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ESSENTIAL OILS OF *THYMUS COMOSUS* HEUFF. EX GRISEB. ET SCHENK (*LAMIACEAE*) COLLECTED FROM DIFFERENT AREAS OF ROMANIA

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Abstract: The factors that determine the composition of the essential oil are numerous and sometimes it is difficult to distinguish since many of them are interdependent. From the category of these factors we can mention seasonal variations, geographic area and growth stages. In the literature there are some data indicating changes in the chemical composition of the essential oil according to the geographical regions in which the plants grow. To highlight any changes that may occur in the chemical composition of volatile oil according to geographical areas, samples of *Thymus comosus* were collected from three different locations with different altitudes. The volatile oil was extracted by hydrodistillation according to European Pharmacopoeia standards. The separation and the identification of the components have been carried out using GC-MS (gas chromatography coupled with massspectrometry). Our analysis led to the identification of a total of 51 chemical compounds, the largest number being found in *Thymus comosus* collected from Braşov (44 compounds) and the lowest number in *Thymus comosus* collected from Păltiniş (12 compound). In conclusion, our studies reveal the fact that geographical area and the altitude can be contributing factors of the quality and quantity of volatile oil.
Keywords: *Thymus*, essential oil**,** geographical areas.

Introduction

Thymus comosus Heuff. ex Griseb. et Schenk (*Lamiaceae*) is a perennial species that grows spontaneously in Romania flora, being spread in rocky meadows, especially in mountain areas (Guşuleac, 1961). The species is sporadic in the Romanian Carpathians, being encountered on grassy and sunny rocks, from the durmast storey until spruce storey, being considered endemic species (Sârbu el al., 2013).

From the available literature it was found that the data regarding the chemical composition of the essential oil of *Thymus comosus* are relatively low. Kisgyörgy and the collaborators (1983) found that the main constituents of the essential oil of *Thymus comosus* are neryl-acetate (24.4%), carvacrol (12.5%) and thymol (10.5%). The study conducted by Pavel et al. (2009) show that the main constituent of the essential oil derived from this species is caryophyllene oxide (54.82%), followed by camphene (10.73%), β-bourbonene (5.90%), limonene (3.84%), eudesmol (3.65%) and α-pinene (3.67%). The very few existing data on the essential oils of *Thymus comosus* shows their chemical variability. The factors that determine the chemical variability of essential oils are numerous; sometimes it is difficult to distinguish one from the other, because many are interdependent (Terblanche, 2000). Among these factors we can mention the seasonal variations and maturation, genetic variation, geographic origin, growth stages, plant parts that are used (Marotti et al., 1994; Stahl-Biskup, 2002, Anwar et al., 2009).

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Taking into account existing studies on environmental factors that may influence the active principles of plants (Uribe-Hernandez et al., 1992; Souto-Bachiller et al., 1997; Van Vuuren et al., 2007; Viljoen et al., 2006 and Chalchat et al. 1995) in this article the authors tried to highlight the changes that may occur in the chemical composition of essential oils of *Thymus comosus*, a species that has been collected from different geographical areas of Romania, areas with different altitude. It should also be noted that analysis of the influence of altitude on volatile oil from this species represents a novelty.

Materials and methods

Plant material

The vegetal material is represented by *Thymus comosus*, a species that grows wild in the Romanian flora. The plant has a woody rhizome, vigorously, emitting numerous roots and suberect stems, ascending and prostrate, the lateral one being elongated and sometimes pseudo-repent, profusely branched at the inferior part. Floriferous branches are on average of 10 cm long, are cylindrical, all around hairy. Leaves are widescreen-ovate or widescreen-elliptic, rounded, the middle almost orbicular, cuneate at base with marginal rib (Guşuleac, 1961), they have a length of about 17 mm and a width of 9 mm. The bracts are like leaves (Jalas, 1972). Species identification was performed by Dr. Ioan Sârbu from Botanic Garden "Anastasie Fătu", Iași. The identification of taxa has been done using the following papers: Flora R.S.R., vol. VIII; Flora Europaea, vol. 3 and Flora ilustrată a României – Pteridophyta et Spermatophyta (Ciocârlan, 2009). The collected material was registered and stored in „Alexandru Ioan Cuza" University's Herbarium from Iaşi.

