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Chemical Composition and Antimicrobial Activity of *Thymus praecox* Opiz ssp. *polytrichus* Essential Oil from Serbia

Nada V. Petrović[1*], Slobodan S. Petrović[1], Mihailo S. Ristić[2], Ana M. Džamić[3] and Ana D. Ćirić[4]

[1]*Development and Production Center BIOSS – PS and others, Bulevar oslobođenja 401 i, 11000 Belgrade, Serbia*
[2]*Institute for Medicinal Plant Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade,Serbia*
[3]*University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Studentski trg 16, 11000 Belgrade, Serbia*
[4] *Institute for Biological Research "Siniša Stanković", University of Belgrade, Despota Stefana 142, 11000 Belgrade, Serbia*

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Abstract: Chemical composition and antimicrobial activity of the essential oil of wild growing *Thymus praecox* Opiz ssp. *polytrichus* were studied. trans-Nerolidol (19.79%), germacrene D (18.48%) and thymol (9.62%) were the main components in essential oil. This study is the first report of the antimicrobial activity of essential oil obtained from the *T. praecox* Opiz ssp. *polytrichus.* Antimicrobial activity of essential oil was investigated on *Bacillus cereus*, *Micrococcus flavus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Salmonella typhimurium, Aspergillus fumigatus, A. versicolor, A. ochraceus, A. niger, Trichoderma viride, Penicillium funiculosum, P. ochrochloron, and P. verrucosum var. cyclopium* strains. In the antimicrobial assays, essential oil showed high antimicrobial potential (MIC 19–150 µg/mL, MBC 39–300 µg/mL for bacteria; and MIC 19.5–39 µg/mL, MFC 39–78 µg/mL for fungi).

Keywords: *T. praecox* ssp. *polytrichus* (A. Kern. Ex Borbàs) Jalas; essential oil; antimicrobial activity.

1. Plant Source

The genus *Thymus* (Lamiaceae) consists of about 215 species of herbaceous perennials and sub shrubs in the world [1]. In the Flora of Serbia the genus *Thymus* is represented by 31 species with more than 60 varieties, most of which found in the grassland and dry sunny rocky limestone or serpentine habitats [2]. Thyme is a constituent of a large number of herbal remedies with a long list of pharmacological and aromatic properties, such as antiseptic, aromatic, antimicrobial, antioxidant, expectorant, stomachic, antispasmodic, carminative and preservative. The two main bioactive

* Corresponding author: E-Mail: biossnada@gmail.com; Phone:+:+381 11 367 74 50; *Fax:*+381 11 367 74 51

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compounds in thyme essential oil are thymol and carvacrol but thymol is believed to be responsible for most of the therapeutic aspects. Internally, thyme oil is used for cough, bronchitis, catarrh, laryngitis and indigestion. Externally, the whole plant and the oil distilled from it have been used to treat fungal infections, minor arthritis, gum disease, and tonsillitis [3,4].

Thymus praecox Opiz ssp. *polytrichus* was collected at the flowering stage during June in 2012, in the south of Serbia (the mountain Pasjača) and determinated as *T. balcanus* Borb. by Prof. Dr M. Veljić. The voucher specimen (No. 16881) was deposited in Herbarium of the Institute of Botany and Botanical Garden"Jevremovac" of the Faculty of Biology, University of Belgrade, Serbia (BEOU). According to Jalas (1972) *T. balcanus* is described as *T. praecox* Opiz subsp. *polytrichus* (A. Kern. ex Borbás) Jalas, as a species with hairs a two stem sides alternating in each internode (alelotrichous) and distributed in mountains of south and south-central Europe[5].

2. Previous Studies

There have been very few studies on the essential oil components of *T. praecox* ssp. *polytrichus*. The essential oils from plants collected in the Tyrolean Alps, FYROM and Bosnia were studied earlier [5-7]. *T. praecox* ssp. *polytrichus* is a rare species according to the characterization of the IUCN [7].

