



GC/MS Analysis of the Essential Oil of *Thymus Longicaulis* Presl. from Kosovo

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Abstract: Three samples of essential oil of *Thymus longicaulis* Presl. were analyzed for their chemical composition. The plants were collected in their natural habitat on three different locations in Kosovo. Aerial parts of the plant were submitted to hydrodistillation using Clevenger apparatus. Gas chromatography – mass spectrometry analysis was performed for each extracted essential oil sample. Essential oil yield resulted with 0.68-1.54% on dried weight. Analyzed essential oil constituents resulted with variation in their quality and quantity. One sample was characterised as thymol/carvacrol chemotype, resulting with 15.26% of thymol and 12.65% of carvacrol as its main constituents. Two other analyzed samples had very small amounts of thymol and carvacrol and resulted with α -terpineol (14.23%), α -terpinyl acetate (11.81%), linalool (9.63%), and myrcene (6.59%) as the main constituents for the first, and α -phellandrene (19.07%), linalool (14.22%), β -caryophyllene (9.27%), and limonene (7.83%) for the second sample, respectively.

INTRODUCTION

The genus *Thymus* (Lamiaceae) is noteworthy for the numerous wild-growing plants biosynthesizing a remarkable amount of volatile compounds. *Thymus* species are perennial, aromatic herbs and sub shrubs native to Europe, North Africa and Asia (Morales, 1997). *Thymus* species, a taxonomically complex group of plants, are well known as medicinal plants containing tannins, bitter substances, and flavonoids, and are aromatic due to their essential oil content. The essential oils themselves are products in demand not only as ingredients in the production of perfumes and cosmetics,

but also for medicinal purposes due to their antiseptic, antispasmodic and antitussive properties (Stahl-Biskup, 2002). The existence of genetically based polymorphisms for secondary compound production has been reported in a range of *Thymus* spp. (Stahl-Biskup, 2002; Thompson, 2002). This variability is caused both by environmental factors and genetic variation (Thompson, Manicacci, and Tarayre, 1998). Numerous chemotypes of wild *Thymus* species have been defined, such as carvacrol and thymol, α -terpineol, thujone, geraniol, linalool, and others (Thompson, Chalchat,

Michet *et al.*, 2003). Thymol and carvacrol low quantity is a characteristic feature of the wild *Thymus* species growing in the Northern European countries (Loziene, Vaiciuniene, Venskutonis, 1998; Raal, Paaver, Arak, *et al.*, 2004; Paaver, Orav, Arak, *et al.*, 2008). Moreover, (*E*)-nerolidol, caryophyllene oxide, myrcene, β -caryophyllene and germacrene D, linalool, linalyl acetate and geranyl acetate were reported as main constituents of wild thyme (Raal, Paaver, Arak, *et al.*, 2004).

In Kosovo, *Thymus* spp. is categorized as a harvestable commercial wild herb found in large quantities, with a potential production of tens of thousands of kg annually (Blakley, 2003). It is used traditionally as folk medicine in Kosovo, as infusion and decoction, mainly for the treatment of respiratory tract inflammation. (Mustafa, Hajdari, Krasniqi, *et al.*, 2012).

The aim of the study was essential oil analysis of three population samples of *Thymus longicaulis* Presl. collected in south-western parts of Kosovo, Sharr mountain area and Rugova mountain area.

EXPERIMENTAL

Plant material and reagents

The plant material was collected from its natural habitat from three different locations in Kosovo at two different areas: Sharr mountain area (south Kosovo) and Rugovë mountain area (west Kosovo). Plants were harvested in full flowering state in June 2012. A voucher specimen of each sample was deposited in Department of Pharmacy, Medicine Faculty, University of Tirana. The geographic area and altitude were recorded for each collection point (Table 1). All reagents used in this study were of the highest purity available and obtained from the Sigma Chemical Company.

Sample preparation

The plants were dried at room temperature. In order to isolate essential oil, hydrodistillation was carried out for 2 hours using 30 g of dried plant material. The oil yield was recorded for each sample and calculated on dried plant weight (Table 1). The essential oil samples were dried over anhydrous sodium sulphate and were kept at -4°C in dark, until analysis.