The aerial parts of this plant were collected in the anthesis period, in 3 different zones (Brașov – 750 m, Păltiniș - 1000 m and Păltiniș - 1400 m). Immediately after harvesting the plant material was dried at room temperature.

Isolation of essential oils

The dried aerial parts of the plant were subjected to hydro-distillation, using a NeoClevenger apparatus, according to the method recommended by the European Pharmacopeia (Maissoneuve, 1983). The obtained essential oils were stored at +4^{o}C until analysis.

Analysis of essential oils

The chemical composition of the essential oil was established by GC-MS analysis with the help of a gas-chromatograph Agilent Technologies tip 6890N coupled to a mass detector (MSD) of the 5975 inert XL Mass Selective Detector type. The conditions for chromatography were: column HP 5MS, mobile phase Helium – discharge: 1 mL/min, injector temperature: 250°C, detector temperature: 250°C, temperature regime from initial 40°C (10 degrees/min.) to 280 degrees, injected volume: 0.1-0.3 µl, spliting ratio-1:100. The DB5 chromatographic column has a length of 30 m an interior diameter of 0.25 mm and a film diameter of 0.25 µm. The separated compounds were identified by means of the Nist spectrum database, and the peak position was confirmed by the Kovats retention index. The analysis of essential oils was performed in the Research Center for the Study of Quality of Horticultural Products, Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine Bucharest, with the support of Prof. Dr. Ioan Burzo.

Results and discussions

The essential oils content from the samples of *Thymus comosus* collected from three different altitudes (750 m, 1000 m and 1400 m) varies between 0.8-1 mL/kg. These results are relatively similar to the results previously obtained by other researchers (Geréd-Csegedi, 1972, Pavel, 2009).

Analyzing the chemical composition of these essential oils, we noticed that there are some differences. A total of 51 chemical compounds were identified, the highest number being found in *Thymus comosus* collected from 750 m (44 compounds) and the lowest number in *Thymus comosus* collected 1400 m (12 compound) (Table 1).

The essential oil from the taxa collected from Brașov (750 m) present as main constituents: farnesol (21.92 %), germacren D (17.04 %) and thymol (11.55 %). In smaller quantities we have identified linalool acetate (5.23 %), β-caryophyllene (4.36 %) and linalool (3.66 %). The essential oil of the taxa collected from Păltiniș at 1000 m altitude has as main components farnesol (31.25 %), germacren D (22.10 %) and β-caryophyllene (18.22 %). In the essential oil from species collected at the 1400 m altitude, it was found the presence of a large amount of farnesol (65.63 %).

Table 1. Chemical composition of the essential oils of *Thymus comosus* taken from three different locations of Romania, locations with different altitude

No.	Compound	Altitude		
		750 m	**1000 m**	**1400 m**
1	α-Pinene	-	1.18	-
2	Camphene	0.20	2.01	-
3	β-Pinene	-	0.22	-
4	Octen-3-ol	0.31	-	-
5	β-Mircene	1.07	-	-
6	3-Octanol	0.10	-	-
7	o-Cimene	1.56	-	-
8	Eucaliptol	0.41	0.85	-
9	trans-β-Ocimene	0.13	0.36	-
10	cic-β-Ocimene	0.65	0.12	-
11	γ-Terpinene	1.05	-	-
12	Izopropil biciclohexan-2-ol	0.89	0.23	-
13	**Linalool**	**3.66**	1.11	2.14
14	Octenil acetate	0.08	-	-
15	Camphor	0.93	-	-
16	Borneol	0.24	0.36	-
17	Terpinene- 4-ol	0.14	0.25	-
18	α-Terpineol	2.10	-	-
19	Estragole	1.92	1.16	0.57
20	metil-timil Eter	0.92	-	-
21	**Linalool acetate**	**5.23**	0.18	-
22	Bornyl acetate	-	0.32	-
23	**Thymol**	**11.55**	2.01	**7.46**
24	Carvacrol	1.67	0.19	0.76
25	α-Terpinyl acetate	0.13	-	-
26	Thymyl acetate	0.23	-	-
27	Neril acetate	0.14	-	-

