VALORISING THE SPECIES *STACHYS OFFICINALIS* (L.) TREVIS. FROM SOUTH-WESTERN ROMANIA

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Abstract: *Banat has a geographical location particularly favorable from the point of view of the climate and of the relief, which favored the development of a large number of grassy species. The Aninei Mountains is the area taken in the study, located in South-West of Romania. It is an area with favorable climatic conditions for a large number of plants also sheds a considerable number of medicinal and aromatic herb, that make the region even more valuable. The genus Stachys belongs, from a systematic point of view, to the Family Lamiaceae. The family contains about 4,000 species spread all over the world, most frequently in Mediterranean area. (Chifu et al., 2001). The genus Stachys groups a large number of species (over 300), with a wide distribution over almost the entire globe. Exceptions to this rule are only Australia and New Zeeland. (Bilusic Vundac et al., 2006) Our research aimed at both identifying the areas where this species thrives and justifies its economic valorisation, and at establishing, through bio-chemical analyses of the material quality. The paper highlights the importance of knowing the real economic potential of spontaneous medicinal species, correlated with biochemical analysis. Determination of chemical composition can recomended or not the use of a medicinal species from certin areas. For the quantitative assessment we used the methodology of economic mapping of medicinal plants in the spontaneous flora indicated by Alexan, Bojor and Craciun in 1983, and improved by Bojor in 1991. Chemical composition was determined after the Official Methods of Analyses. The authors thank the Ministry of Education, Research, Youth, and Sport which, through the National Council of Scientific Research in Higher Education, has financed the present study as part of the research project PN II IDEI nr. contract 1077/2009, project code ID-865. The topic of the project is „Identifying medicinal and aromatic plants from the Aninei Mountains with a view to valorisation''.*

Key words: *medicinal and aromatic plants, Stachys officinalis, Aninei Mountain,quantitaive assessment, chemical composition*

INTRODUCTION

Genus *Stachys*, is classified scientifically in the Phylum Spermatophyta, Sub-phylum Magnoliophytina, Class Magnoliopsida, Subclass Asterideae, Order Lamiales, Family *Lamiaceae*. *Stachys* are herbaceous plants with simple or ramified stems. The leaves are opposed, narrow-lanceolate to broad-ovate, serrated or crenate, sometimes pinnate-divided. The flowers have a bilabiate corolla, a family features, and are grouped in verticils containing 2-20 flowers. The fruits are long, ovoid nucules with 3 edges and rounded tips (Flora R.P.R., vol. VIII). In the Romanian flora, the genus has 11 species (*S. officinalis*, *S. oblique*, *S. alpina*, *S. germanica, S annua, S. angustifolia, S. sylvatica, S. palustris*, *S. maritima*, *S. atherocalyx*, *S. recta*) plus a Mediterranean species cultivated for ornamental purposes – *S. byzantina* – as well as 2 species mentioned in the past but that were not re-confirmed **–** *S. pradica* and *S. arvensis*. (CIOCÂRLAN, 2009)

Stachys officinalis (syn. *Betonica officinalis*) is named commonly bishop's wort, bishopwort, lousewort, purple betony, wild hop, or wood betony. It has been used medicinally from times immemorial. Its aerial parts (*herba*) are used therapeutically in folk tradition; its active principles make it a tonic, astringent, antidiarrhoeic, diuretic, antiseptic, febrifuge, cholagogue, and vulnerary (PÂRVU, 2000). As active principles they mention the following: tannins, bitter substances, choline, betaine, stachydrine, and it is recommended in the treatment of the gout, gallstones, kidney stones, pulmonary catarrh, asthma, diarrhoea (oral use) as well as in the treatment of cuts and varicose ulcer (external use). (ARDELEAN & MOHAN, 2008)

MATERIAL AND METHODS

As we mentioned before, for the quantitative assessment, we used the methodology of economic mapping of medicinal plants in the spontaneous flora indicated by ALEXAN et al. (1983), and BOJOR (1991). The researches were carried on by field observation in different periods of the year. We carried out releves of 1 m^2, following and recording the medicinal species within each frame. According to the methodology, we have calculated average density per m^2 for all medicinal species we came across and that can be recommended for harvesting. Results of quantitative estimates are expressed in kg of dry matter. For a highest accuracy of data recorded in most species analysed, we have established average amounts of dry matter through our own weighing. Results of the study were expressed taking into account the laws of environmental protection and the necessity of recovering area potential (IMBREA *et al*., 2009). Species identification was done using *Flora României*; the species are named after CIOCÂRLAN (2000) and *Flora Europaea* (electronic edition).

The vegetal product necessary for analyses was sampled from the Bozovici area, during flowering, between July 1 and August 20, 2010. After harvesting, the material was dried in a clean room, in the shadow, far from other medicinal species harvested at the same time. The dry product was put into paper bags and labelled with the scientific name, date of harvesting, locality of sampling, features of the biota, etc. A voucher specimen (No. 15 – 1/2010) has been preserved in our laboratory for future reference.

