UNIVERSIDAD DE SANTIAGO DE CHILE

BOLETÍN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
18 (2): 197 - 203 (2019)
© / ISSN 0717 7917 / www.blacpma.usach.cl

Blacpma

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]

Ina Aneva[1], Antoaneta Trendafilova[2], Milena Nikolova[1], Milka Todorova[2] & Kristina Georgieva[1,2]

[1]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria
Contactos | Contacts: **Ina ANEVA** - ***E-mail address:*** **ina.aneva@abv.bg**

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 concencentration of oxygentated 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 ammounts. 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-mirceno (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).

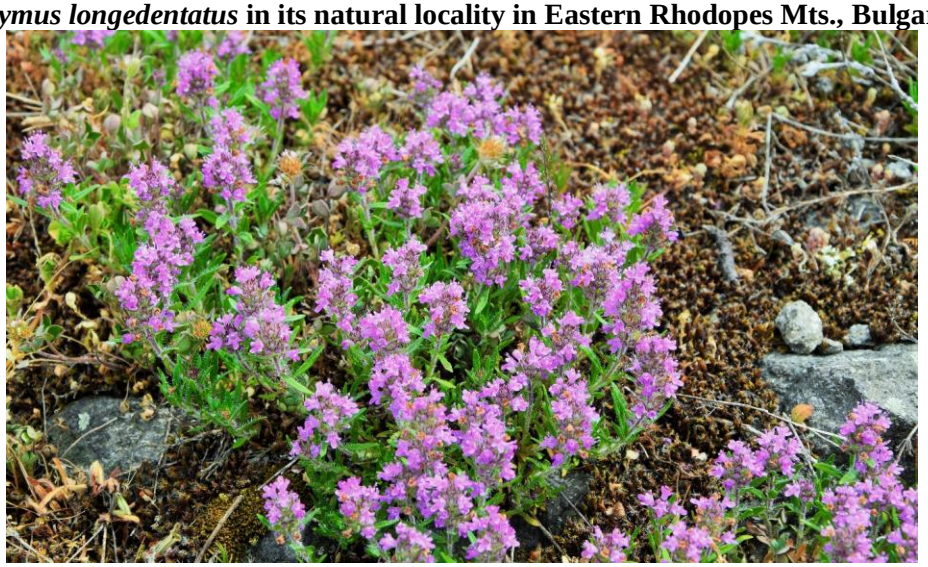
Very typical phenomenon for the genus is the chemical polymorphism, which is due both to the ecological factors, and to genetic variation (Ložienė & Venskutonis, 2005; Rădulescu *et al*., 2009). Many of the species have more than 7 chemotypes (Stahl-Biskup, 2002).

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 Nº 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 μm) was used. Chromatographic conditions were as follows: Helium as carrier gas at a flow rate of 1 mL/min; injection volume was 1 μl, 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 C_8-C_{25} 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	*cis*-β-Ocimene	0.42
1044	1046	*trans*-β-Ocimene	7.32
1065	1068	*cis*-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	*cis*-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.**

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

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

REFERENCES

Adams RP. 2009. **Identification of essential oil components by gas chromatography/mass spectorscopy**, 4th edn, Allured Business Media, Carol Stream, Illinois, USA.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils. **Food Chem Toxicol** 46: 446 - 475.

Baser KHC, Ozek T, Tumen G, 1992. Essential oils of *Thymus cariensis* and *Thymus haussknechtii*, two endemic species in Turkey. **J Essent Oil Res** 4: 659 - 661.

Baser KHC, Kurkcuoglu M, Ozek T, Tumen G, Akgul A. 1995a. Essential oil of *Thymus sipyleus* Boiss. subsp. *sipyleus* var. *Sipyleus.* **J Essent Oil Res** 7: 411 - 413.

Baser K, Ozek T, Kurkcuoglu M, Tumen G. 1995b. Essential oil of *Thymus thracicus* Velen var. *longidens* (Velen) Jalas. **J Essent Oil Res** 7: 661 - 662.

