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Original research paper

ESSENTIAL OIL COMPOSITION OF THE *Thymus serpyllum* L. FROM KOPAONIK MOUNTAIN

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ABSTRACT: *Thymus serpyllum* L. also known as wild or creeping thyme, as well as mother of thyme is a perennial plant from Lamiaceae family indigenous to Europe, especially the Mediterranean region. According to European Pharmacopoeia, dried flowering aerial parts of *T. serpyllum* (*Serpylli herba*), whole or cut, are registered as an official drug. However, it is used in traditional medicine mainly for treating illnesses and problems related to the respiratory and gastrointestinal systems. A total of 88 compounds were isolated from *T. serpyllum* essential oil, among which the dominant were *trans*-caryophyllene with 33.3%, followed by germacrene D (11.5%), *α*-humulene (9.8%), *trans*-*β*-farnesene (6.3%), *α*-pinene (5.3%), myrcene (3.8%), *δ*-cadinene (2.9%), NI (2.9%) and α-farnesene (2.3%). By comparing obtained results and the results from literature, it can be said that *T. serpyllum* from Kopaonik belongs to *trans*-caryophyllene chemotype.

Key words: *wild thyme, GC-MS analysis, trans-caryophyllene.*

INTRODUCTION

Thymus serpyllum L. also known as wild or creeping thyme, as well as mother of thyme is perennial plant from Lamiaceae family indigenous to Europe, especially the Mediterranean region. It is a small bushy herb. The stem is branched, up to about 1.5 mm in diameter, cylindrical or indistinctly quadrangular, green, reddish or purplish, the older stems brown and woody, the younger stems pubescent. The leaves are opposite, 3mm to 12 mm long up to 4 mm wide, elliptical to ovate-lanceolate with an obtuse apex, cuneate and shortly petiolate at the base; the margin is entre and markedly ciliate, especially near the base. Both surfaces are more or less glabrous but distinctly punctuate. The inflorescence is composed of about 6 to 12 flowers in rounded to ovoid terminal heads. The calyx is tubular, two-lipped with the upper lip dividing to form 3 teeth, the lower lip with 2 teeth, edged with long hairs; inner surfaces strongly pubescent, the hairs forming a closed tube after flowering. The corolla is purplish-violet to red, two-lipped, the lower lip with 3 lobes, upper lip notched, inner surface strongly pubescent. There are four stamens, epipetalous, projecting from the corolla tube (Ph Eur, 2010).

According to European Pharmacopoeia, dried flowering aerial parts of *T. serpyllum* (*Serpylli herba*), whole or cut, are registered as an official drug. However, it is used in traditional medicine mainly for treating illnesses and problems related to the respiratory and gastrointestinal systems. Recently, their essential oil is becoming more popular as an important plant derived product, as well as a natural resource for the pharmaceutical industry because of its antioxidative, antimicrobial, anticancerogenic and antihypertensive activities (Mihailovic-Stanojevic et al., 2013; Petrović et al., 2014; Nikolić et al., 2014; Wesolowska et al., 2015). In addition, it can be a source of natural antioxidants, nutritional supplements, or components of functional foods in the food industry (Jarić et al., 2015).

This paper shows the chemical composition of wild *T. serpyllum* essential oil, collected from Kopanik slopes, Brzeće region, in Serbia. Furthermore, comparative analysis of *T. serpyllum* essential oil from Kopanik Mountain (Belo Brdo region) has been given (Stanisavljević et al., 2012).

MATERIAL AND METHODS

Plant material

Aerial parts of *T. serpyllum* were collected in the full flowering stage from natural sites in Kopaonik (Brzeće, around 1193 m a.s.l.) in June 2018.

Extraction of essential oil

The dried samples of *T. serpyllum* were subjected to hydro-distillation using an all glass Clevenger-type apparatus to extract essential oils according to the method outlined by the European Pharmacopoeia (2010). The samples were ground, homogenized and made into a fine powder. In order to extract the essential oils, 100 g of the powder was placed in 1 l conical flask and connected to the Clevenger apparatus. Distilled water in amount of 500 ml was added to the flask and heated to the boiling point. The steam in combination with the essential oils was distilled into a graduated cylinder for 4 h and then separated from aqueous layer. The oil was kept refrigerated until required for further analysis.

