https://doi.org/10.31925/farmacia.2020.2.4 **REVIEW**

THE CURRENT STAGE OF BIOTECHNOLOGICAL APPROACHES AND SYSTEMATIC REMARKS ON SOME SPONTANEOUS MEDICINAL PLANTS IN ROMANIA – REVIEW OF LITERATURE

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Abstract

This review presents the current state of biotechnological approaches for some spontaneous medicinal plants in Romania based on literature published throughout the worlds during the last decade. From a total of 122 species that were listed in 2014 as strictly forbidden for collection, 47 species were selected for a biotechnological investigation (based on both *in vitro* and biochemical studies). For 13 species without such studies, some systematic remarks are presented. In addition to the medicinal aspects, some of these species have other scientific importance (endemics, relicts, etc.) or present other problems such as uncertain taxonomy, very restricted distribution area, low seed germination, etc. In Romania, in the last 5 years, the interest in the biotechnological characteristics of these species has increased. Because medicinal plants represent a valuable source of products, studies on genetic variability, micropropagation, induction and characterisation of desirable metabolites will be helpful to achieve a better management for their sustainable use and conservation.

Rezumat

În acest articol este prezentat stadiul abordărilor biotehnologice pentru unele plante medicinale spontane din România, pe baza literaturii din ultimul deceniu. Dintr-un total de 122 de specii enumerate în 2014 ca fiind strict interzise pentru colectare, pentru 47 din specii au fost publicate, în întreaga lume, diverse studii biotehnologice (studii *in vitro* și studii biochimice). Pentru 13 dintre aceste specii, nu au fost identificate astfel de studii; în cazul lor fiind prezentate câteva remarci sistematice. Pe lângă aspectele medicinale, unele dintre specii au și importanță științifică (endemice, relicte etc.) sau prezintă anumite probleme cum ar fi taxonomia incertă, zona de distribuție foarte limitată, germinația mică a semințelor etc. În țara noastră, în ultimii 5 ani, interesul biotehnologic pentru aceste specii a crescut. Deoarece speciile de plante medicinale reprezintă o valoroasă sursă de produse, studii privind variabilitatea genetică, micropropagarea, inducerea și caracterizarea metaboliților secundari vor fi de ajutor pentru o gestionare mai bună a utilizării și conservării durabile a acestor plante.

Keywords: spontaneous medicinal plant, biotechnology, *in vitro* techniques, secondary metabolites

Introduction

All over the world, medicinal plants, together with aromatic and culinary herbs, include numerous species that can be applied in numerous industries like food, pharmaceutical, chemical and cosmetic based on their constituents. To underline the importance of plant species, experts from the Royal Botanical Gardens Kew have shown that, from the total number of plant species with documented uses, the highest proportion (57.21%) are used as medicines [27].

Medicinal plant species (being natural resources provided by ecosystems) are part of ecosystem services [25] for human well-being. Human well-being is indirectly affected by biodiversity loss due to the disruption of ecosystem function. Loss of biodiversity reduces the resilience of ecosystems, making them more vulnerable to shocks and disturbances and thus less able to supply humans with the services we require [2].

Collection from the wild, loss of the habitat (through residential and commercial development), urbanisation, industrialisation and tourism developments as well as impacts from agriculture represent the most significant threats to these economically important species, affecting nearly half (48%) of plants assessed as Threatened or Near Threatened in the Red List of Medicinal Plants in Europe [1].

Spontaneous medicinal plants collected from the wild, avoiding the pollution from agriculture management [10], represent an undisputable genetic resource, with a vast array of secondary metabolites available; less than 10% of the world's biodiversity has so far been evaluated for potential biological activity [9].

Due to the increasing demand for medicinal plants, there is a pressing need for conservation of these valuable resources. For a sustainable utilisation of wild medicinal plants included in Red Lists, it is important that legal stipulations are followed. The aim of conservation strategies is a sustainable development of valuable germplasm through *in situ* (in natural habitats) and *ex situ* (botanical gardens, seed banks, *in vitro* conservation) techniques. Recently, the larger-

scale cultivation is representing part of conservation strategies [7]. Among the activities of a sustainable conservation programme are: proper identification, collection, characterisation, evaluation, propagation, disease elimination, storage and distribution [28]. For a sustainable use of medicinal plants (both wild/ spontaneous and cultivated), it is necessary to allow modern research technologies for the extraction of pharmacological substances, controlled breeding and conservation.