Table 1: Harvest location, harvest date and essential oil yield of *Thymus longicaulis* Presl.

Sample	Location	Altitude (m)	Yield (%)
L1	Brod (Dragash)	1380 m	1.54
L2	Liqenati Kuqishtës	1500 m	0.68
L3	Bjeshkë e Kuqishtës	1310 m	0.71

Gas chromatography-mass spectrometry

The composition of volatile content was determined by gas chromatography-mass spectrometry technique. GC-MS was carried out on a Shimadzu GCMS-QP2010S gas chromatograph fitted with a fused silica ZB-5 (5% phenyl methyl siloxane) capillary column (30 m x 0.25 mm, 0.25 μ m film thickness), and with an autosampler AOC-20. Column temperature was programmed from 60°C to 240°C at 3°C/min, and helium was used as

carrier gas (1.1 mL/min). Other operating conditions were as follows: inlet pressure 9.43 psi, injector temperature 250°C, detector temperature 280°C, split ratio 1:25, injection volume 1 μ L. Ionization of the sample components was performed in the EI mode, (70 eV), with scan range 40-450 amu.

Qualitative and quantitative analysis

The linear retention indices, RI, for all compounds were determined (Van Den Dool, and Kratz, 1963). The identification of the volatile constituents was accomplished by the visual interpretation, comparing their retention indices and mass spectra with literature data (Adams, 2007), by computer library search (Wiley 8.0, NIST/EPA/NIH Mass Spectral Library 2.0, and Mass Finder 4.2 Computer Software and Terpenoids Library), and in the laboratory own database. Compounds concentrations (as % content) were calculated by integrating their corresponding chromatographic peak areas (TIC mode).

RESULTS AND DISCUSSION

Samples of *Thymus longicaulis* Presl. essential oils yielded from 0.68 to 1.54% of dried weight. Identified compounds in *Thymus* species studied with their relative percentages and retention indices are given in Table 2.

Table 2: Constituents of the three samples of *Thymus longicaulis* Presl. essential oil.

RI	Compound	L1 RA %	L2 RA %	L3 RA %
806	(2E)-Hexenal	-	1.8	0.45
885	α -Thujene	-	0.2	0.29
896	α -Pinene	0.83	-	-
899	Tricyclene	-	0.46	0.27
911	Camphene	0.92	2.44	7.26
935	Sabinene	1.56	1.89	0.35
939	1-Octen-3-ol	0.98	-	-
946	3-Octanone	0.73	1.76	-
950	Myrcene	2.94	6.59	6.32
975	α -Phellandrene	1.2	5.34	19.07
991	<i>p</i> -Cymene	9.45	2.87	1.91
993	Limonene	-	1.49	7.83
996	Sylvestrene	2.31	-	-
998	1,8-Cineole	-	3.97	2.36
1006	Δ -3-Carene	0.2	-	tr
1015	(Z)- β -Ocimene	0.08	-	-
1017	(Z)- β -Ocimene	-	-	0.24
1026	β -Phellandrene	9.67	-	-
1035	<i>cis</i> -Sabinene hydrate	1.83	-	-
1076	Linalool	0.46	9.63	14.22
1091	1-Octen-3-yl acetate	1.34	-	-
1105	Octanol acetate	0.67	1.04	-
1120	Camphor	0.23	2.04	4.69
1142	Borneol	-	0.43	0.41
1153	Terpinen-4-ol	2.62	0.96	-
1166	α -Terpineol	6.42	14.23	0.66
1206	Nerol	-	-	0.66
1211	Thymol methyl ether Carvacrol methyl	0.29	-	0.16
1220	ether	0.93	0.14	0.14
1243	Geraniol	-	0.32	0.28
1263	Bornyl acetate	-	1.3	3.09

