



Artículo Original | Original Article

Essential oil composition of the Balkan endemic *Thymus longedentatus* (Degen & Urum.) Ronniger

[Composición del aceite esencial de *Thymus longedentatus* (Degen & Urum.) Ronniger endemic de los Balcanes]

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Abstract: The chemical composition of essential oil obtained from the aerial parts of the Balkan endemic species *Thymus longedentatus* was studied by GC-MS. Twenty eight compounds, consisting 98.2% of the total components were detected in amounts exceeding 0.1%. The oil was characterized by relatively high concentration of oxygenated monoterpenes (78.7%), among which citral isomers neral (27.5%) and geranial (30.3%) were the principal ones. In addition, 1,8-cineole (7.8%), trans- β -ocimene (7.5%), and β -myrcene (3.7%) were detected in significant amounts. Germacrene D (4.4%) was found to be the main sesquiterpene in the essential oil.

Keywords: *Thymus longedentatus*; Essential oil; Medicinal plant; Aromatic plant

Resumen: Se estudió mediante GC-MS la composición química del aceite esencial obtenido de las partes aéreas de *Thymus longedentatus*, especie endémica de los Balcanes. Se detectaron veintiocho compuestos, que representaban un 98,2% del total de compuestos y que se encontraban en cantidades superiores al 0,1%. El aceite se caracterizó por una concentración relativamente alta de monoterpenos oxigenados (78.7%), entre los cuales los isómeros de citral, neral (27.5%) y geranial (30.3%) eran los principales. Además, se detectó 1,8-cineol (7,8%), trans- β -ocimeno (7,5%) y β -mirceneno (3,7%) en cantidades significativas. Se encontró que Germacreno D (4.4%) es el principal sesquiterpeno en el aceite esencial.

Palabras clave: *Thymus longedentatus*; Acerites esenciales; Planta medicinal; Planta aromática

Recibido | Received: February 4, 2019

Aceptado | Accepted: March 2, 2019

Aceptado en versión corregida | Accepted in revised form: March 15, 2019.

Publicado en línea | Published online: March 30, 2019.

Declaración de intereses | Declaration of interests: This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, Project DN 16/3.

Este artículo puede ser citado como / This article must be cited as: I Aneva, A Trendafilova, M Nikolova, M Todorova, K Georgieva. 2019. Essential oil composition of the Balkan endemic *Thymus longedentatus* (Degen & Urum.) Ronniger. *Bol Latinoam Caribe Plant Med Aromat* 18 (2): 197 – 203.

INTRODUCTION

The species of genus *Thymus* provoke substantial interest worldwide from phytochemical point of view, due to their diverse biological activities with potential for application in pharmaceutical, cosmetic and food industries. They have been used since the ancient times to treat diseases of the respiratory and digestive system, as well as of colds (Zarzuelo & Crespo, 2002). They possess expectorant, antiseptic, fungicide, spasmolytic, carminative, sedative, diaphoretic and diuretic activities (Rustaiyan, 2000; Soliman & Badeaa, 2002; Rasooli & Mirmostafa, 2002; Tepe et al., 2004).

Genus *Thymus* comprises more than 250 species of perennial herbaceous or fruticose plants, classified into 8 sections. Total 66 species with numerous subspecies and varieties are listed in Flora Europaea. The genus is characterized by high degree of hybridization, which was observed between species belonging to different sections, and also between species with different ploidy levels. (Papageorgio, 1980; Baser et al., 1992; Vila et al., 1995; Baser et al., 1998; Guillen & Manzanos, 1998; Lozeine et al., 1998; Saez, 1998; Tumen et al., 1998).

The medicinal activity of the species of genus *Thymus* is due to a large extent to the chemical constituents in the essential oils. Therefore, major part of the studies focused on the determining of chemical composition of the species (Sáez, 2001; Jukic & Miloc, 2005; Cavar et al., 2009; Rădulescu et al., 2009; Vidic et al., 2010).

Very typical phenomenon for the genus is the

chemical polymorphism, which is due both to the ecological factors, and to genetic variation (Ložienė & Venskutonis, 2005; Rădulescu et al., 2009). Many of the species have more than 7 chemotypes (Stahl-Biskup, 2002).

