Bulletin of the Chemists and Technologists of Macedonia, Vol. 15, No. 1, pp. 33 – 38 (1996)
GHTMDD – 269
Received: February 12, 1996
ISSN 350 – 0136
UDC 547.913 : 543.544.3
Original scientific paper

ESSENTIAL OIL ANALYSIS OF SOME TAXA OF GENERA *THYMUS* L. – ENVIRONMENT INFLUENCES –

Svetlana Kulevanova[1], Mihailo Ristić[2], Trajče Stafilov[3], Kiril Dorevski[4], Tomislav Ristov[1]

[1] *Institute of Pharmacognosy, Faculty of Pharmacy, Vodnjanska 17, 91000 Skopje, Republic of Macedonia*
[2] *Institute for Medicinal Plant Research "Dr. J. Pančić", Tadeuš Košćuški 1, 11000 Belgrade, FR Yugoslavia*
[3] *Institute of Chemistry, Faculty of Science, POB 162, 91001 Skopje, Republic of Macedonia*
[4] *Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, Vodnjanska 17, 91000 Skopje, Republic of Macedonia*

The essential oil composition of a few taxa of genera *Thymus* L. wild growing in Macedonia, were investigated using GC and GC-MS methods. Almost the same components were identified in all examined samples but in very different percentages. In the most of the samples thymol, carvacrol, linalool, α-terpineol, terpenyl acetate, *p*-cymene and γ-terpinene were the most abundant components. In the rest of the samples geraniol, linalool and sabynene hydrate were presented in higher amounts. Differences in essential oil composition, obtained from the same taxa, probably due to the differences in environmental conditions of plant growth. Against this, remarkable resemblance was found in composition of essential oils obtained from different *Thymus* taxa from the plants that grow at the same environment.

Key words: *Thymus*; essential oil; GC and GC-MS analysis; environment

INTRODUCTION

The oil of thyme is obtained from *Thymus vulgaris* L., *Thymus zygis* L. or occasionally other related species. It is of great value in pharmacy because of its antibacterial, antimycotic and expectorant activities. These effects are due to the presence of characteristic phenol components, thymol and carvacrol [1]. The content of phenols in commercial thyme oil ranging between 20 to 60% [2–4] depending upon the origin [5, 6], growth stage [7], environmental influences [8] and other factors. Some data points to occurrence of several chemotypes of *T. vulgaris* [9] and *T. zygis* [10] whose presence could be explained by influences from the environment [9, 10]. Besides *T. vulgaris* and *T. zygis*, few other *Thymus* taxa have a potential to be used as a kind of substitution for official thyme oil [11–13]. These taxa contain large amounts of essential oil with high quantity of phenols. However, great chemical polymorphism characterised the taxa of genera *Thymus* L.. In recent years some of these taxa have been submitted to examination of chemotypes and frequently occurrence of different chemotypes have been explained by the environment influences [14–18].

A lot of species of genera *Thymus* occur in flora of Macedonia. Some of them spread widely through the whole territory of Macedonia, like *Thymus tosevii* Velen. while the others are connected to small, limited areas and represent local endemic plants [19].

Examination that has been carried out in last few years include a majority of these taxa. The aim of the presented study is to compare the essential oil composition of some Macedonian *Thymus* taxa to establish similarities and differences in their composition in relation with actual environmental conditions.

EXPERIMENTAL

Plant material

Samples of plants included in the investigation were the following:

A-1: *Thymus tosevii* Velen. ssp. *tosevii*, collected near village Nikolić, Dojran, in the southeast of Macedonia, May 1994.

A-2: *Thymus tosevii* Velen. ssp. *substriatus* (Borbas) Matevski (syn. *T. substriatus* Borb.), collected in the same place and at the same time.

B-1: *Thymus tosevii* Velen. ssp. *tosevii*, collected at Vitačevo, Kavadarci, central part of Macedonia, in June 1994.

B-2: *Thymus tosevii* Velen. ssp. *substriatus* (Borbas) Matevski, collected at the same place and at the same time.

C-1: *Thymus tosevii* Velen. ssp. *tosevii*, collected at grass-lands near the Alšar mine, in the south of Macedonia, in June 1994.