Materials and Methods

The bibliographic screening was performed in different scientific data bases like Web of Science, PubMed, ResearchGate, Google Scholar etc. Some non-indexed citations were analysed to supplement the searches. The literature spanned the period from 2007 to 2017. For this purpose, we used the following keywords: spontaneous, medicinal plant, biotechnology, *ex situ* conservation, *in vitro* techniques, secondary metabolites, systematic remarks, Red List, Romania. The language was limited to English and Romanian.

Results and Discussion

In the last decade (2007 - 2017), the use of biotechnological approaches in medicinal plants was emphasised by numerous published articles and reviews. Data from the Web of Science showed an ascending trend of publications concerning medicinal plants (endemic or not) to 2016 (Figure 1). However, only one article (published in 2012) described studies about species of medicinal plants whose collection is strictly forbidden.

Biotechnological approaches in plant science are represented by the use of *in vitro* techniques (tissue cultures, micropropagation) and molecular tools (characterisation of genetic diversity, genetic maps, marker assisted selection, genomics and genetic modification) [36]. These studies are helpful in formulating plans for management to preserve their genetic diversity and ensure their long-term survival.

For rapid propagation of rare, endemic and/or endangered medicinal plants, micro-propagation has the greatest commercial and economical importance [33]. Propagation of medicinal plants using tissue culture depends on the part of the plant that is collected, the level of threat in the wild and market demand [29].

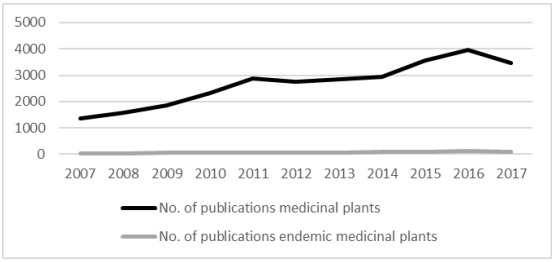

Figure 1.
Number of publications concerning biotechnological approaches of medicinal plant species in the last decade

In Romania, interest in herbal/traditional medicines and dietary supplements has increased for many consumers. In 2016, the value of sales of herbal/traditional products increased by 8%, which represented better performance than the overall consumer health market (growing by 5%) [39].

In Romania, 3,500 species of higher plants (spontaneous or cultivated) have been reported [8]. From the medicinal flora (represented by 900 species) almost 200 have been studied from a pharmaceutical point of view. Around 150 species are systematically harvested for medicinal or aromatic purposes and 50 species are cultivated [34].

In 2014, a list of the main spontaneous medicinal plants from Romania was published, totalling 756 species, of which 126 were threatened with extinction and 122 are strictly forbidden for collection [12].

Examining the most up-to-date literature on biotechnological approaches, of these 122 species, only 25 have recent studies, and 13 species had no *in vitro* or biochemical studies (Table I). For the 58 species where, only biochemical studies are available, *in vitro* techniques (micropropagation) are vital for a future sustainable use of natural resources, without endangering the species in the natural habitats.

While at international level the majority of the studies were regarding the investigation of active constituents and pharmacological activity, in Romania the newest studies were based more on *ex situ* conservation.

Table I
The state of spontaneous medicinal plants with recently biotechnological studies

	No. of species
Total no. of species strictly forbidden for collection	122
No. of species without *in vitro* studies	58
No. of species without biochemical studies	25
No. of species without *in vitro* and biochemical studies	13

From the overall list of species that are strictly forbidden for collection and have no recent biotechnological approaches, 13 examples were selected (Table II), giving the vernacular name (in RO [12]; in ENG [32]) and traditional usage. Where the vernacular name is given in brackets (e.g. [a reedmace or cattail]) the name applies to the genus as a whole, and the species has no unique vernacular name itself.

