

## VALORISING *THYMUS GLABRESCENS* WILLD. FROM THE ANINEI MOUNTAINS

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**Abstract:** The Aninei Mountains are characterised, as well as the entire area of the Banat, by a remarkable floristic diversity. Among medicinal species that are highly abundant and frequent in the area and present on almost all grasslands, the species of the genus *Thymus*. These species are easy to identify after both their morphological aspect and their characteristic aromatic smell, being harvested and used by the inhabitants in the area for both cooking uses and as infusion in respiratory diseases. Of the total 17 species present in Romanian flora, 8 can be found in the studied area. (Flora României – Ciocârlan 2009) The most frequent one are: *Th. glabrescens* Willd., *Th. pannonicus* All. and *Th. pulegioides* L. Though the vegetal product used in phyto-therapy is found as *Serpylli herba*, in which enter different species of the genus, in the present paper we have analysed more thoroughly one of the most frequent species of the genus, with a view to establish the quality of the harvestable vegetal material and of grouping it within admitted standards. The working method is based, in the field stage, species identification starting from botanical determiners (Flora României - vol. III 1961, Flora ilustrată a

României – Ciocârlan 2009). The product we have analysed was harvested at the optimal time and then shadow dried. Later it was analysed in the laboratories of the SC Fares Laboratory Bio Vital in Orăștie, one of the most important producers and traders of medicinal plants – from both spontaneous and cultivated flora – on the Romanian market. Taking into account the large number of both legally and illegally commercialised ecological products on the market, we hope to support the inhabitants in the rural area, that are directly interested in valorising species of the *Thymus* genus. As a result of the analysis of the volatile oil content we can recommend or not the product to be valorised or not. 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, *Thymus glabrescens*, thymol, Aninei Mountains

### INTRODUCTION

Genus *Thymus*, commonly called *thyme* or *common thyme*, is classified scientifically in the Phylum Spermatophyta, Sub-phylum Magnoliophytina, Class Magnoliopsida, Subclass Asteridae, Order Lamiales, Family Lamiaceae (CIOCÂRLAN 2009). It groups 17 species of the Romanian flora and over 200 species worldwide, and is one of the most important genera of the Family Lamiaceae from the point of view of the species and varieties. Among the species found in Romania, *Thymus vulgaris* is cultivated for its uses in the food industry, in the pharmaceutical industry, and in the cosmetics industry. It is a spontaneous plant in Spain, southern France, Greece, and western and southern Italy (MUNTEAN 2007).

Taxonomic interpretation of the species belonging to the genus *Thymus* is very difficult because of the high variability of the populations, with extremely varied morphological features. Its chemical composition is also very different depending on environmental factors and on genetic variability. Antibacterial action of the compounds of the

*Thymus* species has been demonstrated for Gram-positive and Gram-negative bacteria, as its antifungal effect has (MAKSIMOVIĆ *et al.* 2008).

Generally, the undiluted essential oil of thyme species is not to be taken internally. Excessive use of essential oil is toxic, causing such complications as diarrhea, vomiting, nausea, headache, and dizziness, and even slowing the heartbeat, depressing respiration, and lowering body temperature.

Due to its high variability, the vegetal product harvested and traded under the name *Serpylli herba* in Romania represents, in fact, a mixture of several species of the genus: *Th. serpyllum*, *Th. marschallianus*, *Th. pannonicus*, *Th. glabrescens*, *Th. austriacus*, *Th. dacicus*, *Th. pulegioides*, *Th. callieri*, *Th. zygioides*, *Th. pulcherimus*, *Th. marginatus*, *Th. comosus* (GERED – CSEGEDI 1972, cited by CIULEI *et al.* 1993).

### MATERIAL AND METHODS

The vegetal product necessary for analyses was sampled from the Caraşova area, during flowering, between May 24 and June 5, 2009. 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. Determining the species accurately, very difficult in the genus *Thymus*, was done on fresh material with specific determiners, in laboratory conditions, since certain morphological features can be observed only with binocular magnifying glasses. A voucher specimen (No. 9 – 1/2009) has been preserved in our laboratory for future reference. The material was then sent to be analysed to the S.C. Laboratoarele Fares Bio Vital S.R.L. Oraştie to see if they meet quality indicators necessary for the product to be valorised.

Qualitative and quantitative analyses of the *Thymus glabrescens* oil were performed using processes of extraction, separation and batching. Determination of active ingredients was achieved by specific reactions, performed in natural and processed mining solutions. Have been established optimal conditions for extracting the laboratory phase: the degree of grinding of plant material, plant material ratio/solvent, extraction temperature, extraction time, number of successive extractions for exhausted plant material.

To assess essential oil in *Thymus glabrescens*, dry plants were minutely cut and hydro-distilled for 3 hrs using a Clevenger-type apparatus, according to the standard procedure reported in the European Pharmacopoeia.

Determining thymol content was done gas-chromatographically with a Hewlett-Packard S890 series II apparatus, equipped with a flame ionisation detector (FID) and with a SC-INTEGRATOR (Sinicom Oy) to assess quantitatively.

Chromatographic conditions: column gas chromatography – quartz 50 m in length and 0,32 mm diameter; stationary phase: CBWax, 20 m; detector temperature: 250<sup>0</sup>C; and injector temperature: 250<sup>0</sup>C; injected quantity: 0,1 µl; oven temperature programs: 60<sup>0</sup>C (3 minutes); 4<sup>0</sup>/ minutes; 220<sup>0</sup>C. The sample is injected into the carrier gas stream. To identify thymol content in *Thymus glabrescens*, we injected initially standard thymol to establish retention time.

