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POPULATION VARIABILITY IN *THYMUS GLABRESCENS* WILLD. FROM SERBIA: MORPHOLOGY, ANATOMY AND ESSENTIAL OIL COMPOSITION

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Abstract — In five indigenous populations of *Thymus glabrescens* Willd. collected in the region of Banat (Serbia), the variability on leaf morphological traits, leaf and stem anatomy, and composition of the essential oil was studied. The major component in the studied populations was either thymol or γ-terpinene. Distinct differentiation of populations with respect to chemical composition of essential oils might be related to spatial distribution of the studied populations. No correlations between morphology, anatomy, and essential oil yield and composition were determined. Both capitate and peltate glandular trichomes were found on calyces, whereas the latter were noticed on the abaxial and adaxial leaf surface.

Key words: Thymus glabrescens, essential oils, thymol, γ-terpinene, glandular trichomes, cluster analysis

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INTRODUCTION

Thymus L. is one of the most important genera as regards the number of species (more than 200) within the family Lamiaceae. This genus belongs to the tribe Mentheae, subfamily Nepetoideae (Morales, 1986). That there are serious difficulties in the taxonomical interpretation of the taxa belonging to the genus *Thymus* owing to the high variability of populations with respect to many morphological and micromorphological traits, as well as the composition of secondary compounds (Dajić-Stevanović and Šoštarić, 2006). This variabily is caused both by environmental factors and genetic variation due to frequent hybridization leading to variable chromosome number and expressed gynodioecy, a sexual polymorphism in which natural populations contain two type of plants – females and hermaphrodites (Thompson, 2002).

According to Jalas (1971), the genus *Thymus* is divided into eight sections: *Micantes*, *Mastichina*, *Piperella*, *Teucrioides*, *Pseudothymbra*, *Thymus*, *Hyphodromi,* and *Serpyllum*. In Flora of Serbia 31 species of the genus *Thymus* are listed, with more than 60 varieties, most of which grow in various meadow and pasture communities and in dry, sunny, rocky habitats, on both limestone and serpentine (Diklić, 1974).

Lamiaceae genera and species are known for significant variability of the secretory structures responsible for biosynthesis and accumulation of essential oils [with respect to both their type (Ascensao et al., 1998) and their number and distribution within the plant organs (Venkatachalam et al., 1984, Turner et al., 2000)]. Chemical polymorphism is characteristic of the species of *Thymus*; numerous chemotypes have been defined, such as carvacrol and thymol, a-terpineol, thujone, geraniol, linalool, and others (Thompson et al., 1998). Thyme essential oils have been reported to possess antimicrobial activities (Bhaskara et al., 1998; Ložiené et al., 2007), most of which are mediated by thymol and carvacrol, as the phenolic components of the oil. Due to the hydrophobic nature of Lamiaceae

volatiles, the bacterial cell membrane has been proposed as the primary target of their antimicrobial action (Mitić–Ćulafić et al., 2005). Spasmolytic and antioxidant activities (Migul et al., 2004; Sacchetti et al., 2005) have also been reported for the phenolic oil extract of the plants.

Thymus glabrescens Willd. belongs to the section *Serpyllum*, subsection *Isolepides* (Jalas, 1972). It is a perennial herbaceous plant, distributed in Central and Eastern Europe as well as in Asia, inhabiting open dry meadows, grasslands, and rocks with sunny exposure (Jalas, 1972). In Serbia, the species has a scattered distribution being found on the slopes of Mt. Fruška Gora, in the Deliblato Sands, throughout Šumadija, and in Southwest Serbia (Dajić-Stevanović and Šoštarić, unpublished data).

Despite broad scientific interest in the biology, taxonomy, chemotypes, and related biological activity of secondary metabolites of *Thymus* species, there is a general lack of information regarding *Th. glabrescens,* with the exception of two reports from the Western Balkans (Karuza–Stojaković et al., 1989; Kustrak, 1990) and one from Romania (Kisgyörgy et al., 1983). We therefore studied the morphological, anatomical, and essential oils of autochthonous populations of *Th. glabrescens*. The results are here present as a first report on comparative morphology, anatomy, and essential oil composition of this species from Serbia.

MATERIAL AND METHODS

Collection of plant material and study site

Th. glabrescens was collected during the flowering period of June 2004 in the Banat region of Northeast Serbia (Table 1). Voucher specimens were determined in accordance with the Flora of Serbia (Diklić, 1974) and Flora Europaea (Jalas, 1972) and deposited at the Department of Botany, Faculty of Agriculture, University of Belgrade, Belgrade.

Morphology and anatomy

The following morphological features of leaves were analyzed: length (mm), width (mm), and the length/width ratio (N = 30) in all of the collected populations.

In analysis of leaf (N = 45) and stem (N = 30) anatomical traits, plant material was fixed in FAA, subjected to the standard paraffin procedure, and microtome sectioned using a LEICA SM 2000 R microtome, after which sections 7-10 μm thick were stained with safranine and aniline blue. Image analysis was done with LEICA IM1000 software.