3. Present Study

The chemical composition of the essential oil of *T. praecox* ssp. *polytrichus* was determined using GC–FID and GC–MS. The antimicrobial activity of essential oil was evaluated against 16 pathogenic microorganisms using microdilution method. Chemical composition of the essential oil of *T. praecox* ssp. *polytrichus* is shown in Table 1. Yield of essential oil obtained by hydrodistillation was 0.36± 0.053% w/w, with 79 components identified. According to literature data the content of essential oil in wild thyme drug varies to a great extent depending on the origin of the plants and ranging between 0.1 and 0.6% [8], or between 0.1 and 1% [9]. The most represented chemical groups in essential oil are sesquiterpene hydrocarbons (43.5%), and oxygenated sesquiturpenes (28.9%). As can be seen from Table 1. oxygen-containing sesquiterpene trans-nerolidol (24.2%) and sesquiterpene hydrocarbon germacrene-D (16.0%) were the main constituent of *T. praecox* ssp. *polytrichus* essential oil. Comparative analysis of the results of the chemical composition of the *T. praecox* ssp. *polytrichus* essential oil obtained in this study with the results from literature data [5-7] suggest a large differences in terms of the main compounds in the essential oil (Table 2.). Our results show similarity with one of 12 different essential oil types of *T. praecox* ssp. *polytrichus* collected in the Tyrolean Alps [5] which was rich in trans-nerolidol (51.8%) and germacrene-D (9%). Variations in the yield and chemical composition of essential oil *T. praecox* ssp. *polytrichus* could be explained in terms of different origin of plant material, quality of herbs, harvest date, influence of different climatic and other factors on the biosynthesis of these secondary metabolites, and different distillation operating conditions [10]. Also, results of this study are in agreement with the fact of highly complex chemical polymorphism of the genus *Thymus* [5,11].

The results of the determination of antibacterial and antifungal activity of essential oil of *T. praecox* ssp. *polytrichus* and thymol are given in Table 3 and Table 4. The essential oil showed strong activity against both type of microorganisms (MIC 19–150 μg/mL, MBC 39–300 μg/mL for bacteria; and MIC 19.5–39 μg/mL, MFC 39–78 μg/mL for fungi). Investigated essential oil showed better antimicrobial activity than commercial antibiotics streptomycin and ampicillin and commercial fungicides bifonazol and ketoconazole against all tested strains. The fungi appear to be more sensitive compared to the bacteria, which could be explained by their different cell organization. Most of the antimicrobial activity in essential oils from *Thymus* genus is likely due to high content of thymol, carvacrol, linalool and p-cymene [12,13]. From the results in this study it could be assumed that thymol is responsible for strong antimicrobial activity, as well as synergist effect among components in the essential oil. Except that, major components in essential oil trans-nerolidol [14] and germacrene-

D [15] could be also responsible for strong antimicrobial activity of essential oil. The results clearly demonstrated pharmacological activity of the specific chemotype of *T. praecox* ssp. *polytrichus* essential oil and its potential for use in the pharmaceutical and food industry as a new antimicrobial agents.

Table 1. Chemical composition of the essential oil of *T. praecox* ssp. *polytrichus.*