Baser KHC, Kirimer N, Tumen G, Duman H. 1998. Composition of the essential oils of *Thymus canaoviridis* Jalas. **J Essent Oil Res** 10: 199 - 200.

Cavar S, Maksimovic M, Vidic D. 2009. The essential oil of *Thymus aeropunctatus* (Beck) K. Maly. **Nat Prod Comm** 4: 415 - 420.

Chizzola R, Michitsch H, Franz C. 2008. Antioxidative properties of *Thymus vulgaris* leaves: comparison of different extracts and essential oil chemotypes. **J Agric Food Chem** 56: 6897 - 6904.

Ghosh K. 2013. Anticancer effect of lemongrass oil and citral on cervical cancer cell lines. **Pharmacogn Comm** 3: 41 - 48.

Guillen MD, Manzanos MJ. 1998. Study of composition of different parts of a Spanish *Thymus vulgaris* L. plant. **Food Chem** 3: 373 - 383.

Ivancheva S, Stantcheva B. 2000. Ethnobotanical inventory of medicinal plants in Bulgaria. **J Ethnopharmacol** 69: 165 - 172.

Jukic M, Miloc M. 2005. Catalytic oxidation and antioxidant properties of Thyme essential oils (*Thymus vulgarae* L.). **Croatica Chem Acta** 78: 105 - 110.

Katsiotis S, Chatzopoulou P, Svendsen A. 1990. The essential oil of Thymus sibthorpii Ben T. growing wild in Greece. **Scientia Pharmaceut** 58: 303 - 306.

Kulevanova S, Ristic M, Stafilov T. 1999. The essential oil of *Thymus macedonicus* subsp. *macedonicus* (Deg. et Urum.) Ronn. from Macedonia. **Herba Polonica** 45: 80 - 86.

Kulevanova S, Stoeva T, Ristić M. 2000. The essential oil composition of *Thymus tosevii* and *Thymus macedonicus* from Bulgaria. **Boll Chim Farmaceut** 139: 85 - 88.

Ložeine K, Vauciunine J, Venskutonis P. 1998. Chemical composition of the essential oil of creeping thyme (*Thymus serpyllum* L.) growing wild in Lithuania. **Planta Medica** 64: 772 - 773.

Ložienė L, Venskutonis PR. 2005. Influence of environmental and genetic factors on the stability of essential oil composition of *Thymus pulegioides*. **Biochem Syst Ecol** 33: 517 - 525.

Maksimović Z, Milenković M, Vučićević D, Ristić M. 2008. Chemical composition and antimicrobial activity of *Thymus pannonicus* All. (Lamiaceae) essential oil. **Central Eur J Biol** 3: 149 - 154.

Miron D, Battisti F, Silva F, Lana A, Pippi B, Casanova B, Gnoatto S, Fuentefri A, Mayorga P, Schapoval E. 2014. Antifungal activity and mechanism of action of monoterpenes against dermatophytes and yeasts. **Rev Bras Farmacogn** 24: 660 - 667.

Mockute D, Bernotiene G. 1999. The main citral-geraniol and carvacrol chemotypes of the essential oil of *Thymus pulegioides* L. growing wild in Vilnius District (Lithuania), **J Agric Food Chem** 47: 3787 - 3790.

Narasimhan S, Ravi S, Vijayakumar B. 2011. Synthesis and antibacterial activities of citral derivatives. **Int J Chem Appl** 3: 229 - 235.

Nedelcheva A, Pavlova D, Krasteva I. 2010. Medicinal plants biodiversity and their resources of one serpentine site in the Rhodope Mts (Bulgaria). **Natura Montenegrina** 9: 373 - 387.

Onawunmi G. 2008. Evaluation of the antimicrobial activity of citral. **Lett Appl Microbiol** 9:105 - 108.

Papageorgio V. 1980. GLC-MS computer analysis of the essential oil of *Thymus capitatus*. **Planta Medica** 29 - 33.

Pavel M, Radulescu V, Ilies DC. 2009. GC-MS analysis of essential oil obtained from the species *Thymus comosus* Heuff. ex Griseb. (Lamiaceae). **Farmacia** 57: 479 - 484.