In order to describe the number of glandular trichomes on leaves, 30 leaves were taken from individual plants and bright-field microscopy was conducted using a LEICA XTL-3400 D stereo-microscope. For scanning electron microscopy (SEM), small pieces of dry leaves and calyces of 10 plants were sputter-coated with gold for 180 sec at 30 mA using a BAL-TEC SCD 005 instrument and viewed with a JEOL JSM-6460L Velectron microscope at an acceleration voltage of 20 kV.

Isolation and analysis of essential oils

Air dried aerial parts were subjected to hydrodistillation for 3 h using a modified Clevenger-type apparatus with a water-cooled oil receiver to reduce hydrodistillation artifacts. The oil obtained was dried over anhydrous sodium sulfate and stored at 4-6°C until analyzed.

GC-FID analysis was carried out in a Hewlett Packard 5890 II gas chromatograph equipped with FID, a split-splitless injection system (split ratio of 1:30), and a 25 m x 0.32 mm HP-5 fused silica capillary column (film thickness: 0.52 μm). The carrier gas was H_2, the flow rate 1 ml/min. Oven temperature was programmed from 40°C to 260°C at a 4°C/min linear rate; injector and detector temperatures were maintained at 250°C and 300°C, respectively. The injection volume was 1 μl of 1 % (w/v) essential oil in ethanol.

GC-MS analyses were performed with a Hewlett Packard G 1800C GCD Series II (GC-EID) instrument fitted to a 30 mm x 0.25 mm HP-5 MS capillary column (film thickness: 0.25 μm) using He (1 ml/min) as the carrier gas, temperature of the transfer line being maintained at 260°C. Essential oil components were identified by matching their mass spectra with published data (Adams, 1989) and by consulting libraries of mass spectra (Wiley and NIST/NBS).

Table 1. Geographic position and habitat description for studied populations of *Th. glabrescens*

Population	Herbarium voucher code	Locality	Habitat	GPS data
P1	100604-1	Belo Blato	Ruderal habitat	N 45º16′53.6″ E 020º22′33.2″
P2	100604-2	Lukino Selo	Slightly salt-affected meadow	N 45º17′25.0″ E 020º25′53.3″
P3	100604-3	Zrenjanin Melenci	Slightly salt-affected meadow	N 45º28′41.6″ E 020º20′17.7″
P4	180604-1	Devojački Bunar	Meadow on the sand	N 45º00′05.1″ E 020º56′19.5″
P5	180604-4	Jablanka	Steppe meadow	N 45º04′20.6″ E 021º23′04.2″

Data processing

Analysis of variance (ANOVA and Duncan multiple range test) and cluster analysis (based upon Euclidean distances) of all surveyed populations of *Th. glabrecens* were performed using STATISTICA software, version 7.0.

RESULTS AND DISCUSSION

Leaf morphology

The population variability of leaf morphology showed that most of the studied populations differed significantly from each other ($p<0.001$) with the respect to leaf length, leaf width, and the leaf length/width ratio (Table 2). Maximal leaf length and width were determined for P3 and P4, respectively, minimal leaf length and width for P1 and P5, respectively. The highest and lowest leaf length/width ratios were found for P3 and P1, respectively.

The number of secretory glands was practically equal on the abaxial and adaxial leaf surfaces (Table 2) when calculated as the mean for all populations (8.75: 8.76). Nevertheless, among the surveyed populations, the number of glands on both leaf surfaces differed to a great extent. The highest number of secretory glands was determined for P1, followed by P2, and the lowest for P4.

Based upon all tested morphology variables, three distinct clades were determined, one comprising P1, P2, and P5, the other with P3, whereas population P4 was found to be the most distant from all other populations, probably due to the significantly lowest average number of glandular trichomes on both the upper and lower leaf epidermis (Fig. 1).

Leaf and stem anatomy

Leaf anatomical features measured in two different regions (at the central midrib and near the apex) showed that all characters were significant for discrimination of populations (ANOVA, Table 3). The highest and lowest leaf depths were found for P4 and P1, respectively. The height of upper and lower epidermal cells was greatest in P4 and P3, respectively. The height of palisade tissue was greatest in P4 and smallest in P1.

Stem measurements showed that only rib width was not significant for population discrimination. The highest and lowest stem widths were recorded for P4 and P5, respectively, whereas maximal and minimal central cylinder widths were found for P4 and P5, respectively. Based on all measured variables of leaf and stem anatomy, the grouping of populations was in two clades (Fig. 2): the first was made up of P1 and P5 and the second of all the other populations.

Description of secretory structures (SEM microscopy)

As in other species of the family Lamiaceae, *Th. glabrescens* is characterized by the presence of glandular trichomes, which are generally classified as either capitate (clavate) or peltate (subsessile), based on their morphological characteristics (Werker, 1993; Ascensao et al., 1998). Capitate glandular trichomes consist of one or two cells that sit atop a

Table 2. Morphological traits in populations of *Thymus glabrescens* (leaf length and width expressed in mm, number of secretory glands expressed per mm2 of leaf surface). Abbreviations: LL: leaf length; LW: leaf width; L/W ratio: leaf length/width ratio; No. glands ULE: number of glands on upper leaf epidermis; No. glands LLE: number of glands on lower leaf epidermis.