C-2: *Thymus alsarensis* Ronn. (syn. *T. thracicus* Jalas), collected at the same place and at the same time.

D-1: *Thymus tosevii* Velen. ssp. *tosevii,* collected at grass-lands near the village Laki, Berovo, in the east of Macedonia, in June 1994.

D-2: *Thymus macedonicus* (Degen et Urumov) Ronn. (*T. macedonicus* Ronn., *T. sibthropii* Jalas), collected at the same place and at the same time.

The identity of the taxa was confirmed by V. Matevski, from the Institute of Biology, Faculty of Science, Skopje, Macedonia. Voucher specimen for each of the taxa was deposited at the Herbarium in the same Institute of Biology.

All samples collected at flowering stage of plants were air-dried and then submitted to hydrodistillation in Clevenger type apparatus for a duration of 5 hours. After separation from water, essential oil was dried over anhydrous sodium sulphate.

GC and GC-MS analysis

Analyses of the oils were performed by GC-FID and GC-MS on fused capillary column (l = 50 m, ID = 0.2 mm), coated with crosslinked methyl silicone gum (0.5 μm film thickness). A 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 electronic compact: (70 eV). The chromatographic conditions were as above. Transfer line was heated at 280 °C.

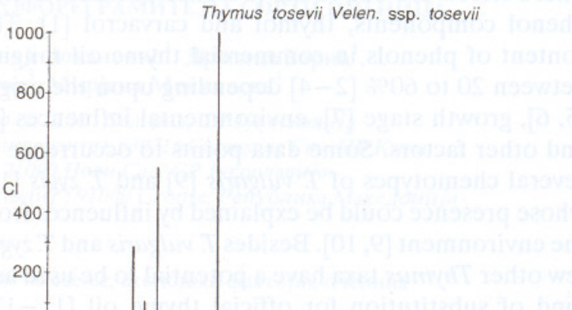
The identification of the components was based on comparison of their retention times with those of analytical standards of available terpenoids, and matching mass spectral data of oil constituents with those from Wiley/NBS library of MS spectra. For the quantification purposes area percent reports obtained by GC-FID were used as a base. Chromatograms of all samples were interpreted in the coordinate system, where concentration indices (CI) were plotted in relation with relative retention times to thymol (RRT).

RESULTS AND DISCUSSION

The hydrodistilation yielded 0.93–1.25% (v/w) of essential oil from *Thymus tosevii* ssp. *tosevii*. The oil was characterised by refractive index n_{20}^{D} ranging between 1.4694–1.4891 and relative density n_{20}^{20} 0.889–0.899. Samples of *T. tosevii* ssp. *substriatus* yielded 1.20–1.40% (v/w) pale, yellow coloured essential oil with n_{20}^{D} 1.491 and n_{20}^{20} 0.89. Two another taxa *T. alsarensis* and *T. macedonicus*, yielded 1.60% and 1.16% (v/w) of essential oil, respectively. The refractive indices were 1.495 and 1.471, and relative density was 0.893 and 0.887 for *T. alsarensis* and *T. macedonicus*, respectively.

Cross-correlation of data recorded by GC-FID and GC-MS showed great resemblance regarding qualitative chemical composition of essential oil samples. Almost the same constituents were identified in all these samples, but in very different percentages.

Samples A-1 (*T. tosevii* ssp. *tosevii*) and A-2 (*T. tosevii* ssp. *substriatus*) contained large amounts of phenols. Thymol was presented in concentration of 19.43% in A-1 and 24.51% in A-2, while 9.72% and 16.35% of carvacrol were determined in samples A-1 and A-2, respectively (Table I). The main constituents in these two samples were the esters with 19.79% of terpenyl acetate in A-1 and 13.03% in A-2. Linalool was the most abundant alcohol with 7.88% in A-1 and 7.42% in A-2. The major important monoterpene hydrocarbons were *p*-cimene and β-pinene. In the fraction of sesquiterpenes the most abundant was *trans*-caryophillene. Normalised gas chromatograms of samples A-1 and A-2 drawn in the form of bar graph pattern are presented in Fig. 1, where concentration indices (CI) were plotted in relation with relative times to thymol (RRT).



