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Essential oil composition of *Thymus tosevii* ssp. *tosevii* var. *longifrons*

SVETLANA KULEVANOVA[1]*
MIHAILO RISTIĆ[2]
TRAJČE STAFILOV[3]
KIRIL DOREVSKI[1]

[1]*Faculty of Pharmacy »St. Cyril and Methodius« University Skopje, Macedonia*

[2]*Institute for Medicinal Plant Research »Dr. J. Pančić« Belgrade, FR Yugoslavia*

[3]*Faculty of Natural Science »St. Cyril and Methodius« University Skopje, Macedonia*

Essential oil composition of *Thymus tosevii* ssp. *tosevii* var. *longifrons* growing wild in Macedonia was examined by the GC-FID and GC-MS methods. Four different types of essential oil were found, one with almost equal contents of thymol and carvacrol, two others with only one of the phenols dominating (thymol or carvacrol) and the last one was a nonphenolic type of the oil. In most of the samples, phenols were the most abundant components followed by *p*-cymene and γ-terpinene. The samples containing phenols only in traces, contained geraniol, linalool and geranyl acetate as main oil components.

Keywords: *Thymus tosevii* ssp. *tosevii* var. *longifrons*, essential oil, GC and GC-MS analysis

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Thymus vulgaris and *T. zygis* have been established as a plant source for commercial thyme oil appreciated for its expectorant, antibacterial, antimycotic and spasmolytic properties (1–7). These effects are due to the presence of phenolic constituents (thymol and carvacrol), although a few recent studies point out that alcohols, especially geraniol, could be also responsible for antimicrobial activity (4). Investigations carried out in the last few years have shown that the content of these phenols vary widely depending on their origin (8, 9), vegetation period (10), means of collection, preparation of plant material and other factors (11). The examinations of some other taxa of the *Thymus* genera, very often point at the presence of other species of these genera which contain large amounts of essential oil with high percentage of thymol and carvacrol (12–14). Some studies on essential oil composition of *T. serpyllum* (15–17) showed that these taxa contain essential oil similar to that of *T. vulgaris* which provides an official use of *Thymi serpylli herba* (18).

Certain number of species of genus *Thymus* L. growing wild on hill and mountain grasslands in Macedonia, have been used for many years in Macedonian folk medicine

* Correspondence

for cough, flu, pulmonary infections, abdominal throes, *etc.* Beside many species of genus *Thymus* L. that occur in flora of Macedonia, only a few of them are collected by local people who all these plants name »majkina duša« (»mother soul«) (19). The plant material *Serpylli herba* (wild thyme), is commonly used as a substitute for official thyme. *Serpylli herba* from Macedonia, represents a mixture of dried herbs from several Macedonian *Thymus* L. species. One of them is *T. tosevii* Velen., taxon of the *Thymus* genera characterized with great polymorphism and complexity, spread through the Balkan peninsula, mostly in Macedonia, Serbia, Bulgaria and Albania. According to Matevski, three subspecies and seven varieties of these taxa occur in Macedonian flora (20). Thus, *Thymus tosevii* ssp. *tosevii* var. *longifrons* represents one of the varieties. These taxa grow on both calcareous and silicate ground at an altitude up to 1600 m. In the present work, the essential oil of *T. tosevii* was examined by GC and GC-MS techniques to determine its composition and check the possibilities for its use in official pharmacy or in some other field.

EXPERIMENTAL

Plant material

Plant materials were collected from different areas in Macedonia, and always in their flowering stage. It was defined as *T. tosevii* Velen. ssp. *tosevii* var. *longifrons* Ronn., collected at Karadžica mountain, Central Macedonia, July 1994, July 1995; Mavrovo, Western Macedonia, July 1955; Mavrovo (Vrben) July 1995; two samples from Osogovski mountain, Eastern Macedonia, July 1995. The identity of the taxa was confirmed by Dr. V. Matevski, from the Institute of Biology, Faculty of Science, Skopje, Macedonia. Voucher specimen for the taxa was deposited in the Herbarium of the same Institute [SKO–174].

Plant material was air-dried and then submitted to steam distillation in Clevenger type apparatus for 5 hours. After separation from water, essential oils were dried over anhydrous sodium sulfate.

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^{-1}. 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 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 a comparison of their retention times with those of analytical standards of available terpenoids, and matching mass spectral data of oil constituents with those from analytical standards as well as those of MS spectra from Wiley/NBS library. For the quantification purposes area percentage reports obtained by GC-FID were used as a base.