Constituents	**KIE**[a]	**KIL**[b]	**EO**	**Constituents**	**KIE**[a]	**KIL**[b]	**EO**
α-Pinene [c,d]	924.9	932	0.3	β-Gurjunene [c,d]	1426.4	1431	0.2
Camphene [c,d]	938.0	946	0.2	6,9-Guaiadiene [c,d]	1432.2	1442	0.1
Sabinene [c,d]	973.1	969	0.1	cis-Muurola-3,5-diene [c,d]	1434.0	1448	0.2
β-Pinene [c,d]	974.0	974	0.5	trans-Muurola-3,5-diene [c,d]	1437.0	1451	0.1
1-Octen-3-ol [c,d]	985.7	974	0.1	α-Humulene [c,d]	1439.8	1452	0.4
Myrcene [c,d]	989.8	988	0.9	allo-Aromadendrene [c,d]	1453.6	1458	0.5
3-Octanol [c,d]	1002.1	988	0.2	(E)-β-Farnesene [c,d]	1457.7	1454	2.7
α-Phellandrene [c,d]	1009.1	1002	0.1	γ-Muurolene [c,d]	1466.0	1478	0.1
α-Terpinene [c,d]	1011.1	1014	0.2	Germacrene D [c,d]	1472.0	1484	18.5
p-Cymene [c,d]	1018.8	1020	1.9	β-Selinene [c,d]	1478.5	1489	0.2
Limonene [c,d]	1021.8	1024	0.5	ar-Curcumene [c,d]	1480.6	1484	0.2
1,8-Cineole [c,d]	1022.7	1026	0.9	epi-Bicyclosesquiphellandrene[c,d]	1481.2	1493	0.3
trans-β-Ocimene[c,d]	1047.1	1044	0.8	Bicyclogermacrene [c,d]	1487.1	1500	0.8
γ-Terpinene [c,d]	1052.4	1054	1.1	α-Muurolene [c,d]	1489.8	1500	0.7
cis-Thujone [c,d]	1099.8	1101	1.5	β-Bisabolene [c,d]	1501.0	1505	5.1
Linalool	1102.6	1095	0.4	trans-Calamenene [c,d]	1510.4	1521	1.1
trans-Thujone [c,d]	1115.0	1112	0.2	δ-Cadinene [c,d]	1512.9	1522	4.7
α-Campholenal [c,d]	1124.9	1122	0.1	Methyl dodecanoate [c,d]	1523.8	1524	0.2
Camphor [c,d]	1134.1	1141	0.6	α-Cadinene [c,d]	1525.8	1537	0.3
Borneol [c,d]	1159.6	1165	0.5	cis-Sesquisabinene hydrate [c,d]	1536.9	1542	0.4
Menthol [c,d]	1170.3	1167	0.1	Elemol [c,d]	1543.1	1548	0.2
Terpinen-4-ol	1172.4	1174	0.3	α-Calacorene [c,d]	1552.5	1544	0.2
α-Terpineol [c,d]	1187.9	1186	0.4	trans-Nerolidol [c,d]	1560.5	1561	19.8
Thymol methyl ether [c,d]	1232.9	1232	0.2	Caryophyllene oxide [c,d]	1569.6	1582	1.0
Carvacrol methyl ether[c,d]	1242.8	1241	0.5	Viridiflorol [c,d]	1577.8	1592	0.8
Thymoquinone [c,d]	1246.7	1248	0.2	β-Copaen-4-α-ol [c,d]	1583.4	1590	0.2
Geraniol [c,d]	1263.0	1257	2.1	Humulene epoxide II [c,d]	1594.7	1608	0.3
Geranial [c,d]	1274.7	1264	0.3	β-Oplopenone [c,d]	1596.5	1607	0.3
Bornyl acetate [c,d]	1279.1	1287	0.1	1,10-di-epi-Cubenol [c,d]	1602.2	1618	0.4
Thymol [c,d]	1303.9	1289	9.6	epi-α-Cadinol (τ-Cadinol) [c,d]	1630.0	1638	1.2
Carvacrol [c,d]	1313.3	1298	0.7	α-Muurolol (Torreyol) [c,d]	1636.1	1644	0.4
α-Cubebene [c,d]	1341.2	1345	0.1	Ledene oxide II**	1640.7	n/a	0.3
Terpinyl acetate [c,d]	1343.9	1346	1.0	α-Cadinol [c,d]	1643.6	1652	1.5
α-Ylangene [c,d]	1360.5	1373	0.1	Helifolenol A [c,d]	1674.7	1674	0.3
α-Copaene [c,d]	1363.8	1374	0.4	Germacra-4(15),5,10(14)-trien-1-α-ol [c,d]	1681.3	1685	0.8
β-Bourbonene [c,d]	1371.4	1387	1.0	(2E,6E)-Farnesol [c,d]	1751.7	1742	0.1
β-Cubebene [c,d]	1379.2	1387	1.1	Hexahydrofarnesyl acetone [c,d]	1848.3	1848	0.9
β-Elemene [c,d]	1382.0	1389	0.6	Manool [c,d]	2044.1	2056	0.2
β-Caryophyllene [c,d]	1408.3	1417	3.4	Oleic acid [c,d]	2108.1	2112	0.2
β-Copaene [c,d]	1415.5	1430	0.5	**Total**			99.7