GC/MS analysis

Gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionization detector (GC-FID) analysis (EI) were performed using an Agilent 7890A GC system equipped with a 5975C inert XL EI/CI MSD and a FID detector connected by capillary flow technology (Agilent Technologies, Santa Clara, California, USA). The separation was achieved using an Agilent HP-5MSI fused silica capillary column HP-5, 5% phenyl methyl siloxane (30 m × 0.25 mm i.d., 0.25 μm film thickness). GC oven temperature was programmed from 60°C to 285°C at a rate of 4°C/min. Helium was used as a carrier gas; inlet pressure was 25 kPa; linear velocity: 1 ml/min at 210°C. Injector temperature: 250°C. Injection mode: splitless. MS scan conditions: source temperature 200°C; interface temperature 250°C; EI energy 70 eV; mass scan range 40–550 amu. Identification of the EOs components was carried out on the basis of retention indices and the comparison with reference spectra (Wiley and NIST databases). The quantitative data are obtained from the electronic integration of the GC-FID peak areas.

RESULTS AND DISCUSSION

A total of 88 compounds were isolated from *T. serpyllum* essential oil, among which the dominant were *trans*-caryophyllene with 33.3%, followed by germacrene D (11.5%), *α*-humulene (9.8%), *trans*-*β*-farnesene (6.3%), *α*-pinene (5.3%), myrcene (3.8%), *δ*-cadinene (2.9%), NI (2.9%) and α-farnesene (2.3%). All compounds isolated from essential oil are listed in Table 1. A typical CG-FID chromatogram is shown in picture 1.
Previous GC/MS analysis of *T. serpyllum* essential oil from Kopaonik (Belo Brdo) registered 26 compounds (98.4% of total oil). The dominant compound was *trans*-caryophyllene (27.7%), followed by *γ*-muurolene (10.5%), *α*-humulene (7.5%), *β*-bisabolol (2.7%) and *trans*-nerolidol (2.4%) (Stanisavljević et al., 2012).

Table 1. Chemical composition of *T. serpyllum* essential oil

No	Compound#	R.T.	RI	%	Stanisavljević et al., 2012
1	tricyclene	5.536	923	tr	-
2	*α*-thujene	5.629	926	0.1	-
3	*α*-pinene	5.818	933	5.3	6.9
4	camphene	6.209	947	1.0	1.0
5	sabinene	6.888	972	tr	-
6	*β*-pinene	6.994	976	0.3	1.8
7	myrcene	7.365	989	3.8	-
	3-octanone	-	*988,8	-	6.6
8	3-octanol	7.488	995	0.1	-
9	*α*-phellandrene	7.824	1005	0.1	0.5
10	*α*-terpinene	8.227	1016	0.1	-
11	*p*-cymene	8.491	1026	0.1	2.0
12	limonene	8.634	1027	1.8	2.7
	1,8-cineole	-	*1030,7	-	2.5
13	*cis*-*β*-ocimene	8.925	1035	0.3	0.7
14	*trans*-*β*-ocimene	9.305	1046	0.2	1.5
15	*γ*-terpinene	9.708	1056	1.8	1.4
16	terpinolene	10.848	1088	0.1	-
17	linalool	11.288	1100	tr	1.2
18	camphor	13.082	1141	0.8	3.6
19	borneol	14.012	1163	0.1	-
20	terpinen-4-ol	14.535	1175	tr	-
21	thymol, methyl ether	17.045	1232	0.1	5.6
22	brnyl acetate	19.280	1283	0.6	-
23	*δ*-elemene	21.565	1335	tr	-
24	*α*-cubebene	22.117	1347	tr	-
25	*α*-copaene	23.266	1373	0.2	-
26	*β*-bourbonene	23.661	1382	0.5	-
27	*β*-cubebene	23.895	1387	0.1	-
28	*β*-elemene	23.986	1390	0.5	-
29	*cis*-caryophyllene	24.627	1405	0.1	-
30	methylindanone	25.005	1413	0.3	-