Variable	P1	P2	P3	P4	P5
Leaf Length	0.85 ± 0.15	1.12 ± 0.09	1.31 ± 0.13	1.28 ± 0.09	1.02 ± 0.09
Leaf Width	0.25 ± 0.05	0.27 ± 0.03	0.24 ± 0.03	0.28 ± 0.04	0.22 ± 0.03
L/W Ratio	3.62 ± 1.18	4.15 ± 0.52	5.51 ± 0.84	4.63 ± 0.56	4.81 ± 0.59
No. Glands ULE	10.00 ± 0.83	11.23 ± 2.14	8.23 ± 1.43	4.70 ± 1.02	9.57 ± 2.61
No. Glands LLE	11.33 ± 1.92	9.53 ± 1.43	7.57 ± 1.45	5.43 ± 1.07	9.97 ± 2.43

ANOVA		
	F	p
LL	80.61781	***
LW	14.22863	***
L/W ratio	24.91103	***
No. Glands ULE	61.89608	***
No. Glands LLE	53.30471	***

Table 3. Anatomical traits of leaf and stem in populations of *Th. glabrescens* (μm). Abbreviations: Upper Epid H: upper leaf epidermis height; Lower Epid H: lower leaf epidermis height; Palisade H: palisade height; CC Width: central cylinder width; variables without asterisk refer to measurments performed at the central leaf midrib; variables with asterisk refer to measurements near the leaf apex.

Variable	P1	P2	P3	P4	P5
Leaf depth	128.86 ± 16.93	186.29 ± 8.74	164.03 ± 16.39	204.83 ± 14.45	174.48 ± 17.02
Upper Epid H	22.68 ± 3.02	19.47 ± 31.26	22.68 ± 3.12	24.22 ± 5.25	18.63 ± 3.03
Lower Epid H	18.07 ± 3.21	17.58 ± 20.56	20.71 ± 3.57	19.90 ± 3.08	17.84 ± 2.87
Palisade H	56.16 ± 10.26	91.08 ± 12.35	73.65 ± 10.61	98.25 ± 9.12	74.37 ± 14.27
Leaf depth*	134.64 ± 14.08	191.10 ± 18.78	161.65 ± 15.51	214.54 ± 16.11	170.18 ± 16.15
Upper Epid H*	23.25 ± 3.12	20.25 ± 2.33	22.69 ± 3.38	24.50 ± 3.47	18.63 ± 3.01
Lower Epid H*	19.26 ± 3.48	19.45 ± 2.75	20.43 ± 2.59	19.69 ± 4.32	17.10 ± 2.49
Pallisade H*	56.42 ± 11.36	87.04 ± 25.52	69.81 ± 14.00	98.31 ± 10.85	74.08 ± 12.86
Stem Diagonal	1022.66 ± 44.47	1165.37 ± 16.65	1201.32 ± 72.54	1132.97 ± 133.19	933.35 ± 49.72
Stem Width	829.12 ± 8.33	944.90 ± 2.88	998.99 ± 84.54	1000.96 ± 106.67	820.29 ± 12.12
CC Width	749.45 ± 29.86	794.86 ± 2.24	779.29 ± 73.73	833.57 ± 85.41	628.13 ± 69.36
Rib Width	57.26 ± 15.63	62.10 ± 26.22	50.77 ± 7.20	61.26 ± 14.77	48.76 ± 3.30

ANOVA		
	F	p
Leaf depth	102.7397	***
Upper Epid H	24.2033	***
Lower Epid H	11.0172	***
Palisade H	80.9541	***
Leaf depth*	129.7449	***
Upper Epid H*	27.2109	***
Lower Epid H*	8.1061	***
Pallisade H*	62.5809	***
Stem Diagonal	11.0129	**
Stem Width	10.3083	**
CC Width	7.9604	**
Rib Width	1.6894	n.s.

stalk of one to several cells (Gang et al., 2001) and are generally considered to have only limited storage capacity, secreting mainly a complex mixture of carbohydrates, lipids, and proteins (Werker et al., 1985). Peltate trichomes of the Lamiaceae are usually composed of several secretory head-cells (up to 16), a wide short stalk, and one basal epidermal cell (Hallahan, 2000). Peltate glands of the Lamiaceae have been shown to be the main site of production of monoterpene and sesquiterpene compounds (Werker, 1993; Hallahan, 2000; McConkey et al.; 2000, Gang et al., 2001).

