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The essential oil of *Thymus tosevii* subsp. *tosevii* var. *degenii* from Macedonia

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Composition of essential oil of *Thymus tosevii* subsp. *tosevii* var. *degenii* from Macedonia was examined by analytical GC and GC-MS techniques. Five different populations of the taxa were investigated, revealing significant variations in the content of certain components. Thymol, carvacrol, geraniol, terpenyl acetate, geranyl acetate, *p*-cymene and γ-terpinene varied mostly. For the majority of samples, phenols were the most prevalent chemical group of all the components identified. Most of the samples contained essential oils with higher amounts of thymol and/or carvacrol, followed by low amounts of geraniol and linalool. Samples with higher content of thymol contained higher quantity of terpenyl acetate. Higher content of geraniol was associated by higher content of geranyl acetate.

Keywords: Thymus, Thymus tosevii subsp. *tosevii* var. *degenii*, essential oil, composition, GC and GC-MS

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For many years, certain species of *Thymus* L. genus, growing wild on hill and mountain grasslands in Macedonia, have been used as remedies for cough, flu, pulmonary infections, abdominal throes *etc.* in Macedonian folk medicine (1). Although there is no *T. serpyllum* L. growing in Macedonia, the mixture of dried herbs from several Macedonian *Thymus* L. species is known as *Serpylli herba* (wild thyme), commonly used as a substitute for *Thymi herba*. One of the species is *T. tosevii* Velen., (genus *Thymus* taxa) which in Macedonian flora occurs with three subspecies and seven varieties (2). *Thymus tosevii* subsp. *tosevii* var. *degenii* (H. Braun.) Ronn. is one of the varieties. Var. *degenii* is a small, strongly scented shrub, with very branchy stems and gland-dotted, ovale and blunt leaves. It flowers in spring with purple coloured calyx whereas its corolla varies in colour from rose to dark rosy-purple. This plant is widely spread throughout the whole territory of Macedonia. While the typical form of *T. tosevii*-complex, var. *tosevii*, occurs in very different localities with various climatic and ecological conditions, the occurrence of var. *degenii* in its natural areal is always connected with the presence of beech-woods.

* Correspondence

In vertical distribution, the area of var. *degenii* is located at higher altitudes if compared to that of var. *tosevii* (2, 3).

Up to now *Thymus tosevii* subsp. *tosevii* var. *degenii* has not been studied yet and the aim of our work is to determine its essential oil composition, by using GC and GC-MS techniques.

EXPERIMENTAL

Plant material

Samples of *Thymus tosevii* subsp. *tosevii* var. *degenii* were collected at five different localities in Macedonia: A (Karadžica mountain, Central Macedonia); B (Mavrovo village, Western Macedonia); C (Lazaropole village, Western Macedonia); D (Pelister, Baba mountain, Southern Macedonia) and E (Osogovo mountain, Eastern Macedonia) during the summer of 1994 and 1995. The identification of the taxa was confirmed by V. Matevski (Department of Botany, Faculty of Sciences, Skopje). Voucher specimens were deposited at the Herbarium of the Department of Botany, Faculty of Sciences, Skopje. [SKO – 176–183]

Dried herbs of plants were water-distilled in a Clevenger apparatus for 5 h. The water was separated from the supernatant oil and the oil was dried over anhydrous Na_2SO_4.

GC and GC-MS analysis

The analyses of oils were performed by GC-FID on a fused silica capillary column PONA (50 m × 0.2 mm), coated with crosslinked methyl silicone gum (0.5 μm film thickness). A Hewlett-Packard, model (grad) 5890 Series II, gas chromatograph equipped with split-splitless injector was used. Instrumental parameters: detector temperature was 300 °C (FID), whereas column temperature was linearly programmed from 40–280 °C, 2 °C min^{-1}. Sample solution in ethanol (1.0%) was injected in split mode (1:100) at 250 °C. The relative amounts of individual components were based on the peak areas obtained, without FID response factor correction (4).