RESULTS AND DISCUSSION

The steam distillation of the samples of *T. tosevii* ssp. *tosevii* var. *longifrons* yielded 0.82–2.30% (V/m) of pale yellow coloured essential oil with pleasant odour and the following characteristics: refractive index from 1.487 to 1.501 and relative density from 0.899 to 0.912.

The composition of the essential oils, analyzed by the GC-FID and GC-MS methods, is presented in Table I. Different samples contained various numbers of components (45–64), but in most of the samples it was possible to identify 34–36 of them, representing 95.87–98.45% of whole oil. Important components in the oil composition were phenols, presenting up to 53.97%, as well as monoterpene hydrocarbons with higher percentages of *p*-cymene and γ-terpinene. Samples collected at Karadžica mountain contained large amounts of both phenols, thus 8.63–21.89% of thymol and 33.06–45.34% of carvacrol were registered. These samples also contained large amounts of *p*-cymene and γ-terpinene (9.89–12.98% and 9.45–11.07%, respectively). Similar percentages of these components were registered in the samples collected at Mavrovo area (Table I). Considering these components, the samples collected at Osogovski mountain had different compositions. One sample contained 33.35% of thymol, only 4.64% of carvacrol, 11.5% of *p*-cymene and 9.54% of γ-terpinene while the other one contained only traces of thymol (0.3%), 10.0% of carvacrol and low quantities of *p*-cymene and γ-terpinene (1.60 and 2.54%, respectively). The last sample differed a lot from the others because of very high percentages of geraniol (37.75%), linalool (25.19%) and geranyl acetate (12.0%). Together with carvacrol these components were most abundantly represented in the essential oil composition of this sample of oil. In the rest of the samples, geraniol and linalool were found in much lower quantities (0.06–7.80% and 0.23–5.86%, respectively). It was also noticed that ester components, especially geranyl acetate, were present in very small amounts in all the samples, except for one sample from Osogovski mountain. The samples originating from the Mavrovo area were different because they contained larger amounts of terpenyl acetate (15.09–22.31%). Twelve sesquiterpene hydrocarbons were identified in most of the samples but only *trans*-caryophyllene and β-bisabolene were registered in higher percentages (0.73–3.17% and 0.88–2.49%, respectively) whereas the others were present in much lower amounts (<0.2%).

As shown, phenols were the main components in most of the samples. Probably four different types of essential oils were also noticed, one with similar content of thymol and carvacrol, two others with dominating thymol or carvacrol and nonphenolic type of essential oil, established in the samples originating from Eastern Macedonia. In our recent study of the essential oil composition of *T. tosevii* ssp. *tosevii* and *T. tosevii* ssp. *substriatus*, we have found the same types of essential oils (21). The samples of essential oils obtained from var. *longifrons* which contained low levels of phenols, on the other hand, contained high amounts of geraniol and linalool followed by high amounts of geranyl acetate (Table I). Samples which contained high percentages of phenols also contained high amounts of *p*-cymene and γ-terpinene (Table I).

Data considering the essential oil composition of *Thymus tosevii* growing wild in Greece, with linalool (35.5%) and geraniol (27.5%) as main components, are available (21). In addition, twelve monoterpene hydrocarbons, nine oxygen components, one ses-

Table I. Composition of the essential oil of Thymus tosevii *ssp.* tosevii *var.* longifrons