In *Th. glabrescens*, both types of glandular tri-

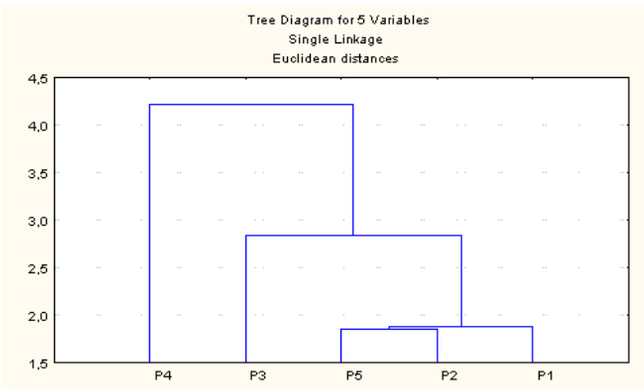

Fig. 1. Cluster analysis of populations of *Th. glabrescens* based on traits of leaf morphology.

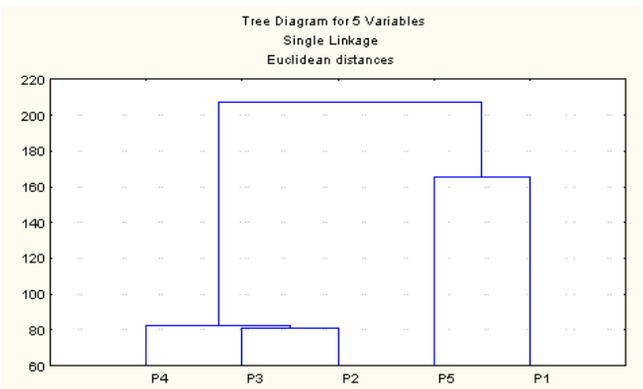

Fig. 2. Cluster analysis of populations of *Th. glabrescens* based on traits of leaf and stem anatomy.

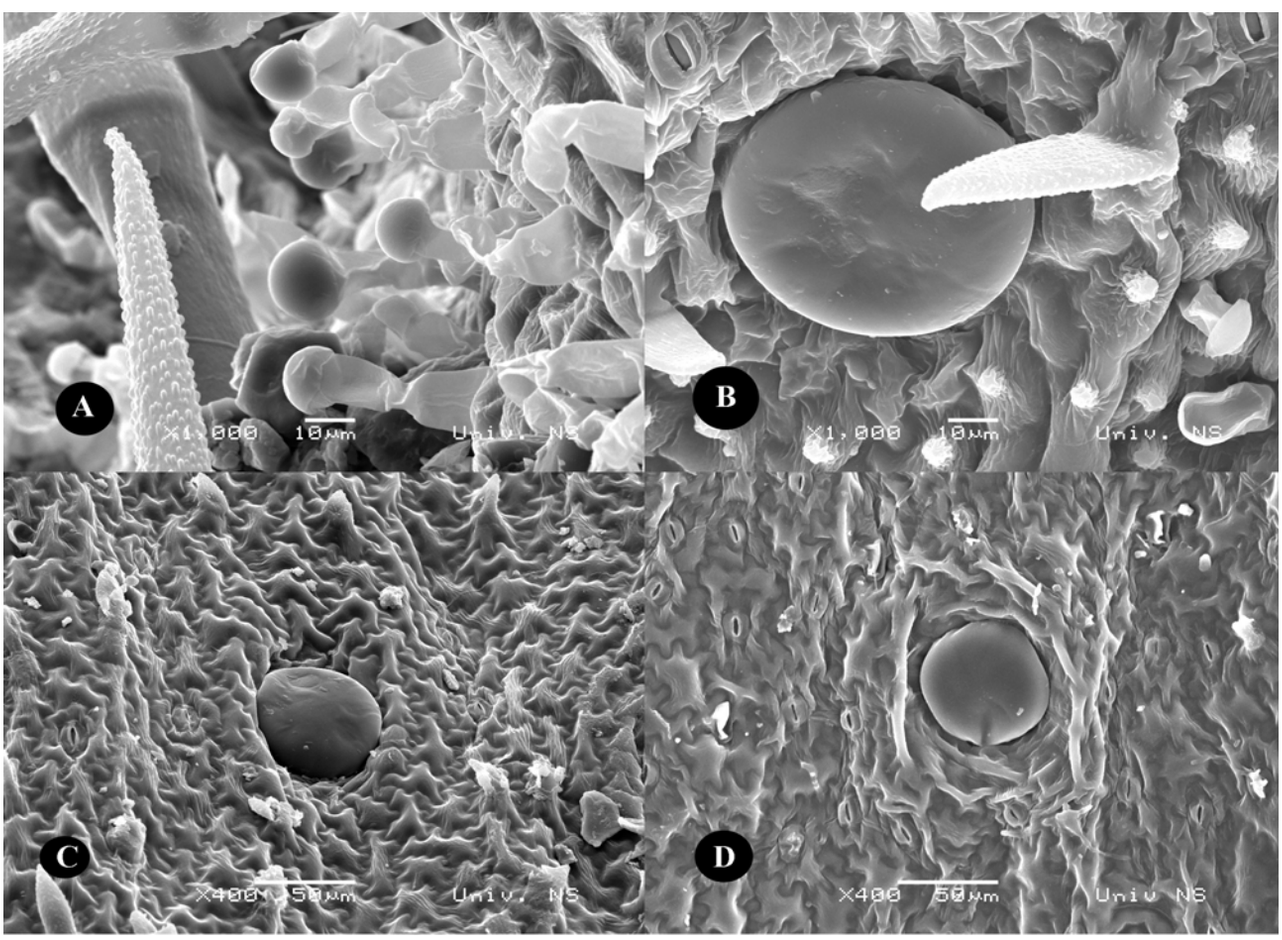

Fig. 3. Glandular trichomes in *Thymus glabrescens*. A - capitate trichome on calyx, B - peltate trichome on calyx, C - peltate trichome on abaxial leaf surface, D - peltate trichome on adaxial leaf surface.

