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The powdered air-dried plant material was hydrodistilled for 4 h using the apparatus according to the British Pharmacopoeia (7). A pale yellow oil was obtained which exhibited the following physical properties: n_D^{25}: 1.4930; $[\alpha]_D^{25}$: $-4.70°$ (*c* 1.06, pentane); d^{25}: 0.875. The sample oil used for TLC, GC, and GC/MS analyses was collected in pentane.

The constituents were tentatively identified on silica gel GF-254 precoated plates (Merck) using some pure authentic samples for comparison. The mobile phase was toluene-ethyl acetate (93:7 by volume), and UV detection was at 254 nm. Further characterization was by vanillin-sulphuric acid reagent and R_f values (8).

The above stationary phase was also used for the identification of camphor, the mobile phase was dichloromethane. After development, the plates were treated with phosphomolybdic acid reagent (9) and heated for 5 min at 100 °C, sprayed with a solution of 0.5 g potassium permanganate in 5 ml concentrated sulphuric acid, and heated again for 5 min. At 100 °C, camphor appeared as a dark blue spot (9).

The oil was examined by capillary GC on a Hewlet Packard 5890 gas chromatograph, fitted with a flame ionization detector. The column was a 30 m × 0.31 mm I.D. high performance fused silica cross linked methyl silicon megabore, film thickness: 3 μm. Carrier gas was nitrogen with a flow rate of 2.5 ml/min. Column temperature was programmed from 70 to 280 °C at 10 °C/min. Injector and detector temperatures were set at 270 °C.

A Kratos Concept 25 spectrometer was used, equipped with a Sun Mash 3 computer data output. The operating conditions were similar to those of GC analysis, but with He as the carrier gas. Mass spectrometer conditions were the following: ionization potential, 70 eV; source temperature, 150 °C; resolution, 1000; scan speed, 1 sec/decade. Identification of the constituents was based on computer matching against the library spectra built up from pure substances and components of known essential oils, and MS literature data (10, 11).

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Composition of the Essential Oil from *Thymus moesiacus* from Macedonia

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Abstract

Composition of the essential oil of *Thymus moesiacus* Velen. from Macedonia was examined by GC and GC-MS methods. The main constituents of the oil were geraniol, linalool, geranyl acetate, and terpenyl acetate.

Thymus moesiacus Velen. is a wild growing endemic plant spread in Bulgaria, Macedonia, Albania, Serbia and Bosnia (1). The taxon creates stable and pure populations on hills and mountain grasslands, mainly spreading on calcerous soil, but always on a thick layer of soil. On mountain Bistra, West Macedonia, the taxon grows with *Poa violacea*, in a stable community with great phytocenologic value. Less frequently, it grows with *Festuca spadicea*. In the Macedonian flora two subspecies are defined: var. *moesiacus* – typical form of the species, and var. *microcalyx*. Morphologically, they are very similar and both varieties are commonly used in folk medicine against cough, flu, pulmonary infection, abdominal throes. The main activities are antiseptic, expectorant, and spasmolytic which are probably due to the content of essential oil and the content of flavonoids. To our best knowledge the content and the composition of its essential oil has not been studied. Therefore, the oil hydrodistilled from *T. moesiacus* Velen. var. *moesiacus*, was examined by GC and GC/MS methods.

Plant material was collected in July 1993 (sample A) and July 1994 (sample B) on mountain Šara, Northwest Macedonia, and July 1994 (sample C) on mountain Bistra, West Macedonia. A voucher specimen was deposited at the Herbarium of the Institute of Biology, Faculty of Natural Sciences, Skopje, Macedonia [SKO 93A, SKO 94B and SKO 94C, respectively]. The identity was confirmed by Dr. Matevski from the Institute of Biology, Faculty of Natural Sciences, Skopje, Macedonia.

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The plant material was air-dried and submitted to hydrodistillation for 5 hours in a Clevenger type apparatus. The oil was dried over anhydrous sodium sulfate to yield 1.7% (v/w) for sample A, 1.0% (v/w) for sample B, and 0.9% (v/w) for sample C, with the following characteristics: refractive index (n_D^{20}) for A 1.470, for B 1.474, and for C 1.475 and relative density (d_{20}^{20}) for A 0.8869, for B 0.8920, and for C 0.8800. The oil was analyzed on a Hewlett-Packard gas chromatograph, model 5890, series II, equipped with split-splitless injector, on a fused capillary column PONA, 50 m × 0.2 mm I.D., 0.5 μm film thickness. The sample solution in ethanol (1.0%) was injected in split mode (1 : 100) at 250 °C. The detector temperature was 300 °C (FID) while the column temperature was linearly programmed from 40–280 °C, with a rate of 2 °C/min. In the case of GC/MS analysis a Hewlett-Packard model HP 5971A MSD operating in EI mode (70 eV) and the same chromatographic condition was used. The transfer line was heated at 280 °C.