Twenty species occur naturally in Bulgaria. As a whole, the essential oil composition of the species of genus *Thymus* in Bulgaria is poorly studied, or not studied at all. In the literature (Ivancheva & Stantcheva, 2000; Nedelcheva et al., 2010) very frequently is used the very general name *Thymus* spp. *diversa*, and this fact confirms the necessity of a profound study and precise identification of the species. In the review paper on the species of genus *Thymus* on Balkans by Zeljkovic & Maksimovic (2014) there are no data about the species from Bulgaria, with the exception of one note on *Thymus tosevii* and *T. macedonicus* (Kulevanova et al., 2000).

The complexity of genus *Thymus* and difficult identification of the species result in lacking of phytochemical studies at species level. Usually samples of different species are bulked together for the purposes of the pharmaceutical industry, and they are designated as *Thymus* sp., which approach does not take into account the peculiarities in the phytochemical composition of the different species.

The aim of this study was to characterize the essential oil composition of *T. longedentatus* in its natural localities in Bulgaria. This is the first phytochemical investigation of this rare and endemic species.

Figure N° 1

Thymus longedentatus in its natural locality in Eastern Rhodopes Mts., Bulgaria



MATERIAL AND METHODS

Plant material and isolation of essential oil

Aerial parts of *T. longedentatus* (Figure No. 1) were collected in May 2018 in Eastern Rhodopes Mts. (GPS coordinates: 41°39'20.63"N/ 25°50'10.91"E, 352 m a.s.l.). A voucher specimen (SOM – 1380) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.

Essential oil was obtained using Clevenger apparatus by water distillation, from sample of 50 g DW, in a flask with 500 ml water, for 2 h.

Gas chromatography mass spectrometry (GC-MS)

The GC-MS analysis was performed on a Thermo Scientific Focus gas chromatograph coupled with Thermo Scientific dual stage quadrupole (DSQ) mass detector operating in electron ionization (EI) mode at 70 eV. ADB-5MS column (30 m x 0.25 mm x 0.25 μ m) was used. Chromatographic conditions were as follows: Helium as carrier gas at a flow rate of 1 mL/min; injection volume was 1 μ l, and the split ratio was 1:50. Column temperature was 60°C for 10 min, and programmed at the rate of 3°C/min to 200°C, and finally held isothermally for 10 min. The injection port was set at 220°C. Significant quadrupole MS operating parameters: interface temperature 240°C; electron impact ionization at 70 eV with scan mass range of 40 to 400 m/z at a sampling rate of 1.0 scan/s. Quantitative data were obtained from TIC of each component. Retention indices (RI) of the oil components were calculated by using retention times of C₈-C₂₅ n-alkanes under the same chromatographic conditions. The individual components were identified by their MS, and RI referring to known compounds from the literature (Tkachev, 2008; Adams, 2009) and also by comparison with those of NIST 98, WILEY and home-made MS databases.

RESULTS AND DISCUSSION

The essential oil obtained from the aerial parts of *T. longedentatus* was studied by GC-MS. Twenty eight compounds chromatographic peaks, corresponding to components in concentration more than 0.1% were

registered (Table No. 1). The results showed that the main class of compounds was monoterpenoids (92.4%), among which oxygen-containing monoterpenoids (78.7%) were prevailing. The essential oil was characterized by a lemon-like odor due to the prevalence of citral isomers - neral (27.5%) and geranial (30.2%). 1,8-Cineole (7.8%), trans- β -ocimene (7.3%), and β -myrcene (3.7%) were also detected in significant amounts. Sesquiterpenoids (6.3%) were in relatively low concentration of in the studied oil.

Germacrene D (4.4%) was the only one, which was detected in substantial amount. It is worth to mention that aromatic compounds such as thymol and carvacrol have not been detected in our sample even in traces.

According to the literature there are different chemotypes based on the dominant compounds in the essential oils – monoterpenes (geraniol, terpineol, linalool, etc.) and aromatic compounds (thymol and carvacrol) (Thompson *et al.*, 2003; Chizzola *et al.*, 2008; Zeljkovic & Maksimovic, 2014). As a rule, the sesquiterpenes are represented by low concentration in the species of the genus *Thymus* (Stahl-Biskup, 2002), but several sesquiterpenoid chemotypes were detected in representatives of the genus, growing on the Balkan Peninsula (Pavel *et al.*, 2009).

Based on described above results *T. longedentatus* essential oil can be classified as geranial chemotype. Its essential oil content is similar to that reported for *T. pannonicus* from Serbia (Zeljkovic & Maksimovic, 2014), whose major components are geranial (41.42%) and neral (29.61%). Other closely related species is *T. sipyleus* spp. *sipyleus* from Turkey with major compounds geranial (36.97%) and neral (25.56%), too (Baser *et al.*, 1995a; Pluhár *et al.*, 2007). Other species with geranial and geraniol chemotypes are listed in Table No. 2.

