [1,24].

In Martinis, 105 medicinal plants/141 species were presented by 35 informants between 2014-2015, which data were confirmed and completed by new herbs in 2016 and by new indications of herbs collected in 2107.

In Petreni, 75 medicinal plants/97 species were listed (number of informants: 15), while 94 medicinal plants/106 species were noted in Sanpaul by 25 informants 2015 and 2016.

Ethnobotanical survey started in Lueta in 2008 was continued, completed and finished between 2013-2017, which include the collection of further local name and use of previously mentioned plants, taking new photos on herbs, habitats, informants, environment, infrastructure and local customs mentioned by more than 140 informants. All records were inserted into an edited monograph (pp 1-289) of the village including data on 250 plants and 15 fungi, relationship between traditional and official medicine, index of local and scientific name of plants and fungi, local customs and maps, as well as summaries in English and Romanian [51].

In addition to the summarized ethnomedicinal description of the selected villages, the results were evaluated and published as the following classifications: ethnobotanical survey of neighbouring settlements or region compared with our data, case study on species, indications and knowledge of informants, beliefs and linguistic aspect connected to the local ethnomedicine, and summary about the relevance, significance and present-day role of Transylvanian ethnobotany compared to those of other European countries. Hereinafter, these types of data presentation will be detailed with our published results as followings:

In addition to the study areas, further ethnobotanical and horticultural data were presented on home gardens in the Homoród-valley – 43 food and 29 ornamental plants [7], in Turulung – 73 plants for 81 diseases [2,41], as a separated book published in Craciunel – 108 wild and 74 cultivated plants [50], and Covasna County – 26 wild and cultivated plants, 2 animals, and 17 other substances used in the ethnoveterinary medicine [8]. The results of these surveys were compared with data obtained from our selected villages in the Homoród-valley.

Case study on species: Ethnomedicinal and ethnobotanical data of *Betula pendula* [6], *Vitis vinifera* [9], *Thymus* [23], *Mentha* and *Salvia* species [12], *Helianthus tuberosus* s.l. [13,35], *Rosa* and *Crataegus* sp. [29], *Helleborus* sp. [39], *Lilium candidum* [37], *Ononis arvensis* [34], *Anthyllis vulneraria, Galium mollugo* and *Veronica beccabunga* [38], as well as the local use of *Fomes fomentarius* and *Piptoporus betulinus* [14] were recorded in the study areas, which results were compared with earlier Transylvanian data and those of other European countries.

Case study on indications: Plants for hypertension – 20 species as a tea [22], and for gastrointestinal disorders – 78 plants with 181 local names – were listed in various preparations in the selected villages [18].

Case study on the knowledge of an informant having widespread knowledge on local plants' use: a special separated issue (pp 1-76) was published based on Anna Mag's memory (1914-2014) in Lueta. She explained many ethnomedicinal data, e.g. more than 100 plants and special cream prescriptions during the conducted 5 interviews [5].

Traditional beliefs connected to medicinal plants were published in referred international journal [3] and as an oral presentation included data from Lueta and other Transylvanian villages [45].

In linguistic aspect, as international cooperation, data of the word 'bear' connected to the local plant terminology were compared among many references of European countries including our data collected in the study areas in Transylvania [15].

Historical data were collected from old herbal books from the monastery era and oriental flora books compared to Transylvanian ethnobotanical records which highlighted some overlapping data among the studied regions [31,32].

Based on an invitation of the Springer Science+Business Media New York, a chapter was accepted on the main Hungarian etnobotanical data in Romania, including the main referred book chapters, books and articles including some of our papers [4]. Other general summaries of the conducted field trips were published in referred journal [20], and as posters and oral presentations in congresses [26,27,40,42,46,47,49].

In the 3rd and 4th schedules of the project, ethnobotanical data of all studied villages were compared with those of earlier reports of Transylvania and other European countries, as well as with new phytochemical and pharmacological results published on each mentioned species. According to these comparison and some of our previosuly investigations, the following species can be reviewed as new samples for further laboratory analyses in the future: herb of *Anthyllis vulneraria* and *Lysimachia nummularia* (locally used for wounds as a tea), herb of *Eryngium planum, Salvia pratensis* and *S. nemorosa* (for respiratory disorders as a tea), tuber of *Lathyrus tuberosus* (food with high sugar content), herb of *Veronica beccabunga* (for rheuma as a tincture), leaf of *Alnus glutinosa* and *A. incana* (for wounds as a foment), and the leaf of *Aristolochia clematitis* (for wounds as a decoction used in the veterinary medicine). These species are underrepresented in scientific databases nowadays possessing few or no enough proved data related to the documented ethnomedicinal use.