1274	Thymol	15.26	-	-
1310	Carvacrol	12.65	0.17	-
1348	α -Terpinyl acetate	11.74	11.81	0.84
1357	Neryl acetate	0.78	0.91	0.36
1379	Geranyl acetate	-	1.9	0.6
1402	β -Caryophyllene	3.88	1.03	9.27
1423	β -Longipinene	0.11	-	-
1433	α -Humulene	0.38	0.26	-
1442	(E)- β -Farnesene	-	-	3.29
1460	γ -Amorphene	0.15	-	-
1461	Germacrene D	0.67	0.86	0.31
1469	(Z)- β -Farnesene	-	0.28	-
1469	6,9-Guaiadiene	tr	-	tr
1484	α -Amorphene	0.11	-	0.23
1496	β -Bisabolene	-	-	0.33
1509	δ -Amorphene	0.63	0.1	0.96
1536	Elemol	-	1.04	0.21
1643	α -Cadinol	-	-	0.91
1643	Khusimone	-	tr	-
1671	epi- α -Bisabolol	0.32	-	-
1703	(2E, 6Z)-Farnesal	1.88	-	-
Aliphatic compounds		3.72	4.60	0.45
Aromatic compounds		38.58	3.18	2.21
Monoterpene hydrocarbons		19.71	18.41	41.70
Oxygenated monoterpenes		24.08	47.50	28.17
Sesquiterpene hydrocarbons		5.95	3.60	14.64
Oxygenated sesquiterpenes		2.20	-	0.91
Total identified		94.24	77.29	88.08

RI= Retention indices calculated on ZB-5 column; RA=Relative area; tr=traces (<0.1%); L1, L2 and L3=samples of *Thymus longicaulis* Presl.hydrodistilled oil.

Fifty-two constituents were identified representing 77.29-94.24% in total. Present compounds were oxygenated monoterpenes (24.08-47.5%), monoterpene hydrocarbons (18.41-41.70%), aromatic compounds (2.21-38.58%), sesquiterpenes (3.60-14.64%), oxygenated sesquiterpenes (0.91-2.20%), and aliphatic compounds (0.45-4.60%). In sample L1 thirty-five constituents were identified. Main compounds were aromatic compounds with 38.58%, oxygenated monoterpenes with 24.08%, and monoterpene hydrocarbons with 19.71%. Main constituents were thymol (15.26%), carvacrol (12.65%), α -terpinyl acetate (11.74%), and β -phellandrene (9.67%). In sample L2 77.29% of compounds were identified, with thirty-one constituents resulted in total. Main compounds were oxygenated monoterpenes (47.50%) and monoterpene hydrocarbons (18.41%). Main constituents were α -terpineol(14.23%), α -terpinyl acetate (11.81%), linalool (9.63%), and myrcene (6.59%). In L3 sample 88.08% of the compounds were identified with thirty-three constituents resulted in total. Main compounds were monoterpene hydrocarbons (41.70%), oxygenated monoterpenes (28.17%), and sesquiterpene hydrocarbons (14.64%). Main constituents were α -phellandrene (19.07%), linalool (14.22%), β -caryophyllene (9.27%), limonene (7.83%), and camphene (7.26%).

In these samples of *Thymus longicaulis* Presl. essential oil, monoterpenoids in general resulted with most abundant levels. Also in the literature monoterpenoids have been reported as the main compounds of the *Thymus longicaulis* essential oil (De Martino, Bruno, Formisano, et al., 2009).

According to the literature, different chemotypes of *Thymus longicaulis* Presl. were reported, providing evidence of intraspecific chemical variability of this plant species.

In a study conducted in Greece, three chemotypes of *Thymus longicaulis* C. Presl subsp. chaubardii (Reichenb. fil.) Jasas were detected: limonene (18.7%) and thymol (19.4%); geraniol (56.8%) and geranyl acetate (7.6%); linalool (63.1%) and α -terpinyl acetate (20.4%). (Tzakou O., Verykokidou E., Roussis V. et al., 1998).