Table II

Selected spontaneous medicinal plant species strictly forbidden for collection and traditional usage

Plant species	Vernacular name RO/ENG	Traditional usage
Adonis volgensis	/[a pheasant's-eye]	Cardiotonic, diuretic, sedative, vasoconstrictor [5]
Aristolochia lutea	*Mărul lupului*/[a birthwort]	Anti-malaria [18]
Calla palustris	*Coada smeului*/Bog arum	Against nettle-rash, skin irritations, wasp stings [30]
Cimicifuga europaea		Menopause symptoms [23]
Cochlearia borzaeana		Stimulates the kidneys, liver activity [30]
Colchicum arenarium	*Brânduşă*/Sand saffron	horticultural trade [4]
Dracocephalum austriacum	*Mătăciune*/Pontic dragonhead	Cytotoxic activity, trypanocidal activity [22]
Erysimum witmannii	*Micsandre sălbatice*/ [a wallflower]	Cough, during acute benign bronchial disease [19]
Jasminum fruticans	*Iasomie*/Wild jasmine	Antioxidant properties [16]
Lycopodium tristachyum	*Brădişor*/Blue clubmoss (or Blue ground-cedar]	Anti-tumour, acetylcholinesterase inhibitory activity [37]
Pyrola carpatica	*Brăbănoi*/Carpathian wintergreen	Treatment of rheumatism, waist pain, knee pain and high blood pressure [24]
Ribes spicatum	*Coacăz*/Downy currant	Antioxidant activity and anti-inflammatory capacity [14]
Typha shuttleworthii	*Papură*/[a reedmace or cattail]	Treatment of intestinal disorders and burns [11]

For most of this selection (11 species), Table III presents information on related species (together with guidance on taxonomic differentiation [31]) and active ingredients in both the species studied and in their relatives. Some of these rare species have very similar morphological characters to more common species, and distinguishing endangered species from their close relatives may be difficult for inexperienced or untrained people e.g. *Typha* species and *Ribes* species.

Table III

Distinguishing characters between medicinal plant species and their relatives, together with active ingredients

Distinguishing characters		Active ingredients of species or their relatives
Plant species	**Related species**	
Adonis volgensis Leaves 2 - 3 times pinnately cut with ultimate segments linear-lanceolate; 1.5 - 3 mm wide	*A. vernalis* Leaves 2 - 4 times pinnately cut with ultimate segments linear; 1 mm wide	convallatoxin, glycosides-cymarine, adonitoxine, saponin phytosterine, adonite, cardiac glycosides [6]
Aristolochia lutea globular tuber; flowers solitary	*A. clematitis* rhizome; flowers in groups of 2 - 6 (-8) in the axils of the leaves	sterols, alkaloids, tannins [18]
Calla palustris Plant aquatic or in boggy places, whose broadly cordate leaves have parallel venation	*Arum maculatum* Plant always terrestrial, whose triangular-hastate leaves have reticulate venation	saponins, cyanogenic compounds, amines, flavonoids, flavanols (quercetin and kaempferol), flavones, luteolin, apigenin [15]
Colchicum arenarium Style almost straight with a capitate stigma; fruiting capsule 10 - 20 mm long and 6 - 7 mm in diameter; leaves (if present) < 2 cm wide.	*C. autumnale* Style curved and with stigma clearly decurrent; leaves (if present) 3 - 5 cm wide - rarely as narrow as 2 cm	colchicine [26]
Dracocephalum austriacum Leaves pinnately cut with 3 - 7 linear lobes	*Salvia pratensis* Leaves ovate-lanceolate, undivided and with a sub-cordate base	rosmarinic and caffeic acids, flavonoids, diterpenes [22]
Erysimum witmannii Perennial; petals large, 18 - 25 mm long, 5 - 9 mm wide; leaves sinuate - dentate	*E. cheiranthoides* Annual; petals 3 - 6 mm long; leaves entire	glucosinolates, mucilages, sugar alcohols, flavonoids, cardiac steroid glucosides, volatile compounds [19]
Jasminum fruticans Yellow flowers; leaves either alternate or opposite, pinnate, with 3 or 5 - 7 (rarely 9) leaflets	*J. officinale* White flowers; pinnate leaves with at least 5 leaflets	alkaloids, carbohydrate, flavonoids, saponins, tannins, terpenoids [16]
Lycopodium tristachyum Stems & branches weakly compressed, 1.2 - 1.5 mm wide; opaque, squamous leaves	*L. complanatum* Stems & branches strongly compressed; 2 - 3 mm wide	alkaloids [37]
Pyrola carpatica Leaf elliptical with cuneate base & petiole longer than the limb	*P. rotundifolia* Leaf round with rounded base and petiole shorter or equalling the limb	tannins, hyperoside, quercentin [38]

