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MANAGEMENT AND ASSESSMENT OF MEDICINAL PLANTS FROM SPONTANEOUS FLORA IN THE REPUBLIC OF MOLDOVA

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Abstract: *The usage of local plants in medicine is a prospective and economically important direction. Flora of the Republic of Moldova includes a vast diversity of species with the opportunity of being applied in the treatment of cardiovascular diseases, infections, oncology etc. But, for their exploitation it is necessary to apply modern technologies of research, which generate new knowledge for the rational breeding of medical plants, extraction of pharmacologic substances and drug production, also determining an economic potential of their application for SMEs in the country.*

Key words: *management, medicinal plants, spontaneous flora.*

1. Introduction

The use of local species, especially from spontaneous flora, in medicine represents a prospective direction and with economic importance for the Republic of Moldova, as the flora shares a wide diversity of major taxons that can be used in the improvement of cardiovascular, infectious, oncologic and other diseases. Various actions have been undertaken in order to assess vegetal resources by studying and describing different species throughout the whole territory of the country. Historically, a set of descriptive methodologies to study the plants were developed, the results being successfully systematized for the first time by Carl von Linne. Nowadays, the application of the classical methods is insufficient as the high effort for the investigation as compared to the amount of results is still low so as to be applied for the right assessment of plant diversity and variability. Along with the development of research at cellular, tissue, biochemical and genetic level, numerous data regarding specific, populational and individual variability were collected. But, until now only Botanic, as one of the classical biologic sciences, is the main source of information regarding medicinal plants and is not developing new methodologies of flora excavation.

As a result, other scientific domains are in trend, offering high throughput data. Thus, taking into consideration the complexity of the spontaneous flora investigation, it's assessment is indispensable without botanical, biogeographic description, molecular and biochemical analyses. All these are to be conducted with the

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exploitation of computer technologies from geoinformatics, bioinformatics and chemoinformatics.

2. Botanical Aspect

The flora of the Republic of Moldova includes about 5513 species of plants [superior plants - 1989 species, including vascular plants - 1832 (among them: pteridophyta - 25, gymnosperma - 1, angiosperma - 1806), briophyta (mosses) - 157 species: inferior plants - 3524 species, inclusive lichenophyta (lichens) - 124, alga - 3400 species]. The richest families of superior plants are the Asteraceae, Poaceae and Cyperaceae. The most variable according to their floristic composition are forest ecosystems (over 850 species), as compared to those of the steppe (over 600 species), meadow (about 650 species), petrophyte (about 250 species), aquatic and palustre (about 160 species) etc.

The number of medicinal plant species encountered on the territory of the Republic of Moldova, according to the most recent sources, is up to 200. According to the Atlas of Medicinal Plants [11], the pharmacological proprieties of 102 species are described. According to other sources [14] 153 species of medicinal plants from indigenous wild and cultivated flora, whose therapeutic activity was verified and scientifically revealed were described.

Each species' description includes popular and scientific names, taxonomy, plant habitat and areal. Hence, there were botanical characterizations for plant recognition, raw material and drug properties. Recent investigations of the local office of the Convention for biological diversity showed the possibility of assessment of about 150 species of aromatic plants from spontaneous flora and 200 species of medicinal plants [6].

3. Biogeographic Aspects

The natural biologic diversity of the Republic of Moldova is conditioned by its geographical position. The territory is situated at the interference of the three biogeographic zones: Central-European - represented by the Central Moldavian Plateau Codri (54.13% or 18.3 ths km^2 of the country territory); Eurasian - by the regions of forest-steppe and steppe (30.28% or 12.23 ths km^2); Mediterranean - by fragments of xerophyte forest-steppe from the south part (15.59% or 5.27 ths km^2), by 90% being situated between Nistru and Prut rivers and with a temperate continental climate. Being placed in the South - East of the European continents and in the zone of interference of the geotectonic and floristic regions, the territory is evidenced by a rich landscape spectra, determined by the variety of natural factors (geologic, orographic, climatic, edaphic, hydrographic etc.). Thus, two natural zones, namely forest - steppe and steppe, which includes 5 landscape regions are emphasized [16].

As an example of the regional description of biodiversity can be the study of natural reservations, as the source of information about medicinal plants.