28	Geranyl acetate	2.11	0.29	-
29	β-Burbonen	0.65	0.89	-
30	β-Elemene	0.20	0.41	
31	**B-Cariophyllene**	**4.36**	**18.22**	2.46
32	α-Farnesene	0.27	1.29	-
33	α-Cariophyelene	0.50	1.29	-
34	**Germacrene D**	**17.04**	**22.10**	2.63
35	elixen	1.87	0.76	0.38
36	β-Bisabolene	2.58	0.33	-
37	δ-Cadinene	0.44	0.30	-
38	Nerolidol	-	0.18	-
39	cis-α-Bisabolene	0.16	-	-
40	α-Bisabolol	0.22	-	-
41	Murolen	2.00	-	-
42	Cariophyelene oxide	0.89	1.28	-
43	Izoaromadendrene epoxide	-	0.14	-
44	Cubenol	0.15	-	-
45	tau Cadinol	1.39	1.79	-
46	α- Cadinol	-	0.87	2.09
47	Leden oxide	-	1.50	0.69
48	tau Muralol	0.44	-	-
49	Farnesal	1.63	1.22	0.34
50	**Farnesol**	**21.92**	**31.25**	**65.63**
51	trans Farnesol	2.38	1.32	2.05
Total (%)		***96.51***	***95.98***	***87.2***

Following analyzes conducted we have seen that farnesol, an acyclic sesquiterpene alcohol, is well represented in all three samples studied, compound that has not been identified in previous studies (Kisgyörgy, 1983; Pavel, 2009). The farnesol is present in many essential oils and is used in perfumery to emphasize the odors of sweet floral perfumes. It is especially used in lilac perfumes. Also, the farnesol is a natural pesticide for mites and is a pheromone for several other insects (http://www.ars-grin.gov/duke).

Germacren D, a hydrocarbon sesquiterpenes class that can be used as a pesticide and pheromone (http://www.ars-grin.gov/duke) are fairly well represented in first two samples. This compound has been identified in previous studies on the volatile oils of *Thymus comosus* (Pavel, 2009), but in much smaller amounts.

Another compound identified by us, in relatively large quantities in samples collected from the 750 m and 1400 m is thymol, a monoterpene specific to genus *Thymus* plants, which has numerous biological activities. The carvacrol, also a specific phenolic monoterpenes for genus *Thymus*, was found in small amounts in the essentials oils analyzed by us. These results obtained by us regarding the thymol and carvacrol, confirm some previous studies (Kisgyörgy, 1983) and also infirm others (Geréd-Csegedi, 1972; Pavel, 2009).

The differences occurring in the chemical composition of volatile oils, both qualitatively and quantitatively may be due to the influence of environmental conditions, the genus *Thymus* being known for chemical variability of essential oils. Thus, Maksimovic and collaborators in 2008 identified in the volatile oil *Thymus pannonicus* All., harvested in northern Serbia, a total of 33 constituents, the main being geranial (41.42%) and neral (29.61). These compounds were not identified in the volatile oil *Thymus pannonicus* collected in Romania (Boz et al., 2009), but traces of geraniol were found, a compound that

forms geranial by oxidation. Other researchers have identified in the volatile oil of this species large amounts of thymol (25-41%) and p-cimen (17-38%) (Pluhar et al., 2007).

Regarding the effect of altitude and geographical location on volatile oils there are in the literature a few works on the species belonging to *Lamiaceae* family (Viljoen A.M. et al., 2006; Maric et al., 2006; Mkaddem et al., 2007), including and species of the genus *Thymus*, other than *Thymus comosus* (Özgüven and Tansi, 1998; Thakuri et al., 2009; Pereira et. al., 2000; Avci, 2011; Pirbalouti et al., 2011).

Our study confirms that also for the species *Thymus comosus*, there may be differences in the chemical composition of volatile oils. These differences may be due to environmental factors such as altitude and geographical position. In the future we intend to analyze the influence of ontogenetic stage on the production of essential oils. The obtained data serve to identify the spontaneous plant populations that have the highest level of aromatic quality and to promote future research with technological transfer.

Conclusions

Analyzing the essential oils from *Thymus comosus*, taxa collected from three different areas of our country, areas with different altitudes were identified a total of 51 chemical compounds. Our results revealed a series of qualitative and quantitative differences of the compounds identified. Farnesol is one of the compounds which have been identified in high percentages in all samples analyzed. It have been also identified a number of differences compared with previous studies, the differences could be due to environmental conditions.

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