Component	Karadžica 1994	Karadžica 1995	Mavrovo 1995	Mavrovo–Vrben 1995	Osogovski mountain 1995	Osogovski mountain – Ponikva 1995
α-Thujene	1.36	1.14	1.23	0.91	1.21	0.15
α-Pinene	0.67	0.78	0.75	0.49	0.94	0.09
Camphene	0.59	0.71	0.88	0.41	1.04	0.09
Octenol-3	–	–	0.57	0.38	0.76	0.02
Sabinene	1.05	0.09	0.91	1.00	1.53	0.26
β-Pinene	1.70	1.90	1.80	6.23	5.47	0.40
α-Phellandrene	0.24	0.17	0.18	0.14	0.20	0.04
o-Cymene	1.95	1.58	1.54	1.45	1.85	0.30
p-Cymene	12.98	9.89	7.76	6.09	11.50	1.60
1,8-Cineol	0.48	0.82	0.33	0.30	0.38	0.06
Limonene	0.36	0.45	0.75	1.23	0.47	0.07
cis-Ocimen	0.05	0.14	0.09	0.05	0.09	0.04
γ-Terpinene	11.07	9.45	9.14	9.61	9.54	2.54
Sabinene hydrate	0.97	2.05	0.67	0.62	4.92	0.15
Linalool	0.23	1.97	2.23	0.44	5.86	25.19
exo-Borneol	1.43	1.46	1.67	0.53	2.50	0.31
endo-Borneol	0.50	0.80	0.47	0.31	1.41	0.05
α-Terpineol	0.17	0.09	1.66	1.45	0.52	0.15
cis-Dihydrocarveol	–	–	0.09	2.69	0.69	0.40
z-Citral	0.23	–	0.29	0.27	0.94	–
Thymol methylether	2.36	–	1.64	1.45	1.51	0.18
Geraniol	0.06	2.94	7.80	0.11	0.57	37.75
E-Citral	–	–	0.25	0.31	0.13	–
Thymol	21.89	8.63	17.25	8.64	33.35	0.30
Carvacrol	33.06	45.34	17.35	21.06	4.62	10.00
Unknown	–	0.06	0.12	5.80	–	0.04
Terpinyl acetate	0.49	0.34	15.09	22.31	1.71	0.56
Geranyl acetate	0.02	0.77	2.30	0.14	0.06	12.00
α-Copaene	0.04	0.08	–	–	–	–
β-Burbonene	0.04	0.05	0.04	–	0.03	–
trans-Caryophyllene	1.31	0.73	1.25	2.22	1.34	3.17
α-Cubebene	0.04	0.05	–	–	–	–
α-Humulene	0.09	0.05	0.08	0.19	0.14	0.12
γ-Muurolene	0.03	0.16	0.05	–	0.17	0.71
β-Cubebene	0.15	0.17	0.23	0.13	–	0.11
β-Bisabolene	2.39	2.49	1.32	1.17	1.28	0.88
γ-Cadinene	0.25	0.32	0.10	0.10	0.21	0.08
δ-Cadinene	0.02	–	–	0.04	–	–
Caryophyllene oxide	0.15	0.10	0.14	0.18	0.17	0.17
Total %	98.42	95.87	98.02	98.45	97.11	97.98
Number of components	64	59	47	54	61	45
Identified components	35	34	36	35	34	34

quiterpene and two terpene esters were identified, which represented about 98% of the oil. Compared to our results, this composition of essential oil seemed to be similar to the essential oil composition of our sample originating from the Berovo area (Eastern Macedonia). Data on *Thymus sibthropii* [Syn. *T. tosevii* (20)], growing wild in Greece, point out that geraniol, thymol and geranyl acetate were the main components of the oil (22). In our investigation, the samples of oils usually contained small quantities of ester compounds, and only one population of *T. tosevii* var. *longifrons* contained larger amounts of geranyl acetate as well as another which contained larger quantities of terpenyl acetate.

CONCLUSION

Thymus tosevii ssp. *tosevii* var. *longifrons* from Macedonia shows a high content of essential oil with varying composition, depending on the plant origin and probably many other factors yet not investigated. The content of total phenols can be very high (up to 53.97%), but there are also samples which contain only the traces of phenol components. These taxa could be used as a substitute for the thyme oil but previously its essential oil composition should be checked, especially if plant material is collected in Eastern Macedonia. The samples which contain larger quantities of geraniol and linalool could be interesting for the use in pharmacy as well as other uses such as in food industry, aroma and flavour production.

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SAŽETAK

Sastav eteričnog ulja *Thymus tosevii* ssp. *tosevii* var. *longifrons*

SVETLANA KULEVANOVA, MIHAILO RISTIĆ, TRAJČE STAFILOV i KIRIL DOREVSKI

Sastav eteričnog ulja *Thymus tosevii* ssp. *tosevii* var. *longifrons* koji raste divlje u Makedoniji ispitan je metodama GC-FID i GC-MS. Nađena su četiri različita tipa eteričnog ulja. Jedno od njih sadržavalo je podjednake količine timola i karvakrola, druga dva pokazivala su dominantan sadržaj samo jednog od fenola (timola ili karvakrola) dok je posljednje bilo nefenolnog tipa. U većini uzoraka fenoli su bili najobilnije zastupljene komponente; slijedili su ih *p*-cimen i γ-terpinen. Uzorci koji su sadržavali fenole samo u tragovima sadržavali su geraniol, linalool i geranil acetat kao glavne komponente ulja.

Faculty of Pharmacy
»St. Cyril and Methodius« University
Skopje, Macedonia

Institute for Medicinal Plant Research
»Dr. J. Pančić«, Belgrade, FR Yugoslavia

i

Faculty of Natural Science
»St. Cyril and Methodius« University
Skopje, Macedonia