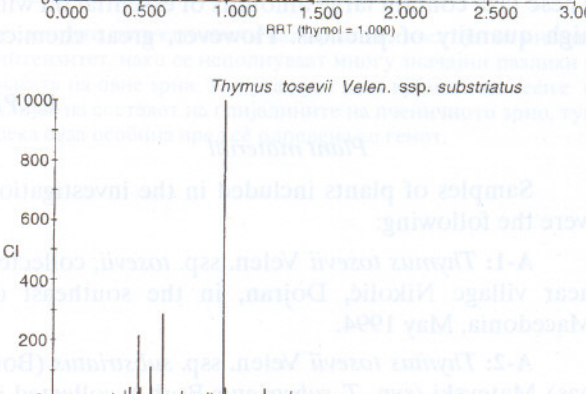


Fig. 1. Bar graph GC-patterns of samples A-1 (*Thymus tosevii* ssp. *tosevii*) and A-2 (*Thymus tosevii* ssp. *substriatus*)

Table I

Composition of essential oil of examined taxa of genera Thymus L.

Component	A-1*	A-2*	B-1*	B-2*	C-1*	C-2*	D-1*	D-2*
Hydrocarbons								
α-Thujene	0.27	0.76	0.78	0.97	1.17	1.06	0.06	0.08
α-Pinene	0.19	0.44	0.51	0.61	0.83	0.85	0.22	0.30
Camphene	0.19	0.27	0.52	0.44	0.51	0.78	0.06	0.08
Sabinene	0.67	1.12	1.53	1.44	0.93	0.74	0.75	3.45
β-Pinene	4.68	4.78	1.70	1.95	1.98	2.22	0.74	8.65
α-Phellandrene	–	0.13	0.14	0.17	0.16	0.16	0.02	0.02
o-Cimene	0.38	1.02	1.13	1.21	1.36	1.14	0.44	0.82
p-Cimene	4.65	5.41	10.89	9.76	7.08	8.92	0.66	0.31
Limonene	0.54	0.49	0.47	0.45	0.74	0.83	0.78	0.89
γ-Terpinene	1.17	4.81	4.02	5.11	4.33	3.56	0.92	1.51
Ethers								
1.8-Cineol	0.66	1.06	1.72	1.94	0.85	0.96	0.14	0.17
Methyl thymol	0.43	0.48	–	1.16	0.47	–	–	–
Alcohols								
Sabinene hydrate	2.10	1.80	0.92	2.11	0.74	1.26	12.66	14.89
Linalool	7.88	7.42	20.92	13.09	9.00	3.21	8.62	11.69
exo-Borneol	0.87	0.83	1.64	1.34	1.54	1.68	0.11	0.11
endo-Borneol	1.51	0.47	1.01	0.64	0.54	0.84	2.53	2.71
α-Terpineol	6.97	0.65	0.33	0.37	1.69	2.46	8.90	8.01
cis-Dihydrocarveol	–	5.13	–	–	1.09	0.81	0.40	0.31
Nerol	–	–	–	–	–	0.04	0.20	0.13
Geraniol	0.53	–	0.24	1.15	–	1.10	21.79	18.50
Phenols								
Thymol	19.43	24.51	38.08	45.57	19.06	18.52	0.58	0.33
Carvacrol	9.72	16.35	1.77	2.03	26.87	33.00	0.05	0.24
Acetates								
Terpinyl acetate	19.97	13.03	0.13	–	9.25	7.36	13.59	11.34
Neryl acetate	9.01	0.03	–	–	–	–	–	–
Geranyl acetate	0.44	0.16	0.04	0.34	–	0.06	5.34	3.85
Sesquiterpenes								
α-Copaene	0.04	0.04	0.03	0.04	0.03	0.05	0.13	0.11
β-Bourbonene	0.09	0.07	0.15	0.10	0.06	0.05	0.06	0.07
trans-caryophyllene	1.94	2.19	2.38	1.90	1.50	1.76	1.01	0.88
α-Cubebene	–	0.10	0.07	0.05	–	0.04	0.03	0.03
α-Humulene	–	0.02	0.09	0.08	–	0.06	0.02	0.05
γ-Muurolene	0.11	0.10	0.17	0.18	0.02	0.13	0.32	0.51
β-Cubebene	0.47	0.15	0.53	0.24	0.24	0.05	1.12	1.20
Calarene	0.46	0.08	0.06	0.08	0.05	0.05	0.08	–
β-Bisabolene	0.33	0.03	1.98	1.52	1.34	1.78	1.02	0.85
γ-Cadinene	0.17	0.41	1.24	0.26	0.15	–	0.71	0.65
δ-Cadinene	0.02	–	0.06	0.02	0.06	0.20	–	–
Caryophyllene oxide	0.41	0.16	0.35	0.31	0.25	0.06	0.99	0.05
Total %	96.30	94.50	95.60	96.63	93.89	94.79	91.03	92.81