31	*trans*-caryophyllene	25.337	1420	33.3	27.7
32	*β*-copaene	25.585	1427	0.2	-
33	*cis*-thujopsene	25.665	1429	0.2	-
34	*trans*-*α*-bergamotene	25.866	1434	0.1	-
35	6,9-guaiadiene	26.227	1443	0.1	-
36	*α*-humulene	26.696	1454	9.8	7.5
37	*trans*-*β*-farnesene	26.824	1457	6.3	-
38	*γ*-muurolene	27.668	1477	0.3	10.5
39	germacrene D	27.893	1482	11.5	-
40	*trans*-muurola-4(14),5-diene	28.262	1491	0.1	-
41	bicyclogermacrene	28.460	1496	1.4	-
42	*α*-muurolene	28.607	1499	0.2	1.6
	β-bisabolene	-	*1509,8	-	1.9
43	germacrene A	28.808	1504	0.2	-
44	*α*-farnesene	28.939	1507	2.3	-
45	*γ*-cadinene	29.177	1513	0.3	-
46	*δ*-cadinene	29.586	1523	2.9	1.3
47	NI	29.923	1531	0.1	-
48	*α*-cadinene	30.144	1536	0.1	-
49	elemol	30.614	1548	tr	-
50	*trans*-nerolidol	31.153	1561	0.1	2.4
51	NI	31.357	1566	0.1	-
52	Germacrene D-4-ol	31.684	1574	0.1	1.1
53	spathulenol	31.771	1575	0.3	-
54	caryophyllene oxide	31.984	1581	1.7	1.3
55	NI	32.235	1587	0.1	-
56	NI	32.604	1596	0.1	-
57	humulene epoxide II	33.017	1606	0.4	-
58	NI	33.128	1609	0.1	-
59	10-epi-*γ*-eudesmol	33.437	1617	0.2	-
60	NI	33.524	1620	0.2	-
61	1-epi-cubenol	33.788	1625	0.1	-
62	NI	33.932	1629	0.2	-
63	NI	34.082	1634	0.3	-
64	*epi*-*α*-cadinole	34.304	1640	0.6	1.1
65	*α*-muurolol (=torreyol)	34.471	1644	0.1	-
66	*β*-eudesmol	34.614	1648	0.1	-
67	*α*-cadinol	34.796	1652	0.9	1.6
68	NI	34.922	1656	0.1	-
69	NI	35.234	1664	2.9	-
70	NI	35.421	1669	0.2	-
	β-bisabolol	-	*1666,9	-	2.6
71	germacra-4(15),5,10(14)-trien-1-α-ol	35.996	1684	0.4	-
72	shyobunol	36.162	1688	0.2	-
73	heptadecane	36.417	1695	tr	-

74	mint sulfide	37.883	1735	tr	-
75	6,10,14-trimethyl-2-pentadecanone	41.685	1841	0.4	-
76	NI	42.937	1878	0.1	-
77	5-*trans*,9-*trans*-farnesyl acetone	44.280	1917	0.1	-
78	geranyl linalool	47.951	2027	0.3	-
79	NI	49.373	2074	0.1	-
80	NI	49.680	2085	0.1	-
81	phytol	50.604	2116	0.9	-
82	NI	51.497	2146	0.1	-
83	tricosane	56.197	2303	tr	-
84	pentacosane	61.779	2500	0.1	-
85	heptacosane	66.870	2701	0.1	-
86	NI	69.993	2832	0.1	-
87	nonacosane	71.535	2901	0.3	-
88	NI	75.942	3098	0.1	-

#Compounds are listed in order of their elution from a HP-5 column, Rt – Retention time, RI – Retention index on an HP-5 column, experimentally determined using a homologous series of n-alkanes, NI – No identified compound, *Experimental Kovats (Retention) index (AMDIS) (Stanisavljević et al., 2012)