chomes were determined (Fig. 3). Capitate glands were noticed on calyces only, but there they were very dense (Fig. 3a). Peltate trichomes were found on both surfaces of the leaf, as well as on the calyces

Table 4. Essential oil composition in populations of *Thymus glabrescens* ; KIE – Experimental Kovats indices; KIL – Literature Kovats indices.

Constituents	KIE	KIL	% w / w					Mean ± SD
			P1	P2	P3	P4	P5	
α-thujene	**923.2**	**924**	1.57	1.48	2.05	1.93	1.69	1.74 ± 0.24
α-pinene	**931.0**	**932**	0.53	0.66	0.81	0.86	1.00	0.77 ± 0.18
camphene	**944.6**	**946**	0.13	0.38	0.19	0.45	0.87	0.40 ± 0.29
sabinene	**973.2**	**969**	1.19	0.82	0.92	1.10	0.90	0.99 ± 0.15
β-pinene	**975.5**	**974**	0.31	0.29	0.43	0.40	0.46	0.38 ± 0.07
1-octen-3-ol	**985.9**	**974**	0.20	0.17	0.27	0.42	0.35	0.28 ± 0.10
β-myrcene	**995.2**	**988**	1.57	1.40	1.82	1.76	1.46	1.60 ± 0.18
3-octanol	**1000.2**	**988**	0.11	-	0.15	0.15	0.37	0.16 ± 0.14
α-phellandrene	**1004.7**	**1002**	0.28	0.23	0.30	0.24	0.22	0.25 ± 0.03
δ3-carene	**1009.6**	**1008**	0.09	0.08	0.10	0.10	0.09	0.09 ± 0.01
α-terpinene	**1016.2**	**1014**	3.00	2.32	3.25	1.31	1.55	2.29 ± 0.86
p-cymene	**1025.6**	**1020**	15.22	14.36	17.75	11.05	11.75	14.03 ± 2.71
limonene	**1029.8**	**1024**	0.60	0.60	0.67	0.54	0.52	0.59 ± 0.06
1,8-cineole	**1032.2**	**1026**	0.79	0.44	1.19	0.93	1.91	1.05 ± 0.55
trans-β-ocimene	**1056.0**	**1044**	0.10	0.12	0.09	0.07	0.07	0.09 ± 0.02
γ-terpinene	**1060.8**	**1054**	33.98	25.21	38.54	6.37	7.50	22.32 ± 14.85
cis-sabinene hydrate	**1067.7**	**1065**	1.04	0.63	0.71	1.01	0.07	0.69 ± 0.39
1-nonen-3-ol	**1081.8**	**n/a**	-	-	-	0.04	-	0.01 ± 0.02
α-terpinolene	**1086.4**	**1086**	0.09	0.10	0.09	0.12	0.18	0.12 ± 0.04
linalool	**1102.7**	**1095**	1.03	0.92	0.46	0.64	0.66	0.74 ± 0.23
nonanal	**1104.5**	**1100**	-	-	-	-	0.09	0.02 ± 0.04
1,3,8-p-menthatriene	**1120.5**	**1108**	-	-	-	0.45	0.35	0.16 ± 0.22
α-camphonelal	**1124.6**	**1122**	-	-	-	0.04	-	0.01 ± 0.02
trans-limonene oxide	**1137.3**	**1137**	-	-	-	0.06	-	0.01 ± 0.02
camphor	**1143.2**	**1141**	-	-	-	0.05	-	0.01 ± 0.02
borneol	**1165.2**	**1165**	0.37	0.85	0.33	1.12	2.36	1.01 ± 0.83
terpinene-4-ol	**1176.7**	**1174**	0.40	0.34	0.38	0.64	1.58	0.67 ± 0.52
dec-1-en-3-ol	**1181.8**	**1177**	-	0.05	-	0.05	0.07	0.03 ± 0.03
α-terpineol	**1191.0**	**1186**	0.23	0.27	0.18	0.20	0.31	0.24 ± 0.06
trans-dihydrocarvone	**1196.0**	**1200**	-	-	-	0.13	0.14	0.05 ± 0.07
trans-carveol	**1219.3**	**1215**	0.10	0.48	-	-	-	0.12 ± 0.21
thymol methylether	**1233.9**	**1232**	2.50	2.33	1.21	3.62	4.27	2.79 ± 1.19
carvacrol methylether	**1243.2**	**1241**	3.01	2.57	2.52	5.95	5.74	3.96 ± 1.73
linalyl acetate	**1254.3**	**1257**	2.45	8.55	0.04	-	-	2.21 ± 3.70
dihydroedulan I	**1285.9**	**1288**	-	0.12	-	-	-	0.02 ± 0.06
thymol	**1292.6**	**1289**	25.96	29.92	22.28	55.12	49.48	36.55 ± 14.77
carvacrol	**1297.2**	**1298**	-	-	-	0.42	0.34	0.15 ± 0.21
δ-elemene	**1335.5**	**1335**	-	-	-	0.04	-	0.01 ± 0.02
eugenol	**1357.9**	**1356**	-	-	-	0.06	-	0.01 ± 0.03
α-copaene	**1374.1**	**1374**	-	0.19	-	-	-	0.04 ± 0.08
β-bourbonene	**1383.7**	**1387**	-	0.12	0.08	0.07	0.12	0.08 ± 0.05
β-caryophyllene	**1419.8**	**1417**	0.77	0.16	0.22	0.26	0.47	0.38 ± 0.25
β-gurjunene	**1433.2**	**1431**	-	0.06	0.04	0.05	0.07	0.04 ± 0.03
α-humulene	**1452.8**	**1452**	0.06	-	-	-	0.05	0.02 ± 0.03
allo-aromadendrene	**1460.4**	**1458**	-	0.04	-	-	0.03	0.01 ± 0.02
γ-muurolene	**1480.4**	**1478**	-	0.05	0.04	0.09	0.12	0.06 ± 0.05
germacrene D	**1489.1**	**1484**	0.75	1.26	1.10	0.25	0.34	0.74 ± 0.45
bicyclogermacrene	**1502.0**	**1500**	1.27	2.15	1.71	1.41	1.70	1.65 ± 0.34
γ-cadinene	**1516.0**	**1513**	-	-	-	0.06	0.06	0.02 ± 0.03
γ-cadinene	**1522.8**	**1522**	-	0.11	0.07	0.13	0.18	0.10 ± 0.07
spathulenol	**1579.6**	**1577**	0.19	0.19	-	0.14	0.20	0.15 ± 0.09
caryophyllene oxide	**1580.9**	**1582**	0.12	-	-	0.08	0.18	0.08 ± 0.08
Identification of components (%)			100.00	100.00	100.00	99.96	99.91	