* Samples of oils (see text)

Samples B-1 (*T. tosevii* ssp. *tosevii*) and B-2 (*T. tosevii* ssp. *substriatus*) contained large amount of phenols. Both samples were characterised by very high percentage of thymol (38.08% for B-1 and 45.57% for B-2) and very low percentage of carvacrol (1.77% for B-1 and 2.03% for B-2). These samples contained a large percentage of linalool (20.92% for B-1 and 13.09% for B-2). Only traces of esters were registered. The most abundant hydrocarbons were *p*-cimene and γ-terpinene (Table I). In fraction of sesquiterpenes the most important were *trans*-caryophyllene and β-bisabolene. Normalised chromatograms of oil samples B-1 and B-2 are presented in Fig. 2.

Phenols were the most abundant components in the essential oils obtained from the samples C-1 (*T. tosevii* ssp. *tosevii*) and C-2 (*T. alsarensis*). Among them, in these samples higher content of carvacrol was recorded. It was 26.87% for C-1 and 33.00% for C-2. The percentages of thymol were also very large, 19.06% for C-1 and 18.52% for C-2. Similar to A-1 and A-2, fraction of esters included 9.25% of terpenyl acetate for C-1 and 7.36% for C-2. The most abundant alcohol in these samples was linalool, the main hydrocarbons were *p*-cimene and γ-terpinene while in fraction of sesquiterpenes that were *trans*-caryophyllene and β-bisabolene (Table I). Normalised chromatograms of these samples are given in Fig. 3.

On the contrary to the samples mentioned before (containing large amount of phenols), in samples D-1 (*T. tosevii* ssp. *tosevii*) and D-2 (*T. macedonicus*) only traces of thymol and carvacrol were registered. Normalised chromatograms of these two oils are presented in Fig. 4.

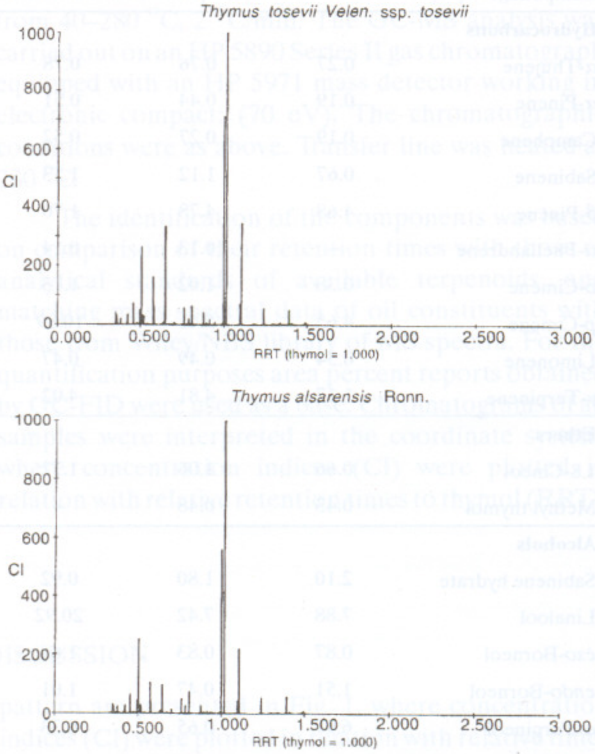


Fig. 3: Bar graph GC-patterns of samples C-1 (*Thymus tosevii* ssp. *tosevii*) and C-2 (*Thymus alsarensis*)