Identification of the components (Table **1**) was based on comparison of their retention times with those of authentic samples on the same column, and matching the mass spectral data with those from the available authentic samples as well as with those from Wiley/NBS library of MS spectra. The quantities of the components were obtained using "peak area 100% method".

Table 1 Composition of the essential oil of *Thymus moesiacus* Velen. (in %).

Compound	CII*	A	B	C
α-Thujene [a,b,c]	938	0.13	0.34	0.24
α-Pinene [a,b,c]	942	0.13	0.23	0.17
Camphene [c]	954	0.16	0.24	0.12
1-octen-3-ol [c]	968	0.30	0.39	–
Sabinene [c]	976	0.49	0.82	1.08
β-Pinene [a,b,c]	981	0.86	1.13	1.36
α-Phellandrene [a,b,c]	1002	–	0.05	–
ortho-Cimene [c]	1018	0.26	0.37	0.52
para-Cimene [c]	1020	2.77	2.93	4.88
1,8-Cineol [a,b,c]	1027	0.06	0.07	0.11
Limonene [a,b,c]	1030	0.33	0.52	0.30
γ-Terpinene [c]	1057	1.62	2.78	3.13
Linalool [a,b,c]	1092	8.14	10.10	25.02
exo-Borneol [a,b,c]	1164	0.56	0.51	0.40
endo-Borneol [a,b,c]	–	0.17	0.27	0.29
α-Terpineol [a,b,c]	1185	2.02	2.07	2.58
Nerol [a,b,c]	1218	0.61	–	0.66
Z-Citral [a,b,c]	1222	–	–	0.45
Thymol methyl ether [c]	–	0.09	–	0.34
Geraniol [a,b,c]	1243	32.06	33.27	14.87
Thymol [a,b,c]	1287	3.03	2.66	7.88
Carvacrol [a,b,c]	1297	12.31	12.81	13.31
Terpinyl acetate [c]	1333	5.20	6.26	1.81
Geranyl acetate [a,b,c]	1364	16.75	11.73	4.11
β-Bourbonene [c]	1406	0.06	0.03	0.04
trans-Caryophyllene [a,b,c]	1428	3.06	0.05	2.68
α-Humulene [c]	1465	0.30	2.56	0.24
γ-Muurolene [c]	1475	0.03	0.27	–
epi-Bicyclosesquiphellandrene [c]	–	0.18	0.16	0.08
Calarene [c]	–	–	0.08	0.07
β-Bisabolene [c]	1501	1.24	0.96	1.31
γ-Cadinene [c]	1518	0.07	0.05	0.08
δ-Cadinene [c]	1524	–	0.03	0.05
Caryophyllene oxide [c]	1563	0.34	0.22	0.37
Total:		92.54%	94.00%	88.79%

* CII – retention index (Kovats)

[a] Compared with retention time of authentic samples.

[b] Compared with mass spectra of authentic samples.

[c] Compared with mass spectra from Wiley/NBS library.

Essential oils obtained from three samples of *Thymus moesiacus* had almost the same composition. It should be noticed that the alcohols geraniol and linalool as well as the esters geranyl acetate and terpinyl acetate were the main constituents of the oils, but they were present in different amounts. The essential oils from samples A and B (mountain Šara) contained 32.06–33.27% of geraniol while sample C (mountain Bistra) contained only 14.87%. However, sample C contained a larger amount of linalool (25.02%). On the other hand, samples A and B contained larger amounts of geranyl acetate (11.73–16.75%) compared to sample C (4.11%). The amounts of phenols were almost equal for samples A, B, and C: cavracrol 12.31; 12.81, and 13.31%, and thymol 3.03; 2.55, and 7.88%, respectively. The essential oil from *T. moesiacus* is interesting because of the high content of geraniol and linalool. It had been shown previously that geraniol possesses a strong antibacterial and antimycotic activity (2). If it is present in esters form, the oils lose the activity (3). It was also shown that essential oils obtained from the same species but from the plant materials collected from the different places, could possess different antibacterial activity (3). The results from our examination show that the content of geraniol is variable depending on the origin of the plant material.

The composition of essential oil from *Thymus moesiacus* Velen. is atypical for species of the genus *Thymus*. High content of linalool was found in *T. leptophyllus*, from Spain (4), the main component being linalyl acetate (68.50%). Large amounts of linalool were also found in essential oil of *T. precox* ssp. *articus*, with large amounts of linalyl acetate (25.83%) (5). *T. beaticus* essential oil from Spain contained 20% of geraniol and 10% of citral and 13.8% of 1.8-cineol (6), components that were identified in trace amounts in *T. moesiacus* oil. Studies of *T. serpyllum* essential oil showed the presence of high contents of linalool but another important component in the oil was myrcene (7). It thus appears that the composition of the essential oil of *T. moesiacus* is quite different from those isolated from other *Thymus* species.

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