One of such natural reservations of medicinal plants is "Rososeni" which represents a scientific object of national importance, which includes genetic stock of medicinal plants and a fond of fauna comprising over 270 species of vascular plants, from which 18 are rare species and 3 are endangered species, included in the Red Book of the Republic of Moldova (*Betula oycoviensis* Bes., *Betula pendula* Roth, *Betula platyphylloides* V.Vassil., *Frangula alnus* Mill., *Rhamnus tinctoria* Waldst. et Kit., *Asparagus tenuifolius* L., *Briza media* L., *Circaea lutetiana* L., *Clematis integrifolia* L., *Clematis recta* L., *Convallaria majalis* L., *Doronicum*

hungaricum (Sapll.) Reichb., *Dryopteris filix-mas* L., *Gladiolus imbricatus* L., *Lilium martagon* L., *Listera ovata* L., *Platanthera bifolia* L., *Platanthera chlorantha* (Cust.) Reichb., *Rhamnus tinctoria* Waldst. et Kit., *Doronicum hungaricum* (Sapll.) Reichb., *Gladiolus imbricatus* L.).

In order to protect the indigenous flora genofond, including those represented by medicinal plants, according to the amendments of the Convention for biological diversity, the system of the natural protected areas of the Republic of Moldova was created [5], regulations regarding the structural organization and domains of activity were elaborated, the classification of state protected natural areas was adopted according to the criteria of the International Union for Conservation of Nature (UICN), which stood at the basis of the „Law regarding state protected natural areas fond", adopted in 1998 (Table 1).

4. Commercial Aspect

Between 1970-1990 the Republic of Moldova was one of the biggest manufacturers of volatile oils and medicinal plants. Cultivated areas of aromatic plants during that period were 18-20 ths ha. Annually 150-160 tons of volatile oils and other products were produced, including lavender - up to 75-80 tons, salvia - 30-35 tons, dill - 25 to 30 tons, mint 25-35 tons, fennel 16 to 20 tons etc. the majority of the products being exported.

The exploitation of medicinal plants had a similar development. Cultivation areas were over 2 ths ha, annually being produced more than 1000 t of products of medicinal plant origin. The major part of medicinal plants was collected from the wild. There were only two specialized agricultural households and seven forests. Raw material from medicinal plants was delivered especially through the pharmaceutical network of the URSS. The local market used only an insignificant quantity of the vegetal products (about 10-15%) [9].

According to the data from the Business Portal, in the Republic of Moldova there are at least 30 companies which are directly or indirectly involved in the commercialization of medicinal plants, all representing SMEs [7], (Table 2).

Though, their commercial activity effect on spontaneous flora is considered negative, because there are no effective mechanisms of natural resource protection. As a result, the collection and commercialization of spring decorative plants (efemeroids) [3], [16] led to their being declared nature monuments and their harvest was thus prohibited.

The area of agriculture lands caused the destruction of the biotopes and determined the decrease of plant areal, restricting their capacity of propagation, being destroyed before maturation and end of lifecycle. Thereby, it essentially diminished the viability of rare species, especially of the Star of Bethlehem (*Ornithogalum),* Saffron (*Crocus*), spring pheasant's-eye (*Adonis*), snowdrop (*Galanthus*), *Hyacinthella*, crocus (*Colchicum*), needle grass (*Stipa*) etc. [10].

From all the steppe species, 18 are included in the Red Book of the Republic of Moldova, nine, such as *Astragalus dasyanthus* Pall., *Belevallia sarmatica* (Georgi) Woronow, *Bulbocodium versicolor* (Ker.-Gawl.) Spreng., *Colchicum triphyllum* G.Kunze, C. Fominii Bordz., *Galanthus elwesii* Hook. fil., *Ornithogalum amphibolum* Zahar., *Ornithogalum oreoides* Zahar., *Stembergia colchiciflora* Waldst. et Kit., being mentioned also in the Red Book of Ukraine (1996) and the Red List of Superior Plants in Romania (1994).