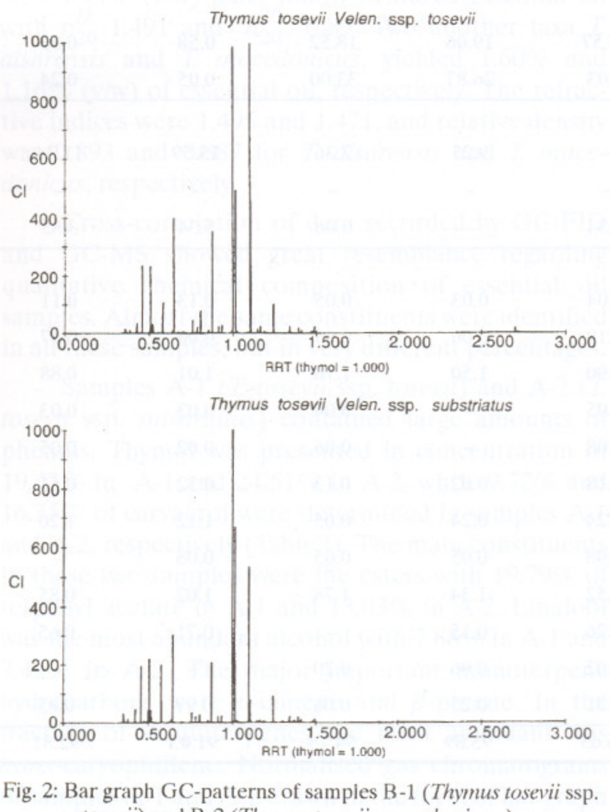


Fig. 2: Bar graph GC-patterns of samples B-1 (*Thymus tosevii* ssp. *tosevii*) and B-2 (*Thymus tosevii* ssp. *substriatus*)

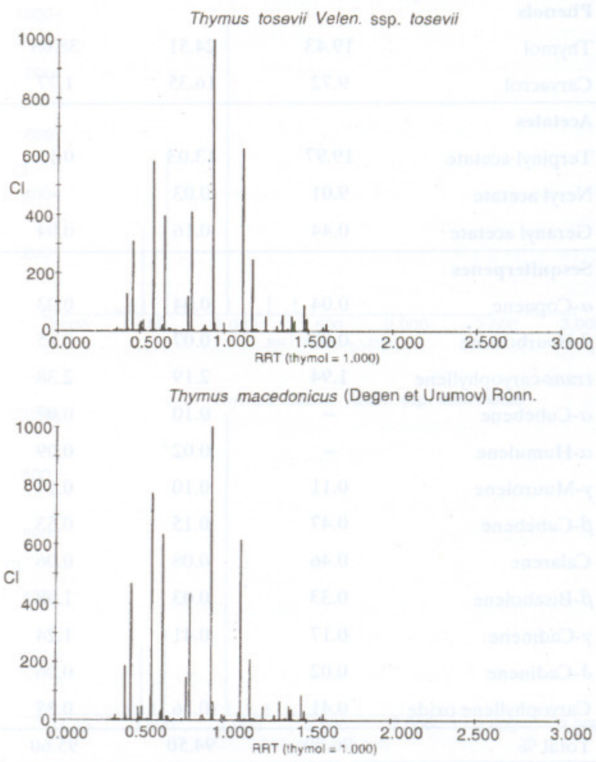


Fig. 4. Bar graph GC-patterns of samples D-1 (*Thymus tosevii* ssp. *tosevii*) and D-2 (*Thymus macedonicus*)