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. The identification of the components was based on comparison of their retention times with those of analytical standards of available terpenoids, and matching mass spectral data of oil constituents with those from the Wiley/NBS library of MS spectra.

RESULTS AND DISCUSSION

The hydrodistillation of samples of *Thymus tosevii* subsp. *tosevii* var. *degenii* yielded 1.00–1.90% (*V/m*) of essential oil, with following characteristics: refractive index (n_D^{20}) 1.4655–1.4978 and relative density (d_{20}^{20}) 0.8900–1.8942.

Gas chromatographic analyses of essential oils of var. *degenii* (Table I) revealed 31–40 identified components representing more than 90% of the oils (except for the sample E, in which 70.16% of oil was identified). These components were ten monoterpene hydrocarbons (α-thujene, α-pinene, camphene, sabinene, β-pinene, α-phellandrene, *o*-cymene, *p*-cymene, limonene, γ-terpinene), two ethers (1,8-cineol, methylthymol ether), one ketone (camphor), two aldehydes (Z-citral, *E*-citral), two phenols (thymol, carvacrol), three esters (geranyl, neryl and terpenyl acetate) and eleven sesquiterpenes (β-bourbonene, *trans*-caryophyllene, α-cubebene, α-humulene, γ-muurolene, β-cubebene, β-bisabolene, γ--cadinene, δ-cadinene, β-nerolidol and caryophyllene oxide). Almost the same components were identified in each of oil samples, but depending on their origin the quantities of the same components significantly varied and thus made these oils different. For the majority of samples, the analysis revealed that the phenols were prevalent chemical group of all the components identified.

From the results presented in Table I, it could be seen that thymol (23.19–20.61%) and carvacrol (15.63–19.41%) were the main components in the oil from sample A, followed by higher amounts of *p*-cymene (7.02–7.71%) and γ-terpinene (8.81–10.21%). Higher amounts of terpenyl acetate (9.50–13.51%) and geraniol (6.80–7.25%) were also determined. Other components were present in much smaller quantities. Sample B contained almost the same composition of essential oil: thymol (14.27–21.29%), carvacrol (6.37–8.34%), *p*-cymene (4.04–9.41%), γ-terpinene (3.95–4.97%) and terpenyl acetate (18.04–25.15%) as its most abundant components. Thymol was the most abundant component in sample C (42.48%) as well, followed by higher quantities of γ-terpinene (10.32%), *p*-cymene (7.90%), terpenyl acetate (8.31%) and lower amounts of carvacrol (6.60%). If compared to the samples A, B and C, the composition of sample D differed due to high content of carvacrol (48.91%). Rather high amounts of *p*-cymene (11.78%) and γ-terpinene (5.16%) were determined too. This sample was characterized by higher amounts of borneol (total borneol was 6.60%), low amounts of other alcohols and traces of esters (Table I). On the other hand, the most abundant components in the essential oil of sample E were geraniol (34.45%) and geranyl acetate (23.12%). These two components represented 57.6% of the oil. We identified only 70.2% of the oil and these two components represented 82.1% of the known part of the oil. In this sample only small amounts of thymol (1.29%) and carvacrol (1.59%) were registered, followed by very low quantities or traces of other components.

A considerable variation of only a few components contributes to certain variation in the essential oil composition of *T. tosevii* subsp. *tosevii* var. *degenii*. Thymol, carvacrol, geraniol, linalool, terpenyl acetate, geranyl acetate, *p*-cymene and γ-terpinene were the most »variable components«. The content variation of other oil constituents, generally, caused no significant variation in the oil composition. The presence of these »variable components« is shown in Fig. 1. For the samples A, B, C and D, the higher content of phenols was characteristic, but it was always associated with very low content of alcohols. On the other hand, in the samples A, B and C, thymol was the most abundant phenol, whereas in the sample D it was carvacrol. In the samples containing much more thymol, the higher amounts of terpenyl acetate were also characteristic. In the sample containing carvacrol, only traces of esters were registered. Four samples: A, B, C and D, contained higher quantities of *p*-cymene and γ-terpinene. It should be notified that these components are biogenetic precursors of thymol and carvacrol. An interesting sample of