3.2. Investigation of the selected plants

The planned analyses of the selected *Galium mollugo, Plantago media* and *Fuchsia* sp. were carried out by the study of their antioxidant and anti-inflammatory effect, completed with phytochemical, antimicrobial and histological investigations of their samples and other selected species, as well.

In our antioxidant study, *G. mollugo*, *P. media* and *Fuchsia* sp. were analysed by DPP, ECL, ORAC, TEAC and FRAP assays [16,52]. Among them, fuchsia species showed the highest antioxidant potential. Each sample showed higher values than those of *Anthyllis vulneraria* and *Veronica beccabunga* as other selected plants from our collections [16,33]. This work was continued in our new developed methods namely ECL and modified ORAC using for the study of further herbs. Among them e.g. *Aristolochia clematitis, Helleborus purpurascens, Polygonum lapathifolium, P. maculosa, Potentilla anserina, Salvia glutinosa* and *S. nemorosa* can be mentioned also from our ethnomedicinal collections [21]. In addition, the samples of *Ajuga reptans*, *Impatiens noli-tangere*, *Lilium candidum*, *Ononis arvensis*, *Rhinanthus serotinus*, and *Thymus serpyllum* showed also high antioxidant potential selected in the study area [30,33,44,52].

In phytochemical aspect, *G. mollugo* and *Fuchsia* sp. were analysed by TLC for their phenolic compounds resulted new data to earlier reports (flavones and 14 flavonoid glycosides in *Fuchsia* sp., 2 phenolic acids and 2 flavonoid glycosides in *G. mollugo*). Flavonoid glycosides and phenolic acids were identified in the aerial part of *A. vulneraria* and *V. beccabunga* by TLC [17,48], as well as 19 polyphenolic compounds in *O. arvensis* and 13 in *Rh. serotinus* by HPLC having also ethnomedicinal role in the region [10,25].

In microbiological aspect, *G. mollugo* and *Fuchsia* sp. were investigated for their antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, MRSA, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*. The leaf extract of fuchsia species showed inhibitory effect against all studied bacteria, while that of *G. mollugo* was effective for *B. subtilis*, *E. coli* and *C. albicans* [11]. In preliminary tests, the extracts of the tepal and the leaf of *Lilium candidum* inhibited the growth of *S. aureus* [37], the herb of *A. vulneraria* and *V. beccabunga* was effective for *B. subtilis* [11], while that of *O. arvensis* against *Salmonella typhimurium, S. aureus* and *E. coli* [19,34].

The histological structure was studied of the used parts providing new data for the selected plants [43], e.g.: the heterogenous and dorsiventral leaf of *P. media*, *G. mollugo* and *Fuchsia* sp. has mesomorphic stoma on both sides [48], similar to the sepal of the flower of *Helleborus odorus* and *H. purpurascens. Helleborus* species have tricolpate pollen and special nectaries as reduced petals [36]. The epidermis cells are covered by thin cuticle in the equifacial leaf of *O. arvensis* [28]. These investigations can complete the phytochemical analyses of the used parts of the ethnomedicinally mentioned herbs and drugs.

4. Conclusions

The documented herbal remedies and home practices play an important role in the studied villages of the Homoród-valley as the following main points:

(a) maintenance, conservation and documentation of the traditional ethnomedicinal knowledge of rural people living in the study areas
(b) to save data of elderly people from disappearing because of the migration and less interest of young people for archaic medical skills
(c) feedback to people about their knowledge in Hungarian papers and posters
(d) data comparison with earlier Transylvanian and other European data, as well as with scientific references to select new drugs and herbs for further analyses – focusing on the similarities and differences can give a more complete overview on the ethnomedicinal knowledge of people in the Homoród-valley
(e) to plan and perform various analyses (e.g. antioxidant, phytochemical, histological, antimicrobial study) on the selected species
(f) to provide new results about the selected plants to wide their phytotherapeutical data these days

(g) to highlight the interdisciplinary significance of ethnobotanical topic including e.g. ethnomedicinal, ethnographic, historical and linguistic aspects of the traditional knowledge of rural people in Translyvanian regions and other countries worldwide [49].

5. Change in the project's course

In the end of the 3rd schedule, I made an application for prolongation with 4th year to fulfill the planned parts of the project. The reasons were the followings: (a) chemicals did not arrived in time for the planned anti-inflammatory study of the selected plants; (b) the edition of the planned monograph on the ethnomedicinal data of Lueta also needed time, because new data were added to the earlier collections (e.g. identification of some new plants by field trips and photos, to clarify data of some drugs by complementary interviews, meeting and discussion with the mayor of Lueta and reviewers on the structure and content of the planned book), which required more work to edit data and illustrations in adequate form. In addition, for comparison, new national and international works were continuously published in the topic, which were also used for the wider explanation of our data.

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