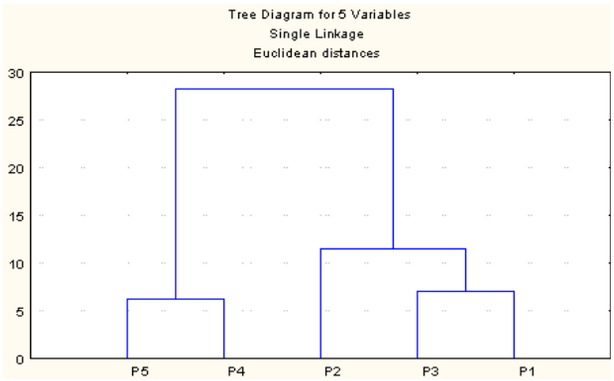

Fig. 4. Cluster analysis of populations of *Th. glabrescens* based on essential oil composition.

Table 4. Essential oil composition in populations of Th. glabrescens ; KIE – Experimental Kovats indices; KIL-Literature Kovats indices.

Major groups of constituents	P1	P2	P3	P4	P5
monoterpene hydrocarbons	43.43	33.68	49.26	15.71	16.87
oxygenated sesquiterpene hydrocarbon derivatives	6.71	12.68	3.29	5.02	7.43
aromatic alcohols	0.00	0.00	0.00	0.06	0.00
aromatic hydrocarbons	15.22	14.36	17.75	11.05	11.75
benzopyrane derivatives	0.00	0.12	0.00	0.00	0.00
phenols	25.96	29.92	22.28	55.54	49.82
phenolic ethers	5.51	4.90	3.73	9.57	10.01
saturated alcohols	0.11	0.00	0.15	0.15	0.37
unsaturated alcohols	0.20	0.22	0.27	0.51	0.42
unsaturated aldehydes	0.00	0.00	0.00	0.00	0.09
sesquiterpene hydrocarbons	2.85	4.12	3.26	2.34	3.15

(Fig. 3, b, c, d).

Essential oil composition

The composition of essential oils in *Th. glabrescens* populations from Serbia was characterized by the presence of a total of 52 components (Table 4). The highest yield of essential oil was found in P2 (0.80%), the lowest in P5 (0.25%). The average essential oil yield for all populations was 0.60 ± 0.22%.

Average values of each component (Table 4) indicated that the major components were thymol (36.55%), γ-terpinene (22.32%) and p-cymene (14.03%). A certain variability of essential oil composition was observed in the analyzed populations of *Th. glabrescens*. While thymol was the major component in populations P2, P4, and P5, γ-terpinene was dominant in P1 and P3.

