THE COMPOSITION OF ESSENTIAL OILS FROM *THYMUS MACEDONICUS* (DEGEN ET URUMOV) RONN. *SUBSP. MACEDONICUS* AND *THYMUS TOSEVII* VELEN. *SUBSP. TOSEVII* GROWING IN MACEDONIA

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SUMMARY. Essential oil composition of two taxa of genera *Thymus L.*, was examined by GC-FID and GC-MS methods. The main components of *Thymus macedonicus* essential oil were geraniol, α-terpineol, linalool, terpinyl acetate and sabinene hydrate. The taxa belongs to the group of nonphenolic taxa of the genera *Thymus*. One sample of *Thymus tosevii* essential oil contained almost the same components in similar percentage ratio. Another sample of this taxa had quite different composition with thymol, carvacrol, terpinyl acetate, neryl acetate, α-terpineol and linalool as main components. Differences in the composition of essential oil between two samples of *T. tosevii* point at possibility that these are different chemotypes of the same taxa.

Key Words: *Thymus, T. macedonicus, T. tosevii,* Essential oil, GC and GC-MS methods

Hills and mountains in Eastern Macedonia are covered by beautifully spread grass-lands containing very different taxa of genera *Thymus L. (Lamiaceae)*. Some of the taxa are strongly connected to silicate kind of ground, which is very often in the east of Macedonia. One of them is *Thymus macedonicus* (Degen et Urumov) Ronn., completely defined species with two lower taxa: *subsp. macedonicus* and *subsp. kortiathicus*. The main morphological characteristics of this species are ramified flowering branches and a lot of fibers thickly covering the whole plant. The taxa creates pure and stable populations on hills, besides the roads, besides the oakwoods, etc. Also, mixed populations with *Thymus tosevii* Velen. *subsp. tosevii* are frequently presented. *T. tosevii subs. tosevii*, wild growing taxa, equally presented on silicate or calcerous ground, is the most presented taxa of the genera *Thymus* in Macedonian flora. The plant is characterized by ramified flowering branches without fibers and flowers jointed in a „small-heads" [1]. Both plants have been used in folk medicine for many years against cold, flu, pulmonary infection, abdominal throes, etc. [2].

Essential oils from this two taxa of genera *Thymus* have not been studied up to now. Those oils have been examined by GC-FID and GC-MS methods.

Material and Methods

Samples of *T. macedonicus* were collected near Berovo, before blooming (sample A) and after 10 days in a flowering period, in June 1994 (sample B). One sample of *T. tosevii* was collected at the same place and at the same time (sample C) while the other one was collected near Dojran, at the end of May 1994 (sample D). The voucher specimen of both taxa were deposited in the Herbarium of Institute of Biology, Faculty of Science, Skopje, Macedonia. The identity of the taxa was confirmed by Dr. V. Matevski from the same Institute.

Distillation of oil

Content of essential oil was determined in leaves and herbs of the examined taxa. The essential oils were hydrodistilled for 5 hours in a Clevenger type apparatus.

GC and GC-MS analysis

Analyses of the oils were performed by GC-FID and GC-MS of fused capillary column (l=50 m, ID=0.2 mm), coated with crosslinked methyl silicone gum (0.5 μm film thickness). Hewlett-Packard model 5890 Series II gas chromatograph equipped with split-splitless injector was used. Sample solution in ethanol (1.0 %) was injected in split mode (1:100) at 250 °C. Detector temperature was 300 °C (FID), while column temperature was linearly programmed from 40–280 °C, 2 °C/min. The GC-MS analysis was carried out on an HP 5890 Series II gas chromatograph equipped with an HP 5971 mass detector working in electron impact mode (70 eV). The chromatographic conditions were as above. Transfer line was heated at 280 °C.

Identification of the components was based on comparison of their retention indices with those of authentic samples and matching mass spectral data with those from Wiley/NBS library of MS spectra.

Results and Discussion

Two different taxa of genera *Thymus* examined in this work, contained almost equal amounts of pale yellow colored essential oil with pleasant odor. The results are presented in Table 1.

The composition of essential oils, analyzed by GC-FID and GC-MS, was almost identical for A, B and C, samples that were collected near Berovo. Alcohols were the most important components of these oils. They represented 58.62; 55.91 and 55.20 % of oil with 24.72; 18.50 and 21.79 % of geraniol for samples A, B and C, respectively (Table 2). Another important components were hydrocarbons with β-pinen and sabinene as main, presented in higher quantities (6.58–8.64 % and 2.28–3.45 %, respectively). High percentage of terpinyl acetate was also recorded in these oils (10.33–13.59 %). On the other hand, only traces of typical phenols (thymol and carvacrol) were registered.