Fond of the natural areas protected by state Table 1

TYPE OF PROTECTED AREAS	UICN CATEGORY	NUMBER	AREA [ha]
I. Scientific reservations	I	5	19378
II. Nature monuments	III	130	2906.8
a) Geographic and paleontological		86	268.8
b) Hydrologic		31	99.8
c) Botanic		13	125.2
III. Natural reservations	IV	63	8009
a) Forest		51	5001
b) of medicinal plants		9	2796
c) Mixt		3	212
IV. Landscape reservations	V	41	34200
V. Reservations of resources	VI	13	523
VI. Areas with multifunctional management	VII	32	1030.4
a) Representative sectors of steppe vegetation		5	148
b) Representative sectors of grassland vegetation		25	674.7
c) Forest belts		2	207.7
VII. Botanical gardens		1	105
VIII. Park		2	104
IX. Monuments of landscape architecture		20	191,1
X. Zoological gardens		1	20
TOTAL		308	66467.3

5. Biochemical and Molecular Aspects

All techniques used in genome based research of medicinal plants can be divided in two basic groups [9]. The first set of methods includes determination of nucleotide sequences of one or more genetic loci and identification of the interested fragments, characteristic to the studied species. Such techniques are PCR based, such as Allele specific PCR, ARMS (amplified refractory mutation system), MARMS (multiplex amplification refractory mutation system), ADN microarray and DNA sequencing. The second set of methods uses polymorphic intraspecific variations of the DNA sequences, randomly spread in the whole genome, for genomic DNA imprinting. The techniques referred to in this set of methods are AFLP (amplified fragmented length polymorphism), AP-PCR (arbitrarily primed PCR), DALP (direct amplification of length polymorphism), RAPD (randomly amplified polymorphic DNA), RFLP (restriction length polymorphism), SSR (simple sequence repeat polymorphism).

Plant DNA comprises three independent replicative genomes. Moreover, the nuclear genome is organized in chromosomes, chloroplast and mitochondrial genome being circular. The Chloroplast genome of angiosperms varies between 120 and 220 kb [8] and the mitochondrial genome varies between 200 kb at Brassica, up to 2.5 Mb at *Citrullus lanatus* and is much bigger than the mitochondrial genome in animals, which varies in the limits of 15-18 kb.

Yield of the additional products [tons] Table 2

Product	1995	1996	1997	1998
Fruits and berries	366	250	317	226
Medicinal plant	12	17	29	44
Forage	1706	2041	1220	2007

The application of the genome based methods for the authentication of medicinal plants must be done in accordance with phylogenetic plant research and plant barcoding [12], [14], [15]. Common genetic loci used for the medicinal plants' authentication include ITS (internal transcribed spacers) regions, which separates coding regions of the 5.8S, 18S and 26S nuclear genes [1], [2] and intergenic spaces, that separates repetitive multiple copies of 5S ARNr gene [4]. At the same time, some of the loci used in phylogenetic studies include chloroplast genes [13] e.g., *atp*F, *mat*K, *rbc*L, *rpo*B and *rpo*C1, also the intron of the trnL gene and intergenic spaces between *trn*C-*trn*D, *trn*L-*trn*F, *trn*H-*psb*A and *psb*K-*psb*KI genes.

6. Conclusions

The combination of the above discussed aspects is supposed to reflect modern strategies to assess natural resources of plants, on the whole, and of the medicinal plants in particular, that open future perspectives for the development of multilateral research of medicinal plants in the Republic of Moldova:

• The country's territory represents a relatively small geographic space, that allows an easy and exact mapping of medicinal plants by the application of geoinformatics, by a description of the climate conditions, also with a periodic monitoring of the area. For the fast data coverage, it is necessary to create an *on-line* service, where all of the researchers who conduct studies in the wild, can present their data, while the registration in such a public database would be one of the conditions for the results to be accepted for publication in specialized journals. As a result, the database will be filled in rapidly with the data, thus regional monitoring could be possible in studied and unstudied territories.

• The number of identified species varies between 150-200, and all of them constitute an object of scientific study. Investigation of these species should be important not because of their production and commercial value, but also from the point of view of research and conservation of biodiversity.

• The application of modern research techniques offers the opportunity to rapidly implement new species in culture, for breeding and application in biotechnology, due to their valuable qualities of biochemical content and quality. So, molecular biology methods ensure not only data on the plant's taxonomy or inter and intraspecific polymorphism, but also efficient tools for monitoring breeding.

• The multilateral nature of scientific activities must result in the creation and development of databases or of *on-line* public services for data storage, information and investigation results in an organized and systematic way, but also for data extraction and other uses. Thus, it is possible to store the information in a bioinformatic database regarding genome and genetic data of the studied species, protein structures, gene expression data etc. Data regarding the structure of the chemical substances identified in medicinal plants, their structure and variability can be stored in chemoinformatic resources. Altogether, these resources offer information about

biosynthetic pathways of important chemical compounds and genetic mechanism of their regulation, for the stimulation of the production of biologic active compounds.

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