Table I. Essential oil composition of Thymus tosevii *subsp.* tosevii *var.* degenii *(in %)*

Components	A		B		C	D	E
	1994	1995	1994	1995	1995	1995	1995
α-Thujene[a, b, c]	0.96	0.84	0.54	0.58	1.20	1.55	0.08
α-Pinene[a, b, c]	0.62	0.64	0.41	0.37	0.57	1.59	0.08
Camphene[c]	0.70	0.92	0.48	0.28	0.24	2.13	0.08
1-Otenol-3[c]	0.27	0.52	0.36	0.83	0.22	0.06	0.02
Sabinene [c]	0.69	0.94	0.91	0.73	1.09	1.40	0.12
β-Pinene[a, b, c]	1.40	1.42	5.37	6.63	1.93	1.45	0.50
α-Phellandrene[a, b, c]	0.15	0.12	0.08	0.09	0.21	0.17	–
o-Cymene [c]	1.32	1.18	0.65	0.83	1.79	1.15	0.08
p-Cymene [c]	7.71	7.02	9.41	4.04	7.90	11.78	1.20
1,8-Cineol[a, b, c]	0.39	0.33	0.31	0.26	0.30	0.07	0.04
Limonene[a, b, c]	0.54	0.71	1.00	1.08	0.73	2.15	0.10
cis-Ocimene [c]	0.06	0.08	0.07	0.11	0.12	0.04	0.07
γ-Terpinene [c]	7.81	10.21	3.95	4.97	10.32	5.16	0.56
Sabinene hydrate[a, b, c]	0.73	0.59	–	1.04	1.04	1.88	0.13
Linalool[a, b, c]	0.45	1.35	5.36	5.29	4.10	0.77	0.02
Camphor[a, b, c]	–	0.53	–	0.11	0.08	0.66	–
exo-Borneol[a, b, c]	1.40	1.96	1.03	0.49	0.52	5.55	0.19
endo-Borneol[a, b, c]	0.35	0.44	0.35	0.36	0.62	1.05	0.02
α-Terpineol[a, b, c]	1.19	2.64	2.15	3.14	0.81	0.24	0.30
cis-Dihydrocarveol [c]	–	–	3.61	3.94	–	–	0.15
Methyl thymol ether [c]	0.16	0.21	0.73	–	–	–	0.60
Nerol[a, b, c]	0.24	–	–	0.09	–	–	–
Z-Citral[a, b, c]	1.88	0.23	1.02	0.24	0.41	0.32	0.45
Geraniol[a, b, c]	6.80	7.25	1.44	2.47	0.42	1.87	34.45
E-Citral[a, b, c]	0.21	0.63	0.19	–	0.37	0.08	1.59
Thymol[a, b, c]	23.19	20.61	21.29	14.27	42.48	1.25	1.29
Carvacrol[a, b, c]	19.41	15.63	6.37	8.34	6.60	48.69	1.59
Unidentified	9.50	0.04	5.90	6.90	0.05	0.02	0.35
Terpenyl acetate[c]	–	13.51	18.04	25.15	8.31	0.07	1.38
Neryl acetate[a, b, c]	0.04	0.06	0.06	0.09	0.02	0.03	0.09
Geranyl acetate[a, b, c]	3.62	3.42	0.47	0.24	0.23	0.72	23.12
β-Buorbonene [c]	0.09	0.07	0.05	0.04	0.03	0.05	0.06
trans-Caryophyllene[a, b, c]	1.89	0.97	1.93	1.98	2.19	1.73	2.46
α-Cubebene [c]	0.05	–	0.01	–	–	0.03	–
α-Humulene [c]	0.14	0.07	0.20	0.23	0.23	0.10	0.13
γ-Muurolene [c]	0.17	0.05	0.04	0.04	0.13	0.08	–
β-Cubebene [c]	0.39	0.26	0.16	0.20	–	0.11	0.41
β-Bisabolene [c]	0.03	1.89	1.60	1.47	2.26	0.97	0.75
γ -Cadinene [c]	2.82	0.12	0.11	0.10	0.19	0.14	–
δ-Cadinene [c]	0.01	–	0.15	–	–	–	–
β-Nerolidol[a, b, c]	0.05	–	–	–	0.07	–	0.06
Caryophylleneoxide [c]	0.19	0.17	0.37	0.13	0.19	0.38	0.16
Total %	97.62	97.63	96.17	97.15	97.97	95.49	72.68