Quite different essential oil was obtained from *T. tosevii* collected near Dojran, at the southeast of Mace-

Table 1

Content of essential oil in different organs of *Thymus macedonicus* and *Thymus tosevii*, in % (v/w)

Plant organ	*T. macedonicus* before blooming Berovo	*T. macedonicus* flowering stage Berovo	*T. tosevii* flowering stage Berovo	*T. tosevii* flowering stage Dojran
Herbs	0.80	1.16	1.25	1.10
Leaves	1.10	1.40	1.60	1.40

donia. This sample (D) contained 19.44 % of thymol, 9.72 % of carvacrol, 19.74 % of terpinyl acetate, 9.02 % of neryl acetate, 7.88 % of linalool and 6.97 % of α-terpineol as main components. Comparing with samples A, B and C, for which geraniol was the most abundant component, sample D contained only traces of geraniol (0.52 %).

Differences in the composition of essential oils from the same taxa (*T. tosevii)* obtained from the plant material collected from different places (Berovo and Dojran) were probably due to the differences in the extrinsic factors that has an influente on plant's metabolism. On the other side, very similar composition of essential oils obtained from two different taxa (*T. tosevii* and *T. macedonicus)* but from the plant material with same origin (Berovo) confirmed our first conclusion. However, differences in oil composition between two samples of *T. tosevii,* point at possibility of different chemotypes of the taxa that should be confirmed by further examination.

In accordance with Matevski [1], *T. tosevii* is wide spread though the whole Balkan peninsula. Diklić has been described this taxa in Flora of SR Serbia [3]. *T. macedonicus* is spread in a smaller, limited area, in the east of Macedonia, till the border with Bulgaria [1]. In Flora of SR Serbia this taxa is not mentioned. Both these taxa Stojanov *et al.* [4] have been included in taxa *T. sibthropii* as a synonym, in Flora of Bulgaria.

In this work, for the first time, these taxa were examined on the essential oil composition. According to the obtained results it could be concluded that *Thymus macedonicus* belongs to the group of nonphenolic taxa of the genera *Thymus,* what is similar to few other taxa like *T. camphoratus, T. capitellatus* and *T. vilossus* [5], *T. leptophyllus* [6], *T. hyemalis* [7] and *T. beaticus* [8], that are also nonphenolic. But, even there are no phenols in the essential oil of *T. macedonicus,* it is still interesting because of the content of geraniol. Another examined taxa, *T. tosevii* has different composition of essential oil depending on the differences in plant origin. Determination of chemotypes of this taxa should point at possibilities of certain official use of the taxa.

Table 2

The composition of essential oils from *Thymus macedonicus* and *Thymus tosevii* (given in %)

Component	CI[a]	A[b]	B[b]	C[b]	D[b]
Hydrocarbons					
α-Thujene	938	0.05	0.09	0.06	0.27
α-Pinene	942	0.18	0.30	0.22	0.19
Camphene	954	0.05	0.08	0.06	0.19
Sabinene	976	2.28	3.45	2.74	0.66
β-Pinene	981	6.58	8.64	6.74	4.68
α-Phellandrene	1002	0.02	0.02	0.02	0.04
o-Cymene	—	0.53	0.85	0.44	0.38
p-Cymene	1020	0.26	0.31	0.66	4.65
Limonene	1030	0.71	0.90	0.78	0.54
γ-Terpinene	1057	1.02	1.51	0.92	1.17
Ethers					
1.8-Cineol	1021	0.13	0.17	0.14	0.66
Alcohols					
Linalool	1092	9.81	11.70	8.62	7.88
exo-Borneol	1146	0.11	0.11	0.11	0.87
endo-Borneol	—	2.05	2.71	2.53	1.51
α-Terpineol	1185	12.06	8.00	8.90	6.97
cis-Dihydrocarveol	—	0.31	0.27	0.39	—
Nerol	1218	0.43	0.32	0.20	—
Geraniol	1243	24.72	18.50	21.79	0.52
Sabinenen hydrate	—	9.13	14.30	12.66	2.10
Phenols					
Thymol	1287	0.60	0.33	0.58	19.44
Carvacrol	1297	0.34	0.24	0.05	9.72
Acetates					
Terpinyl acetate	1333	10.33	11.34	13.59	19.74
Neryl acetate	1343	—	—	—	9.02
Geranyl acetate	1398	6.20	3.85	5.34	0.44
Sesquiterpens					
α-Copaene	—	0.11	0.11	0.07	0.04
β-Burbonene	—	0.13	—	0.13	0.09
trans-Caryophyllene	1406	1.02	0.88	1.01	1.95
α-Cubebene	1428	0.03	0.02	0.02	0.05
α-Humulene	—	0.06	0.05	0.02	0.09
γ-Muurolene	1465	0.07	0.29	0.31	0.11
β-Cubebene	—	1.27	1.20	1.12	0.47
Calarene	1475	0.04	0.03	0.04	0.02
β-Bisabolene	—	0.14	0.85	1.02	0.33
γ-Cadinene	1501	0.92	0.68	0.71	0.17
Caryophyllene oxide	1524	0.72	0.05	0.99	0.41
Total %:	—	92.49	92.15	92.35	95.37

[a] Kovats's retention index; [b] Samples of oils (see text)

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