Based on all identified components of the essential oil, *Th. glabrescens* populations were grouped into two clades, the first made up of P4 and P5, the second of P1 and P3 and the close to them P2 (Fig. 4). Such grouping might be related to spatial distribution of the populations, populations P1, P2, and P3 being found in the central part of the Banat region and populations P4 and P5 in the southern part of the study area (Table 1).

Regarding the presence of particular chemical compounds in populations of *Th. glabrescens* (Table 5), it was found that the dominant compounds were monoterpene hydrocarbons (especially in P1, P2, and P3), followed by phenols (thymol was present in all populations except P3), aromatic hydrocarbons, and phenolic ethers.

Previously reported results on essential oil composition in *Th. glabrescens* from Croatia indicated that the major components were 1,8-cineole (29.4%); myrcene, camphene, α-pinene, β-pinene, and thymyl acetate (14.3%); and carvacrol, p-cimene and thymol, depending on the population (Kustrak et al., 1990). For *Th. glabrescens* from Bosnia and Herzegovina, the main component was α-terpinyl acetate (32%), followed by terpenene-4-ol, thymol, myrcene, and α-pinene components (Karuza-Stojaković et al., 1989). Our results indicate the existence of one major chemotype within populations of *Th. glabrescens* from Serbia - the thymol chemotype – but two "sub-chemotypes" could be distinguished, the strict thymol sub-type and the γ-terpinene sub-type. However, the presence of thymol, carvacrol, γ-terpinene, and p-cimene cannot be considered independently, since all these terpenes are closely connected in biogenetic processes, where γ-terpinene and p-cimene are known as precursors in the biochemical pathway of phenols (Stahl–Biskup, 2002).

As for possible relations between morphological and anatomical features and chemical characterization of the essential oil (yield and composition), no

correlations were determined between the composition (e.g., the content of monoterpenes, phenols, and thymol) and yield of essential oil and the number of glands or the leaf length/width ratio (r = 0.49); between the number of glands and leaf depth (r = 0.64); between the leaf length/width ratio and leaf depth (r = 0.38); etc. Although a relatively high correlation was found between leaf length and average gland number (r = 0.77), such a possible relation should be tested on a larger number of populations. According to published data and the results presented in this study, several chemotypes exist within *Th. glabrescens*, which is in accordance with the assertion that taxa of the section *Serpyllum* (and the section *Thymus*) exhibit a high number of chemotypes (Sáez and Stahl–Biskup, 2002).

Essential oils containing large amounts of thymol have been shown to possess high antioxidant activity (Farag et al., 1989; Aeschbach et al. 1994; Ložiené et al., 2007), and our further research will therefore be focused on biological activity of the essential oil of *Th. glabrescens* as well as analysis of vacuolar flavonoids, since they have been reported to be an important taxonomic character of the tribe Menthae (Marin, 1996).

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REFERENCES

Adams, R. P. (1989) *Identification of Essential Oils by Ion Trap Mass Spectroscopy.* Academic Press, San Diego, California.

Aeschbach, R., Loliger, J., Scott, B. C., Murcia, A., Butler, J., and *B. Haliwell* (1994). Antioxidation actions of thymol, carvacrol, 6-gingerol, zingerone, and hydoxytyrosol. *Food Chem. Toxicol.* **32**, 31–36.

Ascensao, L., Figueiredo, A. C., Barroso, J. G., Pedro, L. G., Schripsema, J., Deans, S. G., and *J. C. J. Scheffer* (1998). *Plectranthus madagascariensis* – morphology of the glandular trichomes, essential oil composition, and its biological activity. *Internat. J. Plant Sci.* **159**, 31–38.

Bhaskara, M. V., Angers, P., Gosselin, A., and *J. Arul* (1998). Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. *Phytochemistry* **47**, 1515–1520.

Dajić Stevanović, Z., and *I. Šoštarić* (2006). Review of some useful methods in taxonomical interpretation of difficult taxa of medicinal and aromatic plants. Case: *Thymus* L., In: *Proceedings of the 4th Conference on Medicinal and Aromatic Plants of South-East European Countries,* 28-31 May, Iasi, Romania, 63-71.

Diklić, N. (1974). *Thymus* L., In: *Flora R. Srbije* (Ed. M. Josifović), 475-509. SANU, Belgrade.

Farag, R. S., Badei, A. Z. M. A., and *G. S. A. El Baroty* (1989). Influence of thyme and clove essential oils on cottonseed oil oxidation. *J. Am. Oil Chem. Soc.* **66**, 800–804.

Gang, D. R., Wang, J., Dudareva, N., Nam, K. H., Simon, J. R., Lewinsohn, E., and *E. Pichersky* (2001). An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Physiol.* **125**, 539-555.

Hallahan, D. L. (2000). Monoterpenoid biosynthesis in glandular trichomes of Labiatae plants. In: *Advances in Botanical Research: Plant Trichomes* (Eds. D. L. Hallahan and J. C. Gray), 77-120. Academic Press, Amsterdam.