Chemical composition was determined after the Official Methods of Analyses:

The chemical analysis to determine sample composition was made using standard procedures. Water content was determined after air drying, fat content was determined through extraction with a Soxhlet apparatus, sugars were determined through difference, ash content was determined through incineration, raw fibber through incineration, after acid and base digestion, after acid digestion of the samples and protein with the Kjeldahl method (AOAC, 1990).

Assessing energetic value. Caloric power was assessed (in kcal) by multiplying the percentage of crude protein, crude lipids and carbohydrates, with the recommended factor (2.44, 8.37, and 3.57, respectively), use in analysing vegetables. Calorimetric value was determined with the Atwater factor (FAO, 2006a). Dry matter content was determined by drying at 105°C. Crude ash content was determined by calcinating the sample at 450°C. protein content was determined by dosing total nitrogen (N) with the Kjeldahl method (AOAC, 2000). The value of total nitrogen x factor = value of protein N x 6.25 (1/0.16 = 6.25). Lipid content was determined through the gravimetric method (AOAC, 2000). Available carbohydrate content was determined through difference (total sugars – dietary fibbers).

Total sugars (AOAC, 2000)

100 – (weight in grams [proteins + fats + water + ash + alcohol] in 100 g)

Available sugars

100 – (weight in grams [proteins + fats + water + ash + alcohol] in 100 g)

Ascorbic acid (vitamin C) is determined titrimetrically taking into account the fact that it is a strong reducing agent that loses hydrogen atoms easily, turning into dehydroascorbic acid that also has vitamin activity. Vitamin activity is lost when the alctonic cycle of the dehydroascorbic acid is hydrolysed forming dicetogulonic acid. The method we used is based on the titering of the ascorbic acid from vegetal extracts with 2,6-dichlorindophenol until persistent pink for 5 seconds. Establishing 2,6-dichlorindophenol solution titre is done with an exact concentration solution of vitamin C freshly prepared and titered under the same conditions as the samples.

Analysis of polyphenol and flavone content of the vegetal material. The method is based on the reducing effect of polyphenols on the hexavalent molybdenum from the polyphosphomolybdate contained by the Folin-Ciocâltău reactive. In a strongly acid medium, hexavalent molybdenum is partially reduced by the polyphenols to lower states (+4 and +5). The compounds thus obtained are blue in an alkaline medium (molybdenum blue) with specific absorption stripes. These absorptions are due to the charge transfer bands specific to transitional metals in low valence states. The sample with a 1-10 mL extract volume containing phenol substances was introduced in a graded balloon of 100 mL. We added in the balloon 1 mL of Folin-Ciocâlteu solution and, after 15 minutes, 10 mL of saturated solution of Na_2CO_3. The solution was diluted with 20 mL of water and left to rest for 15 minutes, and then it was completed with distilled water. We measured solution absorbance at 670 nm. We obtained the control solution by diluting 1 mL of the Folin-Ciocâlteu reactive and 10 mL of Na_2CO_3 up to 100 mL with distilled water.

Vitamin content was determined according to the method presented by STEPHANE et al. (2005).

RESULTS AND DISCUSSIONS

Stachys officinalis (bishop's wort) is an herbaceous, perennial, cosmopolite species found in haymaking fields, in clearings, shrubberies, and forest borders. The stem is tall (up to 70-100 cm), pubescent, and usually not ramified. Basal leaves are long petiolate, rosette-shaped, prolonged-ovate, and hairy on both sides. The red-purplish flowers are gathered in verticils grouped in terminal ears. The nucules are brown, ovoid, and smooth. The species blooms from June to August and is common all over Romania (Flora RPR, vol. VIII, 1961).

Together with bishop's wort, other species of the genus also thrive in the area, such as: *Stachys sylvatica, Stachys recta, Stachys germanica*. But these species are less known and less used as medicinal plants.

After visiting the area at different times of the year, we could also identify and assess quantitatively the main harvestable medicinal species of the area. As for the species *Stachys officinalis*, it can be found mainly on the meadows located in the south-eastern area of the Aninei Mountains.

The species was also found on the other meadows, but with low abundance-dominance and frequency; this is why we do not recommend harvesting the species from those areas.

In the south-western of the Aninei Mountains (the Taria-Globul Taria area), on certain areas, the species makes up true covers with spots totalling 20 individuals/m^2. The large number of individuals in certain areas looks like medicinal crops (Foto 1).

From these areas, one can harvest 2000 kg of dry matter while observing the laws of species protection (IMBREA *et al*., 2009).

Stachys officinalis absorbs mineral substances from which, through chlorophyll assimilation, are bio-synthesised organic substances.

The analysis of the chemical composition of *Stachys officinalis* pointed out a high content of dry matter due to a more intense photosynthesis allowing the accumulation of a higher amount of carbohydrates.

Sulphur is absorbed by the plants from the soil through the roots under the form of ions SO_4^{2-}, and in smaller amounts from the air through the leaves, as SO_2. *Stachys officinalis* needs sulphur for the biosynthesis of some organic sulphur compounds (cystine).