The high content of citral isomers in the essential oil of *T. longedentatus* is a prerequisite for antioxidant (Bakkali *et al.*, 2008), antimicrobial (Onawunmi, 2008; Narasimhan *et al.*, 2011), antifungal (Miron *et al.*, 2014) and antitumor activities (Ghosh, 2013; Zeng *et al.*, 2015).

Table No. 1
Volatile components from *Thymus longedentatus*

RI*	RI**	Compound	%
932	930	α -Pinene	0.76
946	948	Camphene	0.82
969	971	Sabinene	0.33
974	973	β -Pinene	0.38
979	978	1-Octen-3-ol	0.4
988	988	β -Myrcene	3.68
1026	1028	1,8-Cineole	7.77
1032	1035	<i>cis</i> - β -Ocimene	0.42
1044	1046	<i>trans</i> - β -Ocimene	7.32
1065	1068	<i>cis</i> -Sabinene hydrate	0.68
1095	1095	Linalool	1.01
1141	1143	Camphor	2.12
1155	1157	Isoborneol	0.48
1160	1162	Isocitral	2.16
1174	1175	Terpinen-4-ol	0.17
1186	1188	α -Terpineol	0.76
1227	1229	Nerol	1.6
1235	1237	Neral	27.47
1249	1250	Geraniol	0.45
1264	1265	Geranial	30.25
1359	1359	Neryl acetate	1.81
1379	1380	Geranyl acetate	1.97
1387	1388	β -Bourbonene	0.25
1440	1442	<i>cis</i> - β -Farnesene	0.56
1480	1480	Germacrene D	4.43
1574	1576	Germacrene D-4-ol	0.72
1652	1653	α -Cadinol	0.38
		Total	99.15

RI* Literature retention indices; RI** Experimentally determined retention indices to C8–C30 n-alkanes on HP-5MS column

Table No. 2
***Thymus* species with geranial and geraniol chemotype.**

<i>Thymus</i> species	Country	Major constituents	References
<i>Thymus longedentatus</i>	Bulgaria	Geranial (30.25%), neral (27.47%), eucalyptol (7.77%), <i>trans</i> - β -ocimene (7.32%), germacrene D (4.43%), β -myrcene (3.68%)	this study
<i>Thymus longicaulis</i>	Greece	geraniol (56.8%), geranyl acetate (7.6%), nerol (6.3%)	Tzakou <i>et al.</i> , 1998; Zeljkovic & Maksimovic, 2014
<i>Thymus macedonicus</i> spp. <i>macedonucus</i>	Macedonia	geraniol (43.26%), geranyl acetate (37.61%), linalool (9.98%)	Kulevanova <i>et al.</i> , 1999; Zeljkovic & Maksimovic, 2014
<i>Thymus pannonicus</i>	Serbia	geranial (41.42%), neral (29.61%)	Maksimović <i>et al.</i> , 2008; Zeljkovic & Maksimovic, 2014

<i>Thymus sibthorpii</i>	Greece	geraniol (30.09%), linalool (22.95%), citronellyl acetate (9.89%)	Katsiotis <i>et al.</i> , 1990
<i>Thymus sipyleus</i> <i>spp. sipyleus</i>	Turkey	geranial (36.97%), neral (25.56%)	Baser <i>et al.</i> , 1995a; Tumen <i>et al.</i> , 1995
<i>Thymus praecox</i>	Hungary	geraniol (18-23%), germacrene D (14-16%), β -caryophyllene (3.7-12.2%)	Pluhár <i>et al.</i> , 2007
<i>Thymus pulegioides</i>	Lithuania	geraniol (27%), geranial (19.5%), β -caryophyllene (6%), neral (8.8%), nerol (8.5%)	Mockute & Bernotiene, 1999
<i>Thymus thracicus</i>	Turkey	geraniol (47.3%), geranyl acetate (18.25%), camphor (3.6%)	Tumen <i>et al.</i> , 1995; Baser <i>et al.</i> , 1995b

CONCLUSION

The chemical composition of the volatile extracts of *T. longedentatus* could be an important source for the manufacture of pharmaceutical, cosmetic, flavoring and pesticide industries.

ACKNOWLEDGEMENTS

This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, Project DN 16/3.

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