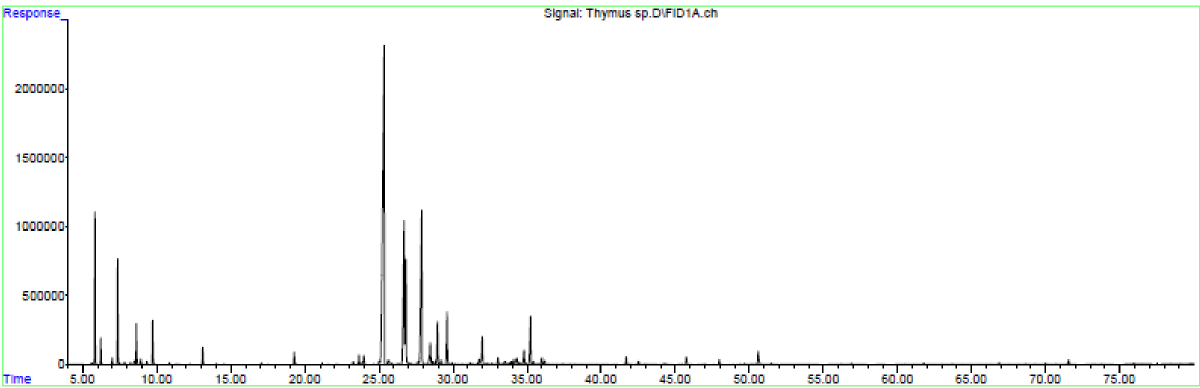

Figure 1. A typical GC-FID chromatogram of *T. serpyllum*

The composition of *T. serpyllum* essential oil is affected by geographic region, the development stage of the plant, the harvest season, habitat and climatic conditions, as well as the extraction technique (Wesolowska et al., 2014; Jarić et al., 2015).

Twenty-six samples of *T. serpyllum* ssp. *serpyllum* were collected from 14 habitats in Lithuania, revealed remarkable intraspecific variations in the oil composition. Five main groups of the plants were observed using hierarchical cluster analysis based on the major essential oil constituents. Consequently, five chemotypes of *T. serpyllum* have been defined, namely 1,8-cineole, germacrene B, trans-β-ocimene, α-cadinol and cis-p-menth-2-en-1-ol (Ložienė and Venskutonis, 2006).

Furthermore, analysis of essential oil composition of *T. serpyllum* originating from 20 different natural places in Estonia, registers 55 components. The main compounds were *trans*-nerolidol, caryophyllene oxide and myrcene (Raal et al., 2004). Similarly, the main components of essential oil of *T. serpyllum* from Serbia (Pasjača Mountain) were: *trans*-

nerolidol (24.2%), germacrene D (16.0%), thymol (7.3%), δ-cadinene (3.7%) and *β*-bisabolene (3.3%) (Petrović et al., 2014).

However, intraspecific variability of *T. serpyllum* occurring in Poland can be classified into three chemotypes: geranyl acetate+β-terpineol+β-myrcene, geranyl acetate+β-terpineol+borneol and pure linalool type (Baczek et al., 2018).

In *T. vulgaris,* seven genetically distinct chemotypes are described that can be distinguished on the basis of the dominant monoterpene produced in the glandular trichomes. It is established that the monoterpene variations in *T. vulgaris* plants may represent an adaptive strategy in relation to the environmental variations. The different chemotypes show a geographic and localized distribution. The phenolic chemotypes (carvacrol and thymol) dominate thyme populations in hot dry sites close to the Mediterranean Sea, whereas the non-phenolic (geraniol, α-terpineol, thuyanol-4 or trans-sabinene hydrate and linalool) chemotypes dominate sites further inland, particularly above 400m elevation, i.e. in wetter, cooler climates. Within the non-phenolic chemotypes, it is the α-terpineol chemotype which is most abundant on the wettest soils. For the two phenolic types, carvacrol is limited to the driest conditions, whereas thymol is less specialized and can occur on moist soils. The phenolic types, particularly carvacrol, are absent on sites which experience sub-freezing temperatures (Thompson et al., 2003).

CONCLUSIONS

Both analyzed samples of *T. serpyllum* from Kopaonik (Brzeće and Belo Brdo) show presence of *trans*-caryophyllene as the main compound with 33.3% and 27.7%, respectively. According to this, it can be concluded that *T. serpyllum* from Kopaonik belongs to the *trans*-caryophyllene chemotype. However, it may be the adaptive strategy of *T. serpyllum* population in relation to the environmental conditions at Kopaonik Mountain.

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