Chemical profile of thymus longicaulis from Serbia consisted of α -terpineol acetate (67.52%), thymol (4.47%), limonene (3.58%), α -terpineol (3.43%) and γ -terpinene (2.07%) as main constituents, with a content of monoterpene hydrocarbons (11.20%), oxygenated monoterpenes (80.07%), sesquiterpenes (2.94%), oxygenated sesquiterpenes (0.79%), and the rest 4.86%. The oil was poor in sesquiterpene hydrocarbons (3.66%), of which only small amounts of β -caryophyllene (1.54%), β -bisabolene (0.70%), and germacrene D (0.50%) were present. (Grujić Jovanović S., Marin P.D., Dzamić A. et al., 2009).

Thymol, α -terpinyl acetate, geraniol were the predominant components in each of the three different chemotypes detected in Turkey (Baser, Ozek, Kirimer, et al., 1993).

Thymol and carvacrol were the main components in the oils of *Thymus longicaulis* according to another study report from Turkey, with other major components identified as *p*-cymene, γ -terpinene and borneol (Azaz, Irtem, Kurkcuoglu, et al., 2004). The same plant species from southern Italy had cymene, γ -terpinene, myrcene and limonene as the main constituents (De Martino, Bruno, Formisano, et al., 2009). In a study conducted in Croatia, thymol (46.3%), γ -terpinene (16.2%), thymol methyl ether (11.4%) and *p*-cymene (9.4%) were identified as the most abundant compounds followed by borneol, β -caryophyllene, δ -3-carene and linalool ranged from 1.4% to 2.2%. It is clear that *Thymus longicaulis* originating from Croatia belongs to a thymol chemotype (Kuštrak, 2005; Vladimir-Knežević, Kosalec, Babac, et al., 2012). The oxygenated monoterpene α -terpinyl acetate present as the main constituent in essential oil samples L1 and L2, was reported in literature as α -terpinyl acetate chemotype of *Thymus* spp. (Čavar, Maksimović, and Vidic, 2009; Mockute, and Bernotiene, 2011).

CONCLUSIONS

Essential oil of *Thymus longicaulis* from Kosovo resulted with a quantitative and qualitative variation of constituents. Phenolic and non-phenolic chemotypes were detected within the analyzed essential oil samples, thus being in accordance with the literature data.

The observed differences in essential oil profile of investigated species confirm the influence of origin of the plant material, as well as environmental conditions on the nature of plant chemical composition.