### RESULTS AND DISCUSSIONS

*Thymus glabrescens* Willd. Is distributed in Central and East Europe and in Asia (JALAS 1972). It is a herbaceous, perennial species with pseudo-repens stems ended by spike-like racem. The leaves are uneven and grow in size towards the inflorescence. They can be glabrous on both sides (subsp. *glabrescens*) or, together with the stems, covered by long hairs (subsp. *pilosus*). The flowers are pink-purple, while the fruit is a nutlet. The species is common

in Romania on grassland, shrubbery, and grassy, sunny spots (CIOCĂRLAN 2009).

The species has been used since ancient times in food flavouring. The aerial part of the plant (*herba*) is important phyto-therapeutically, particularly due to its volatile oil and to its flavonoids with stomachic, choleric-colagogue, carminative, anthelmintic, antiseptic, anti-inflammatory, antispastic, wound healing, anti-diarrhoeic, antiviral (PĂRVU 2000; PAVEL 2009).

Most of the compounds found in plants belong to rather few families of substances. Only small chemical modifications like methylations, hydroxylations, intercalations with metal ions *etc.* lead to a wide spectrum of functionally different substances.

Generally, the *Thymus* species essential oil was found to be rich in the active monoterpene phenols (thymol and carvacrol) and their corresponding monoterpene hydrocarbon (HC) precursors ( $p$  – cymene and  $\gamma$  – terpinene), which collectively showed synchronized patterns of variation during the different regions.

Though there are numerous studies on the chemical composition of the species belonging to the genus *Thymus*, there are relatively few studies on the species *Th. glabrescens* carried out in Serbia, western Balkans, and Romania (DAJIĆ-STEVAŃOVIĆ *et al.* 2008).

In this study, the authors have identified 52 components of the volatile oil in *Th. glabrescens*. Among them are thymol (minimum 22.28 – maximum 55.12%, i.e. a mean of 35%), followed by  $\gamma$ -Terpinene (22.32%) and *p*-cymene (14.03%).

The main components of *Th. glabrescens* vary largely from one country to another, depending on environmental factors, which made most authors call it “genetic and chemical polymorphism” (LOŽIENĖ & VENSĀKUTONIS 2005). Generally, the production of phenolic compounds is favoured in warmer and drier climatic regions (MAKSIMOVIĆ *et al.* 2008).

The vegetal material was harvested in the Caraşova area, during flowering, from dry and sunny grassland, on limey substratum. The soils are limey rendzines with short horizon, with frequent rock fragments on the surface.

According to the analysis bulletin released by the S.C. Laboratoarele Fares Bio Vital S.R.L. Oraştie, the vegetal product *Thymus glabrescens* has the following physical and chemical features:

- aspect, colour, taste, and smell are in accordance with literature;
- advanced purity of the raw material;
- the loss generated by drying meets the standards of the Farmacopeea Română Ed. X (1998), cap. IX.C.15;
- positive identification of the volatile oil;
- the dosage of the volatile oil is 0.7% (v/d.w.), F.R. ed. X, cap. IX.D.10;
- insoluble ashes content in chlorhydric acid 100g/l was 5.1%: FR X, cap. IX.C.7.

Through chromatographic analysis, we determined thymol (mean amount 42.23%) in the volatile oil from *Th. glabrescens*. Thus, essential oil in *Thymus glabrescens* in the Caraşova area (Banat, Romania) belongs, as well as that of the Serbian Banat (DAJIĆ-STEVAŃOVIĆ *et al.* 2008), to the chemotype T (thymol + *p*-cymene +  $\gamma$ -terpinene).

The essential oil from Caraşova area was obtained from air-dried plant material in a yield of 0.7% v/d.w. A similar amount (0.73%) was obtained from *Thymus glabrescens* in the Gorj County (Romania) by Pavel *et al.* (2009), but their chemical composition is different: geraniol (55.5%), neryl acetat (11.1%) and  $\beta$ -bisabolene (6.7%) while thymol was identified in small amounts (1.5%).

The vegetal material that can be harvested from the grassland between Reşita, Caraşova and Anina, from a total area of 2,133 ha (areas covered by the species *Pteridium aquilinum* were not included) reaches about 8,000 kg dry matter (7,962 kg d.m.), taking into account the laws of environmental protection. This amount refers to *Thymus herba* since, as we

have mentioned previously, species identification is extremely difficult and the vegetal product traded under the name *Serpylli herba* is, in fact, a mixture of several species of the genus. We consider that the species analysed represent about 40% of the total harvestable amount.

### CONCLUSIONS

Field observations aiming at identifying medicinal and aromatic plants harvestable from the area as well as establishing the amount of plants to be harvested, together with the sampling, point out a high share of the species of the genus *Thymus* in the area. Thus, analysing the grassland between Reșița, Carașova and Anina, in northern Aninei Mountains, we can see that, from a total area of about 2,133 ha of grassland (we did not include areas covered by the fern *Pteridium aquilinum*) we can harvest a total amount of herba of about 8,000 kg dry matter (7,962 kg d.m.), taking into account the laws of nature protection. This amount refers to *Thymus herba* since, as we have explained above, identifying species is extremely difficult and the product marketed under the name *Serpylli herba* is but a mixture of several species of the genus. We can say that the species analysed shares about 40% of the total harvestable amount. Analysis results confirm the chemical features of the product obtained from one of the most common species of the genus in the area, while high content of thymol recommends its introduction into cultivation. The essential oil content is a significant selection factor for plant breeders.

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