Distinguishing characters		Active ingredients of species or their relatives
Plant species	**Related species**	
Ribes spicatum lack of spines and glandular hairs, possession of red fruit and bisexual flowers	*R. rubrum* saucer-shaped hypanthium and anther lobes distinctly separated by connective	flavanols, phenolic acids, monoterpenes, vitamin C, catechins [14]
Typha shuttleworthii Female part of inflorescence is markedly longer than the male; bristles at base of ovary as long as stigma; seed 0.7 - 0.9 mm long; leaves < 10 mm wide.	*T. latifolia* Male and female parts of inflorescence of similar length when fresh; stigma longer than bristles at base of ovary; seed 1.4 - 1.7 mm long; leaves > 10 mm wide	quercetin- 3- neohesperidosid, quercetin- and kaempferol-3-glucoside, quercetin- and kaempferol-3-galactoside [17]

Focussing on the presence of these species in the Romanian market, two of them are already used as part of the composition of some supplements. Uncontrolled exploitation will have a great impact on the species if the essential parts for survival (underground parts, flowers, seeds) are harvested. Removal or damaging of roots will affect the plant regeneration by reducing the water uptake, increasing the susceptibility to fungal infections. In the case of the spontaneous medicinal species listed by Dihoru & Boruz [12], the most frequently used parts were whole plants (42.85%), followed by underground organs (33.33%), leaves (23.80%), inflorescences (14.28%) and seeds (4.76%). In this context, the application of *in vitro* techniques may assure the multiplication of plant material used for secondary metabolites extraction and conservation of important plant genotypes.

Some of these medicinal plants also have scientific importance due to their toxic properties (*Adonis volgensis)*, problems with taxon determination (*Cochlearia borzaeana*) or rarity *(Dracocephalum austriacum*). *Adonis volgensis* is confined to a restricted area with steppic conditions (in Romania), whilst *Ribes spicatum* and *Cochlearia borzaeana* are glacial relicts. *C. borzaeana, Pyrola carpatica, Erysimum witmannii* and *Colchicum arenarium* are endemic to Romania or to the Carpathians [13], being of biodiversity conservation importance internationally; their extinction could represent the loss of novel ecological interactions [20].

Some medicinal species present problems with seed viability. In the cases of *Cimicifuga europaea* and *Calla palustris*, seeds start to lose their viability soon after storage [40]. *Adonis volgensis* also has propagation problems, showing low seed production, failure of vegetative propagation and occasional attack by microscopic fungi [3].

A further insight into the species selected for the present paper is provided by Figure 2, which summarises their ecological distribution in Romania. Information on taxonomy, synonymy, local and international nomenclature can be useful in ensuring sustainable use of medicinal plants.

At the national level, spontaneous medicinal plants whose collection is strictly forbidden according to Ciocarlan, 2009 [8] are rare or sporadic (as measures of abundance) over more than one vegetation zone - only a few (e.g. *Adonis volgensis* and *Jasminum fruticans*) are narrowly distributed and confined to one vegetation zone or level (Figure 2).