Pluhár Z, Héthelyi É, Kutta G, Kamondy L. 2007. Evaluation of environmental factors influencing essential oil quality of *Thymus pannonicus* All. and *Thymus praecox* Opiz**. J Herbs, Spices Med Plant** 13: 23 - 43.

Rădulescu V, Pavel M, Teodor A, Tănase A, Ilieş DC. 2009. Analysis of essential compounds from infusion and hydrodistillate obtained from the species *Thymus pulegioides* L. (Lamiaceae). **Farmacia** 57: 282 - 289.

Rasooli I, Mirmostafa SA. 2002. Antibacterial properties of *Thymus pubescens* and *Thymus serpyllum* essential oils. **Fitoterapia** 73: 244 - 250.

Rustaiyan A, Masoudi S, Monfared A, Kamalinejad M, Lajevardi T, Sedaghat S, Yari M. 2000. Essential constituents of three *Thymus* species grown wild in Iran. **Planta Medica** 66: 197 - 198.

Sáez F. 1998. Variability in essential oils from populations of *Thymus hyemalis* Lange in southeasten Spain. **J Herbs Spices Med Plants** 5: 65 - 76.

Sáez F. 2001. Essential oil variability in *Thymus serpylloides* ssp. *gadorensis* growing wild in Southeastern Spain. **Biochem Syst Ecol** 29: 189 - 198.

Soliman KM, Badeaa RI. 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. **Food Chem Toxicol** 40: 1669 - 1675.

Stahl-Biskup E. 2002**. Essential oil polymorphism in the genus *Thymus***. In Stahl-Biskup E, Sáez F, Eds. Thyme: The genus Thymus, Taylor & Francis, London, UK.

Tepe B, Daferera D, Sokmen M, Polissiou M, Sokmen A. 2004. In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigii* M. Zohary et P.H. Davis. **J Agric Food Chem** 52: 1132 - 1137.

Thompson JD, Chalchat JC, Michet A, Linhart YB, Ehlers B. 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotype. **J Chem Ecol** 29: 859 - 880.

Tkachev A. 2008. **Issledovanie letuchikh veshchestv rastenii** (Study of volatile substances of plants), offset. Novosibirsk.

Tumen G, Kirimer N, Baser K. 1995. Composition of the essential oils of *Thymus* species growing in Turkey. **Chem Nat Comp** 31: 42 - 46.

Tumen G, Koyuncu M, Krimer N, Baser KHC. 1998. Composition of the essential oil of *Thymus cilicicus* Boiss. & Bal. **J Essent Oil Res** 6: 97 - 98.

Tzakou O, Verykokidou E, Roussis V, Chinou I. 1998. Chemical composition and antibacterial properties of *Thymus longicaulis* subsp. *chaoubardii* oils: three chemotypes in the same population, **J Essent Oil Res** 10: 97 - 99.

Vidic D, Cavar S, Solić ME, Maksimović M. 2010. Essential constituents of two rare subspecies of *Thymus praecox*. **Nat Prod Comm** 5: 1123 - 1126.

Vila R, Freixa B, Cañigueral S, Adzet T, Tomas X, Molins J. 1995. Composition and study of the variability of the essential oil and *Thymus funkii* Cosson. **Flav Frag J** 10: 379 - 383.

Zarzuelo A, Crespo E. 2002. **Themedicinal and non-medicinal uses of thyme**. In Thyme: The Genus *Thymus:* Medicinal and Aromatic Plants-Industrial Profiles. E. Stahl-Biskup, F Saez Eds. Taylor & Francis, New York, USA.

Zeljkovic S, Maksimovic M. 2014. Chemical composition and bioactivity of essential oil from *Thymus* species in Balkan Peninsula. **Phytochem Rev** 14: 1 - 18.

Zeng S, Kapur A, Patankar M, Xiong M. 2015. Formulation, characterization, and antitumor properties of Trans- and Cis-citral in the 4T1 breast cancer Xenograft mouse model. **Pharm Res** 32: 2548 - 2558.