Photo 1: *Stachys officinalis* in the Taria Hills (original photo)

Lack of sulphur in plant nutrition results in growth slowing down and even stopping. Sulphur lack results in an increase of soluble nitrogen (ammonia, nitric, aminic, and amidic) and in a slowing down of protein substance formation. *Stachys officinalis* takes over the chlorine in the soil through its root system and from the atmosphere through leaves stomata. Chlorine is an important activator of some enzymes (cytochrome oxidase). Oxidation of cytochrome C reduced by coenzyme I is speeded up by the presence of the chlorine.

The physic-chemical analysis of *Stachys officinalis* plants are presented in table 1.

Table 1

Physic-chemical analysis of *Stachys officinalis* plants

Nr.	Physical or chemical parameters			*Stachys officinalis*
1	Physical state			Dried powder
2	Colour			Grey
3	Ash pH			11,01±1,32
4	Conductibility of ash solution (MS / cm)			769±54,47
5	% loss through drying			0,468±0,01
6	% ash content			7,5±0,19
7	Chloride mg / l			16,43±2,69
8	Sulphate mg / l			11,23±1,12
9	%of ash	alcohol	Soluble substances	33,58±1,09
10			Insoluble substances	66,42±9,36
11		water	Soluble substances	28,43±6,87
12			Insoluble substances	71,57± 1,36
13		acid	Soluble substances	98,00± 4,26
14			Insoluble substances	2,00±0,11
The values represent the mean of three chemical measurements ± the standard deviation.				

Analyses made to determine the content of organic N (turned into proteins) show that the species was efficient in absorbing both nitrogen and other mineral elements from the soil. (PIZZI & BRUNO 2011).

The chemical composition of *Stachys officinalis* plants is presented in table 2.

Table 2

Chemical composition of *Stachys officinalis* plants

Nr.	Physical or chemical parameter	*Stachys officinalis*
1	Dry matter %	86±6,6
2	Lipids %	7,03±1,69
3	Proteins %	14,4±0,12
4	Total fibber (g/100g)	19,5±1,02
5	Soluble fibber (g/100g)	9,0±0,32
6	Insoluble fibber (g/100g)	6,82±0,58
7	Carbohydrates %	49,0±0,01
8	Energy (kcal)	5,25±0,4
9	Vitamin C (mg/100g)	5,21±1,47
The values represent the mean of three chemical measurements ± the standard deviation.		

Fibber content shows that *Stachys officinalis* is efficient in holding water, particularly insoluble ones. (WANG et al. 2008) Soluble fibbers make up some compounds that fix substances similar to cholesterol and, due to a cycle of biochemical reactions, they play an important role in the decrease of cholesterol in the human body (SLAVIN 2003).

Though volatile oil is the dominant and specific product of inflorescences, ***herba*** is sued due to its content in different active principles (polyphenols, flavones, cumarines, triterpenes, phyitosterols, and tannins).

Metanolic extracts from the plants we investigated phytochemically were analysed quantitatively and qualitatively to assess polyphenol and flavones content and to identify their fractions.

Polyphenols-*samples*: caffeic acid, chlorogenic acid, ferrulic acid, cumaric acid; Flavones-*samples*: quercetol, rutoside, apigenol, luteolin.

To characterise and analyse phenolic substances, we used the Folin–Ciocâlteu method (MATKOWSKI & WOŁNIAK, 2005).

In table 3 we presents the values of some substances with biological activity.

Table 3

Value of some biological activity substances from *Stachys officinalis* plants

Nr. crt.	Chermical compounds	*Stachys officinalis*
1	Polyphenols (cafeic acid g% s.u.)	2,41
2	Flavones (rutozid g% s.u.)	1,890
3	Vitamin E mg	3,23
4	Vitamin B1 mg	2,58
5	Vitamin B2 mg	0,29
6	Nicotinic acid mg	10,71
7	Vitamin B6 mg	0,55
8	Biotin mg	0,06
9	Folic acid mg	0,20
The values represent the mean of three chemical measurements ± the standard deviation.		

Compared to the values found in other researches, the plants of *Stachys officinalis* in the Bozovici area are remarkable for their higher content of polyphenols and flavones. (BILUŠIĆ VUNDAĆA *et al*. 2007)

The mixture of polyphenol compounds together with hydrosoluble and liposoluble vitamins from *Stachys officinalis* make it a strong antioxidant (KATERERE&LUSEBA, 2010).

CONCLUSIONS

The study brings forth the need to know the real potential of valorising different areas and also the importance of knowing the biological value of the harvested material taking into account the fact that lots of people market medicinal plants without mentioning the area of origin and with no previous chemical analysis.

Harvesting spontaneous medicinal plants without knowing the potential of the area or the proper harvesting method can result in a series of negative consequences and, therefore, in serious ecological unbalance (it can favour the explosive development of species detrimental to other species thus changing the floristic composition or even determine species extinction).

Results of analyses show the quality of the material analysed compared to that of similar materials presented in literature. High values of polyphenols, flavones, and vitamins recommend the species to be harvested and even to be super-sowed or introduced into cultivation.

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