Jalas, R. (1972). *Thymus* L. In: *Flora Europaea, Vol. 3* (Eds. T. Tutin, V. H. Haywood, N. A. Burges, D. M. Moore, and D. Valentine), 172-182. University Press, Cambridge

Karuza-Stojaković, L., Pavlović, S., Živanović, P., and *B. Todorović* (1989). Composition and yield of essential oils of various species of the genus *Thymus* L. *Arh. Farm.* **39**, 105-111.

Kisgyörgy, Z., Csedö, K., Hörster, H., Gergely, J., and *G. Racz* (1983). The volatile oil of the more important indigenous *Thymus* species occurring in the composition of Serpylli herba. Rev. Med. (Tirgu-Mures, Rom.) 124-130.

Kustrak, D., Martinis, Z., Kuftinec, J., and N. Blazevic (1990). Composition of the essential oils of some *Thymus* and *Thymbra* species. *Flavour Fragr. J.* **5**, 227-231.

Ložiené, K., Venskutonis, P. R., Šipailiené, A., and *J. Labokas* (2007). Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. *Food Chem.* **103**, 546-559.

Marin, P. D. (1996). A chemotaxonomic study of vacuolar flavonoids from some Balkan *Micromeria* species (Lamiaceae). *Arch. Biol. Sci. (Belgrade),* **48** (1-2), 49-54.

McConkey, M. E., Gershenzon, J., and *R. B. Croteau* (2000). Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. *Plant Physiol.* **122**, 215-224.

Miguel, G., Simöes, M., Figueiredo, A. C., Barroso, J. C., Pedro, L. G., and *L. Carvalho* (2004). Composition and antioxidant activities of the essential oils of *Thymus caespititus*, *Thymus camphoratus*, and *Thymus mastichina*. *Food Chem.* **86**, 183–188.

Mitić-Ćulafić, D., Vuković-Gačić, B., Knežević-Vukčević, J.,

Stanković, S., and *D. Simić* (2005). Comparative study on the antibacterial activity of volatiles from sage *(Salvia officinalis* L.*) Arch. Biol. Sci. (Belgrade),* **57** (3), 173-178.

Morales, R. (1986). Taxonomía de los géneros *Thymus* (excluida la seccion *Serpyllum*) y *Thymbra* en la Península Ibérica. Ruizia **3**, 1-324.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., and *M. Radice* (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antibacterials, and antimicrobials in foods. *Food Chem.* **91**, 621–632.

Sáez, F., and *E. Stahl-Biskup* (2002). Essential oil polymorphism in the genus *Thymus*, In: *Thyme: The Genus Thymus* (Eds. E. Stahl-Biskup and F. Sáez), 124-143. Taylor and Francis, London and New York.

Stahl-Biskup, E. (2002). Essential oil chemistry of the genus *Thymus* – a global view. In: *Thyme: The Genus Thymus* (Eds. E. Stahl-Biskup and F. Sáez), 75-124. Taylor and Francis, London and New York.

Thompson, J. D. (2002). Population structure and the spatial dynamics of genetic polymorphism in thyme, In: *Thyme: The Genus Thymus* (Eds. E. Stahl-Biskup and F. Sáez), 44-74. Taylor and Francis, London and New York.

Thompson, J. D., Manicacci, D., and *M. Tarayre* (1998). Thirty-five years of thyme: a tale of two polymorphisms. Why so many females? Why so many chemotypes? *BioScience* **48**, 805–815.

Turner, G. W., Gershenzon J., and *R. B. Croteau* (2000). Distribution of peltate glandular trichomes on developing leaves of peppermint. *Plant Physiol.* **124**, 655–664.

Venkatachalam, K. V., Kjonaas R., and *R. Croteau* (1984). Development and essential oil content of secretory glands of sage (*Salvia officinalis*). *Plant Physiol.* **76**, 148–150.

Werker, E. (1993). Function of essential oil-secreting glandular hairs in aromatic plants of the Lamiaceae – a review. *Flavour Fragr. J.* **8**, 249–255.

Werker, E., Ravid, U., and *E. Putievsky* (1985). Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae. *Israel J. Bot.* **34**, 31–45.

ПОПУЛАЦИОНА ВАРИЈАБИЛНОСТ УНУТАР ВРСТЕ *THYMUS GLABRESCENS* WILLD. ИЗ СРБИЈЕ: МОРФОЛОГИЈА, АНАТОМИЈА И САСТАВ ЕТАРСКИХ УЉА

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У пет аутохтоних популација *Thymus glabrescens* Willd. сакупљених у региону Баната, Србија, испитивана је варијабилност морфолошких карактеристика листа, анатомске грађе листа и стабла и састава етарских уља. Главне компоненте етарских уља проучаваних популација биле су тимол, γ-терпинен и p-цимен. Уочена је просторна диференцијација популација у односу на састав етарских уља. Није установљена корелација између морфолошких и анатомских карактеристика популација и приноса и састава етарских уља. Присуство и изглед жлезданих трихома су утврђени посматрањем надземних делова на светлосном и скенинг електронском микроскопу. Пелтатне трихоме су уочене на чашици, лицу и наличју листа, док су капитатне нађене само на чашицама.