REFERENCES

- Adams, R. P. (2007) *Identification of Essential oil of Components by Gas Chromatography/Mass Spectrometry*. (4th Ed) Allured Publishing Corporation.
- Azaz, A. D., Irtem, H. A., Kurkcuoglu, M., Baser, K. H. C. (2004). Composition and the *in vitro* Antimicrobial Activities of the Essential Oils of some *Thymus* Species. *Zeitschrift für Naturforschung C*. 59(1-2), 75-80.
- Baser, K. H. C., Özek, T., Kirimer, N., Tümen, G. (1993). The Occurrence of Three Chemotypes of *Thymus longicaulis* C. Presl. subsp. *longicaulis* in the Same Population. *Journal of Essential Oil Research*. 5(3), 291-295.
- Blakley, T. (2003). *Marketing Analysis for Medicinal Herbs, Essential Oils, and Other Specialty Products*. (1st Ed) Kosovo Business Support, KOS012.
- Ćavar, S., Maksimović, M., Vidic, D. (2009). The Essential Oil of *Thymus aureopunctatus* (Beck) K. Malý. *Natural Product Communications*, 4(3), 15-420.
- De Martino, L., Bruno, M., Formisano, C., De Feo, V., Napolitano, F., Rosselli, S., Senatore, F. (2009). Chemical Composition and Antimicrobial Activity of the Essential Oils from Two Species of *Thymus* Growing Wild in Southern Italy. *Molecules*, 14(11), 4614-4624.
- Grujić Jovanović S., Marin P.D., Dzamić A., Ristić M. (2009). Essential oil composition of *Thymus longicaulis* from Serbia. *Chemistry of Natural Compounds*. 45(2), 265-266.
- Kuštrak, D. (2005). Posebni dio fitofarmacije: Etericna masla. In Golden marketing, Tehnicka knjiga (Ed.) *Farmakognozija-Fitofarmacija*, Zagreb. p.p. 299-308).
- Loziene, K., Vaiciuniene, J., Venskutonis, P. R. (1998). Chemical composition of the essential oil of creeping thyme (*Thymus serpyllum*) growing wild in Lithuania. *Planta Medica*, 64(8), 772-773.
- Mockute, D., Bernotiene, G. (2001) The alpha-terpenyl acetate chemotype of essential oil of *Thymus pulegioides* L. *Biochemical Systematics and Ecology*, 29(1), 69-76.
- Morales, R. (1997). Synopsis of the genus *Thymus* L. in the Mediterranean area. *Lagascalia*, 19(1-2), 249-262.
- Mustafa, B., Hajdari, A., Krasniqi, F., Hoxha, E., Ademi, H., Quave, C. L., Pieroni, A. (2012). Medical ethnobotany of the Albanian Alps in Kosovo, *Journal of Ethnobiology and Ethnomedicine*, 8(6), 1-14.
- Paaver, U., Orav, A., Arak, E., Mäeorg, U., Raal, A. (2008). Phytochemical analysis of the essential oil of *Thymus serpyllum* L. growing wild in Estonia. *Natural Product Research*, 22(2), 108-115.
- Raal, A., Paaver, U., Arak, E., Orav, A. (2004). Content and composition of the essential oil of *Thymus serpyllum* L. growing wild in Estonia. *Medicina (Kaunas)* 40(8), 795-800.
- Stahl-Biskup, E. (2002). Essential oil chemistry of the genus *Thymus* – a global view. In Stahl-Biskup, E. & Saez (Ed.), *F. Thyme: the Genus Thymus*. (p.p. 75-354).Taylor & Francis, London.
- Thompson, J. D. (2002). Population structure and the spatial dynamics of genetic polymorphism in thyme. In Stahl-Biskup, E. & Saez (Ed), *F. Thyme: the Genus Thymus*. (p.p. 44–74). Taylor & Francis, London.
- Thompson, J. D., Chalchat, J. C., Michet, A., Linhart, Y. B., Ehlers, B. (2003). Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *Journal of Chemical Ecology*. 29(4), 859-80.
- Thompson, J. D., Manicacci, D., Tarayre, M. (1998). Thirty-five years of thyme: a tale of two polymorphisms. *BioScience*. 48(10), 805–815.
- Tzakou O., Verykokidou E., Roussis V., Chinou I. (1998). Chemical composition and antibacterial properties of *Thymus longicaulis* subsp. *chaubardii* oils: Three chemotypes in the same population. *Journal of Essential Oil Research*. 10 (1), 97-99.
- Van Den Dool, H, Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*. 11, 463-471.
- Vladimir-Knežević, S., Kosalec, I., Babac, M., Petrović, M., Ralić, J., Matica, B., Blažeković, B. (2012). Antimicrobial activity of *Thymus longicaulis* C. Presl essential oil against respiratory pathogens. *Central European Journal of Biology*. 7(6), 1109-1115.

Summary/Sažetak

Ispitivan je hemijski sastav tri uzorka esencijalnog ulja *Thymus longicaulis* Presl. Biljni material je prikupljen sa prirodnog staništa na tri različite lokacije na Kosovu. Nadzemni dijelovi biljaka su podrvgnuti hidrodesitlaciji uz korištenje Clevenger aparature, te analizirani kombinovanom tehnikom gasna hromatografija-masena spektrometrija. Prinosi esencijalnog ulja su varirali od 0.68 do 1.54%. Jedan od uzoraka se pokazao kao predstavnik timol/karvakrol hemotipa, sa 15.26% timola i 12.65% karvakrola. Preostala dva uzorka su pokazala veoma nizak sadržaj timola i karvakrola. Prvi uzorak sadržavao je 14.23%, α -terpineola 11.81% α -terpinil acetate, 9.63% linalola i 6.59% mircena, a drugi 19.07% α -felandrena, 14.22% linalola, 9.27% β -kariofilena i 7.83% limonena.