In these two samples, the most important was the fraction containing alcohols derived from monoterpene hydrocarbons (56.21% for D-1 and 55.76% for D-2). The most abundant alcohol was garaniol (21.79% for D-1 and 18.50% for D-2). High percentages of linalool (8.62% for D-1 and 11.69% for D-2) and α-terpineol (8.90% for D-1 and 8.01% for D-2) were determined too. Sabinene hydrate (thujanol-4) occurred in these two samples in much higher quantities (12.66% for D-1 and 14.30% for D-2) than in other samples where it was present only in very low concentration. High contents of esters (especially terpenyl acetate and geranyl acetate, which was more significantly increased in D-1 and D-2 than terpenyl acetate) were also established. In fraction of hydrocarbons, β-pinene was the most abundant constituent while in fraction of sesquiterpenes the most abundant were *trans*-caryophyllene, β-bisabolene and β-cubebene.

Summarising the obtained results, it could be noticed that different taxa of genera *Thymus* growing under the same environmental conditions contained essential oil with almost the same composition. On the other hand, changes in these conditions could cause the changes in essential oil composition. Samples A-1 and A-2 as well as C-1 and C-2 were collected from the area being under the strong influence of the Mediterran climate. Although these samples represented different *Thymus* taxa (*T. tosevii* ssp *tosevii, T. tosevii* ssp. *substriastus* and *T. alsarensis*) similar environmental conditions influenced similarity in their essential oil composition. This could be seen from the Fig. 1 and Fig. 3. The area where samples B-1 and B-2 were collected differs from those two areas because of the higher altitude and other conditions characteristic for the continental climate. Essential oil composition of B-1 and B-2 was different from those of A-1 and A-2, although A-1 and B-1 as well as A-2 and B-2 belonged to the same taxa. The most noticeable effect of the environment was reflected on essential oil composition in samples D-1 and D-2. These two samples were collected in an area with rough climatic conditions, with hot summers, strong, very cold and snowy winters. Growing in the same environment these two very different taxa, *T. tosevii* ssp. *tosevii* (D-1) and *T. macedonicus* (D-2) contained qualitatively the same essential oil, without phenols in their composition. It is very important to point out that chemical composition of essential oil of D-1 much differs from those in A-1, B-1 and C-1, although all these samples belonged to the same taxa (*T. tosevii* ssp. *tosevii*).

In our study *T. tosevii* ssp. *tosevii* was chosen as a kind of marker. This taxa is very common in Macedonian flora and its collection together with other taxa of genera *Thymus* was possible to achieve during almost every sampling. From the results of our examination, it appears that essential oil composition of *T. tosevii* ssp. *tosevii* could be very different, depending strongly upon the environmental conditions of plant growth. The main constituents of the oil could be thymol and carvacrol, or only thymol and only carvacrol together with terpenyl acetate, *p*-cymene and γ-terpinene. Samples of *T. tosevii* that contained geraniol and linalool, as the most abundant component were also registered which was similar to *T. tosevii* essential oil originated from Greece [20]. Data considering the variation of essential oil composition of some other taxa of genera *Thymus*, caused by the environmental influences could be found in the literature. Thus, Falchi Delitala et al. [21] found close relations between environmental stress condition and production of essential oil and phenols in *Thymus capitatus* and *Thymus herba-barona*, Cabo et al. [22] found big variations in essential oil composition of *Thymus zygis* between the plants that grew in different environment and Piccaglia and Marotti [23] found several different ecotypes of Italian *Thymus vulgaris.*

REFERENCES

[1] C. O. Van den Broucke, *Fitoterapia* **54**, 171 (1983).

[2] J. Passet, *Dragoco Rept.*, **10**, 166 (1980).

[3] D. Fehr, G. Stenzhorn, *Pharm. Ztg.* **124**, 2342 (1979).

[4] B. M. Lowrence, The existence of intraspecific differences in specific genera in the Labiatae family, Paper No. 35, *8th International Congress of Essential Oils*, Cannes, 1980.

[5] M. S. Karawaya, J. S. Hifnawy, *J. A. O. A. C.* **57**, 997 (1974).

[6] J. D. Miguel, H. M. Richard, F. G. Sandret, *J. Agric. Food Chem.*, **24**, 833 (1976).

[7] J. Lami, *Parfum. Cosmet. Aromes,* **51**, 73 (1983).