* Legend:
[a] KI_E = Kovats index, experimentally determined by calibrated AMDIS (uncorrected).
[b] KI_L = Kovats index, value taken from the literature [16]
[c] = Identified by MS.
[d] = Identified by KI.
[**] = Tentative identification.
EO = Essential oil obtained by hydrodistillation.

Table 2. Comparative analysis of the chemical composition of the essential oil of *T. praecox* ssp. *polytrichus.*

Literature	Results in this study	Vidic et al., 1999 [7]	Kulevanova et al., 1998 [6]	Bischof-Deichnik et al., 2000 [5]
Isolation of the essential oil	*Hydrodistillation -Clevenger*	*Hydrodistillation -Clevenger*	*Hydrodistillation -Clevenger*	*Hydrodistillation -Karlsruher*
Origin	*Serbia*	*Bosnia*	*FYROM*	*Austria*
Monoterpene hydrocarbons	6.7%	0.5%		Very polymorphous, 12 essential oil chemotypes: thymol type, geraniol /geranyl acetate type, trans-sabinene hydrate/terpinen-4-ol, trans-nerolidol type, hedycaryol type, germacra-1(10),4-dien-6-ol, germacra-1(10),5-dien-4-ol, linalool type, τ-cadinol type, α-terpineol type, linalool/linalyl acetate type, borneol type
Oxygenated monoterpenes	19.5%	29.9%		
Sesquiterpene hydrocarbons	43.5%	24.2%		
Oxygenated sesquiterpenes	28.9	38.7%		
Other compounds + unidentified	1.4%	1.9%		
Main compounds	trans-Nerolidol (19.8%), Germacrene D (18.5%), Thymol (9.6%), β-Bisabolene (5.1%), δ-Cadinene (4.7%), β-Caryophyllene (3.2%)	Linalool (13.9%), Nerolidol (10.4%)	Main components: trans-Caryophyllene (22.4%), β-Pinene (12.5%), α-pinene (5.6%)	
The total number of identified compounds	79	73		
Total identified	99.7%	95.2%		

Table 3.Antibacterial activity of the essential oil of *T. praecox* ssp. *polytrichus* (MIC and MBC / μg/mL)

	Bacterial strain		**EO**	**Thymol**	**Streptomycin**	**Ampicillin**
			MIC MBC	MIC MBC	MIC MBC	MIC MBC
Gram (+)	*Bacillus cereus*	MIC	28 ± 1.00^{a}	25 ± 0.00^{a}	100 ± 0.67^{c}	300 ± 1.67^{d}
		MBC	39 ± 0.33^{a}	50 ± 0.33^{b}	200 ± 1.00^{d}	400 ± 0.00^{e}
	Micrococcus flavus	MIC	150 ± 1.67^{c}	25 ± 0.67^{a}	200 ± 2.67^{c}	300 ± 1.33^{d}
		MBC	300 ± 3.33^{c}	50 ± 0.67^{a}	300 ± 2.67^{c}	400 ± 1.33^{d}
	Staphylococcus aureus	MIC	19 ± 0.33^{a}	25 ± 1.67^{b}	50 ± 0.67^{c}	300 ± 0.67^{c}
		MBC	39 ± 1.33^{a}	50 ± 0.00^{b}	100 ± 1.33^{c}	400 ± 1.33^{e}
	Listeria monocytogenes	MIC	100 ± 0.00^{a}	100 ± 1.00^{a}	200 ± 1.33^{b}	400 ± 0.67^{c}
		MBC	150 ± 1.67^{b}	100 ± 2.00^{a}	300 ± 0.67^{c}	500 ± 3.33^{d}
Gram (-)	*Escherichia coli*	MIC	28 ± 0.67^{a}	100 ± 0.00^{c}	200 ± 5.00^{d}	300 ± 0.67^{e}
		MBC	39 ± 1.00^{a}	150 ± 0.67^{b}	300 ± 0.00^{c}	500 ± 1.67^{d}
	Pseudomonas aeruginosa	MIC	78 ± 0.67^{a}	100 ± 1.00^{b}	200 ± 1.33^{c}	800 ± 1.33^{d}
		MBC	150 ± 1.67^{a}	150 ± 1.67^{a}	300 ± 1.33^{b}	1250 ± 1.67^{c}
	Enterobacter cloacae	MIC	19 ± 1.33^{a}	50 ± 1.67^{b}	300 ± 0.00^{d}	400 ± 4.33^{e}
		MBC	39 ± 1.33^{a}	100 ± 1.33^{b}	500 ± 3.67^{d}	800 ± 1.33^{e}
	Salmonella typhimurium	MIC	19 ± 1.00^{a}	50 ± 0.67^{b}	200 ± 3.67^{c}	300 ± 1.00^{d}
		MBC	39 ± 1.00^{a}	100 ± 1.33^{c}	300 ± 1.67^{d}	500 ± 2.00^{e}