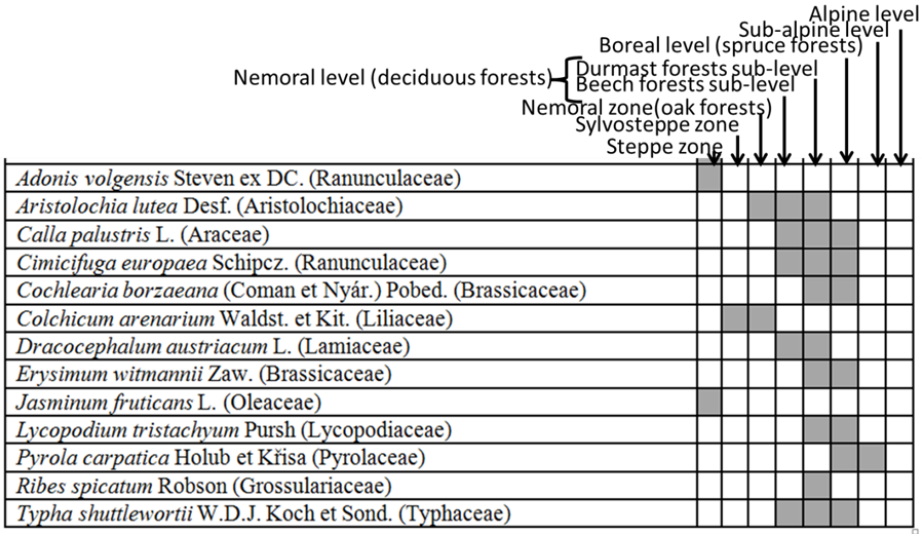

Figure 2.
The distribution of spontaneous medicinal plants strictly forbidden for collection according with Romanian vegetation zones and levels

In terms of phenology (specifically flowering time), these species flowers in [8]: February - March: *Bulbocodium versicolor* (Ker Gawl.) Spreng; April - May: *Adonis volgensis* Steven ex DC., *Aristolochia lutea* Desf., *Ribes spicatum* Robson; May - June: *Jasminum fruticans* L.; May - August: *Calla palustris* L.; June - July: *Cochlearia borzaeana* (Coman et Nyár.) Pobed., *Dracocephalum austriacum* L.; June - August: *Erysimum witmannii* Zaw., *Pyrola carpatica* Holub *et* Křisa, *Typha shuttlewortii* W.D.J. Koch *et* Sond.; July - August: *Cimicifuga europaea* Schipcz., *Lycopodium tristachyum* Pursh; September - October: *Colchicum arenarium* Waldst. *et* Kit.

There are synonyms for some species that should be borne in mind by specialists working with these plant species. We used Ciocârlan (2009) [8] to highlight the synonyms: *Cochlearia borzaeana* (Coman *et* Nyár.) Pobed. is synonymous with *C. pyrenaica* DC. var. *borzaea* Coman *et* Nyár; *Lycopodium tristachyum* Pursh is synonymous with *Diphasiastrum tristachyum* (Pursh) Holub and *Diphasium tristachyum* (Pursh) Rothm.); *Ribes spicatum* Robson is synonymous with *R. schlechtendalii* Langer, *R. rubrum* sensu Jancz. *et* auct. non-L. and *R. heteromorphum* Ţopa.

The nomenclature used in Romania [12] sometimes differs from that used internationally [21, 35]: *Bulbocodium versicolor* (Ker Gawl.) Spreng. is a synonym of *Colchicum bulbocodium* subsp. *versicolor* (Ker Gawl.) K. Perss.; *Cimicifuga europaea* Schipcz. is a synonym of *Actaea europaea* (Schipcz.) J. Compton; *Cochlearia borzaeana* (Coman & Nyaudy) Pobed. is a synonym of *Cochlearia pyrenaica* DC.

Conclusions

This paper presents a review, based on literature published since 2007, of the current status of biotechnological approaches of some spontaneous medicinal plants in Romania, with an emphasis on plant species whose collection is forbidden.

Taking into account that the demand for medicinal plants (cultivated and spontaneous) is increasing, there is a priority for the identification and integration of conservation measures (utilising all the tools available including the *in vitro* methods).

There is a need to expand studies on medicinal plants (whether protected or not) to enable future strategies for increasing knowledge and discovering new valuable compounds.

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Conflict of interest

The authors declare no conflict of interest.

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