[8] J. Cabo, J. Jimenez, A. Revert, L. Bravo, *Ars. Pharm.*, **22**, 188 (1981).

[9] P. Rovesti, *Parfum, Cosmet. Savon, France* **1**, 138 (1971).

[10] D. Garcia-Martin, M. C. Garcia-Vallejo, Chemotypes of *Thymus zygis* (Lofl.) L. of Guadaramma Sierra and other places in Castile (Spain), *9th International Congress of Essential Oils*, Singapore, 1983.

[11] C. S. Mathela, J. Agarwal, J. Taskinen, *J. Indian Chem. Soc.*, **57**, 1249 (1980).

[12] M. Sattar Shafiq Malik, A. K. Shafiq, *Pak. J. Sci. Ind. Res.*, **34**, 119 (1991).

[13] A. Herriset, J. Jolivet, P. Rey, *Plantes Med. et Phytotherap.*, **7**, 114 (1973).

[14] A. Fleischer, Z. Fleischer, S. Abu-Rukun, *J. Sci. Food Agric.*, **35**, 495 (1984).

[15] B. Bellomaria, K. Hruska, G Valentini, *G. Bot. Ital.,* **115**, 17 (1981).

[16] I. Weisner, J. Novak, *Zborník Vysoké Školy Zemedělske v Praze,* **41**, 45 (1984).

[17] B. Bellomaria, G. Valentini, N. Arnold, H. J. Arnold, *Pharmazie,* **49**, 684 (1994).

[18] R. Piccalia, M. Marotti, *Flavour Fragrance J.*, **6**, 241 (1991).

[19] V. Matevski, Ph. D. Thesis, Faculty of Science, Skopje, 1987.

[20] S. Katsiotis, N. Iconomou, *Planta Med.*, **0** (4), 334 (1986)

[21] L. Falchi Deitala, V. Solinas, C. Gessa, *Fitoterapia,* **54**, 87 (1983).

[22] J. Cabo, J. Jimenez, A. Revert, L. Bavo, *Ars Pharm.*, 22, 188 (1981).

[23] R. Piccaglia, M. Marotti, *Flavour Fragrance J.*, **6**, 241 (1991).

Резиме

АНАЛИЗА НА ЕТЕРИЧНОТО МАСЛО ОД НЕКОИ ТАКСОНИ НА РОДОТ *THYMUS* L. – ВЛИЈАНИЈА НА НАДВОРЕШНАТА СРЕДИНА –

Светлана Кулеванова[1], Михаило Ристиќ[2], Трајче Стафилов[3], Кирил Доревски[4], Томислав Ристов[1]

[1]*Институт за фармакогнозија, Фармацевтски факултет Скопје, Водњанска 17, 91000 Скопје, Република Македонија*
[2]*Институт за проучување на лековити билки "Др. Јосиф Панчиќ", Белград, 11000 Белград, С.Р. Југославија*
[3]*Институт за хемија, Природно-математички факултет, Скопје, п. фах 162, 91001 Скопје, Република Македонија*
[4]*Институт за фармацевтска хемија, Фармацевтски факултет, Скопје, Водњанска 17, 91000 Скопје, Република Македонија*

Клучни зборови: *Thymus;* етерично масло; GC и GC-MS анализа; надворешна средина

Составот на етеричното масло од неколку таксони од родот *Thymus* L., кои диво растат во Македонија, е испитуван користејќи ги методите GC и GC-MS. Во сите испитувани примероци беа идентификувани, главно, исти компоненти, но со многу различна содржина. Во повеќето примероци тимол, карвакрол, линалол, α-терпинеол, терпенил ацетат, *p*-цимен и γ-терпинен беа најмногу застапените компоненти. Во останатите примероци гераниол, линалол и сабинен хидрат беа присутни во поголеми количества. Разликите во составот на етеричните масла, добиени од истиот таксон, веројатно се должат на разликите во надворешните услови во кои растат растенијата. Наспроти ова, голема сличност е најдена во составот на етеричното масло добиено од различни таксони од родот *Thymus*, од растенија кои растат во исти надворешни услови.