[a] Compared with retention time of authentic samples;
[b] Compared with MS spectra of authentic samples;
[c] Compared to MS spectra from Wiley/NBS library.

var. *degenii* was the sample E, characterized by traces of phenols followed by traces of *p*-cymene and γ-terpinene. The most abundant constituents of this oil were geraniol and geranyl acetate, as we have already mentioned (Fig. 1).

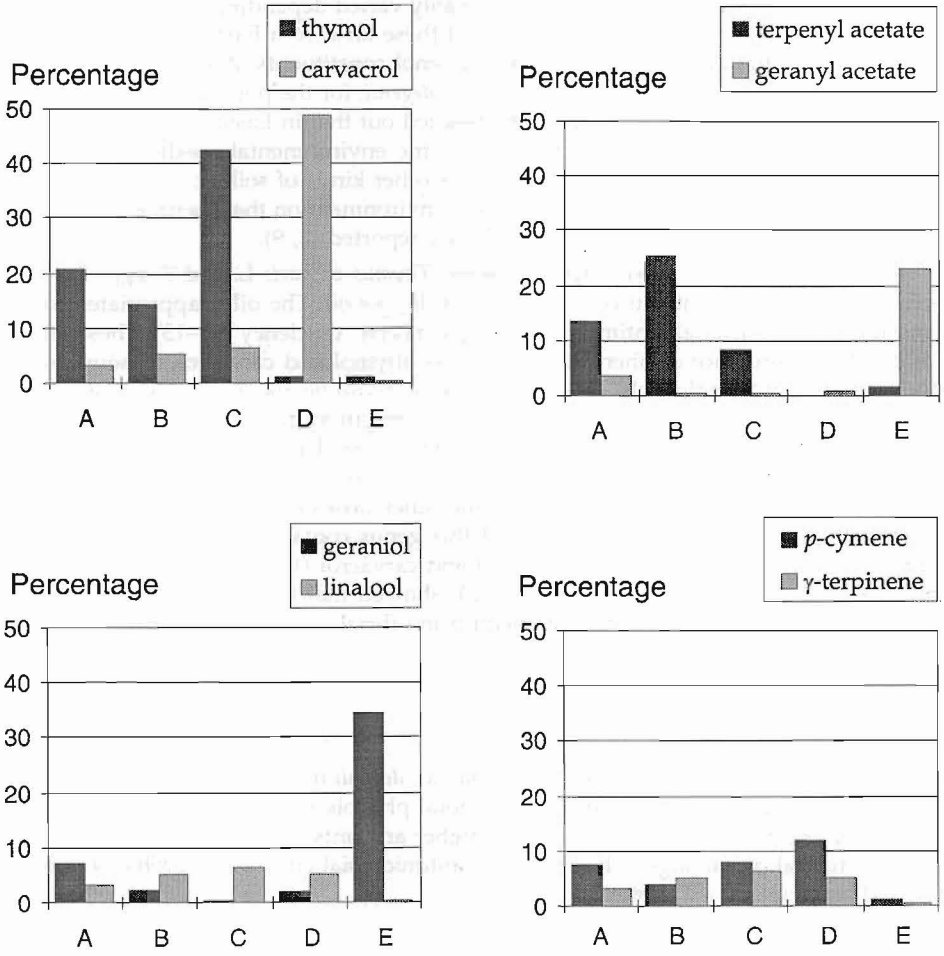

Fig. 1. Comparative histogram of »variable components« in the oils of *T. tosevii* subsp. *tosevii* var. *degenii*; samples: A-Kitka (1995); B-Vrben 1995); C-Lazaropole (1995); D-Pelister (1995); E-Ponikva (1995).