Table 4. Antifungal activity of the essential oil of *T. praecox* ssp. *polytrichus* (MIC and MBC / µg/mL).

Fungi	EO	Thymol	Bifonazol	Ketoconazole
	MIC MFC	MIC MFC	MIC MFC	MIC MFC
Aspergillus fumigatus	19.5 ± 0.17^{a}	25 ± 0.67^{b}	150 ± 0.00^{c}	200 ± 1.33^{d}
	39 ± 1.00^{a}	500 ± 1.33^{d}	200 ± 1.33^{b}	500 ± 0.00^{d}
A. versicolor	19.5 ± 0.17^{a}	10 ± 0.67^{a}	100 ± 1.33^{c}	500 ± 3.33^{d}
	39 ± 0.33^{b}	10 ± 0.67^{a}	200 ± 2.33^{d}	1000 ± 5.00^{e}
A. ochraceus	19.5 ± 0.67^{a}	10 ± 1.00^{a}	150 ± 1.33^{b}	2500 ± 6.67^{c}
	78 ± 1.00^{c}	20 ± 1.33^{a}	200 ± 2.67^{d}	3000 ± 0.00^{e}
A. niger	39 ± 0.33^{b}	10 ± 0.00^{a}	150 ± 2.67^{d}	200 ± 1.33^{e}
	78 ± 0.33^{b}	20 ± 0.67^{a}	200 ± 1.33^{d}	500 ± 3.33^{e}
Trichoderma viride	39 ± 0.33^{b}	10 ± 0.67^{a}	150 ± 0.67^{c}	2500 ± 6.67^{d}
	78 ± 1.00^{c}	10 ± 1.00^{a}	200 ± 1.33^{d}	2500 ± 3.33^{e}
Penicillium funiculosum	39 ± 0.00^{b}	10 ± 0.67^{a}	200 ± 1.33^{c}	200 ± 2.67^{c}
	78 ± 0.33^{b}	25 ± 1.67^{a}	250 ± 0.00^{d}	500 ± 3.33^{e}
P. ochrochloron	39 ± 1.33^{b}	25 ± 1.00^{a}	200 ± 5.00^{c}	1500 ± 5.00^{d}
	78 ± 1.00^{b}	25 ± 1.00^{a}	250 ± 1.67^{c}	2000 ± 2.67^{d}
P. verrucosum var.	19.5 ± 0.17^{ab}	10 ± 1.00^{a}	200 ± 1.33^{c}	1500 ± 2.67^{d}
cyclopium	39 ± 1.33^{ab}	25 ± 0.00^{a}	300 ± 2.67^{c}	2000 ± 3.33^{d}

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP/

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