Comparing our results to some data on the essential oil composition of *T. tosevii* from Macedonia, great resemblance has been found. Thymol, carvacrol and terpenyl acetate were found to be the main components of the essential oil of *T. tosevii* subsp. *tosevii* (5). Carvacrol has been previously determined as the most abundant component in the essential oil of *T. tosevii* subsp. *substriatus* (5), and now the same has been found for cer-

tain populations of var. *degenii*. Higher content of geraniol associated with higher content of thymol or carvacrol, followed by higher content of terpenyl acetate was characteristic for *T. longidens* var. *lanicaulis* Ronn. (6). Recently, we have reported the data on the essential oil composition of *T. tosevii* subsp. *tosevii* var. *longifrons* from Macedonia (7). The oil composition of var. *longifrons* considerably varied depending upon the plant origin. It has been found that the populations of these taxa from Eastern Macedonia contained the essential oils with only traces of phenol constituents. A similar composition of the essential oil now has been found for var. *degenii*, for the populations of these taxa grown in Eastern Macedonia. It should be pointed out that in Eastern Macedonia a silicate kind of soil is characteristic thus making the environmental conditions for plant growth different from those with calcareous or other kinds of soils covering the rest of the Macedonian territory. The influence of the environment on the essential oils composition of some *Thymus* taxa has been previously reported (8, 9).

It is well known that two *Thymus* species, *Thymus vulgaris* L. and *T. zygis* L., have been established as plant source for the official thyme oil. The oil is appreciated for its expectorant, antibacterial, antimycotic and spasmolytic efficiency (10–13). These effects are due to the presence of phenolic constituents, thymol and carvacrol, although some studies point out that alcohols, especially geraniol, could be also responsible for antimicrobial activity (14). These last few years an investigation has been carried out which showed that the content of these phenols varied widely depending on their origin (15, 16), vegetation period (17), way of collection, preparation of plant material and some other factors (18). The examinations of some other taxa of genus *Thymus*, very often point to the occurrence of other species of this genus containing large amounts of essential oil with high percentages of thymol and carvacrol (19–20). Some studies on essential oil composition of *T. serpyllum* (21–23) showed that these taxa contain essential oil similar to that from *T. vulgaris*. The plant is in official use as *Thymi serpylli herba* (24).

CONCLUSION

The essential oil of *T. tosevii* subsp. *tosevii* var. *degenii* from Macedonia could be considered as a thyme oil rich in phenol, with total phenols ranging from 22.6% to 49.9% (except for one population which contains higher amounts of alcohols). Because of that, the essential oil of var. *degenii* has potential antimicrobial effects and probably it could be used as a kind of antiseptic.

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SAŽETAK

Eterična ulja *Thymus tosevii* subsp. *tosevii* ver. *degenii* iz Makedonije

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Uporabom GC i GC-MS određivan je sadržaj eteričnih ulja u *Thymus tosevii* subsp. *tosevii degenii* iz Makedonije. Istraživnja u pet različitih populacija pokazala su značajne varijacije u sadržaju pojedinih komponenata, kao što su timol, karvakrol, geraniol, terpenil-acetat, *p*-cimen i γ-terpinen. Najznačajnija kemijska skupina koja je identificirana su fenoli. Većina uzoraka sadrži veće količine timola i/ili karvakrola, te niske koncentracije geraniola i linalola. Uzorci s većim sadržajem timola imali su i više terpenil-acetata, dok je količina geraniola u korelaciji sa sadržajem geranil-acetata.

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