CHEMICAL COMPOSITION OF *Hypericum perforatum* L. ESSENTIAL OIL

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The essential oil isolated from fresh aerial parts of *Hypericum perforatum* L. was analyzed by GC and GC/MS. One hundred and thirty four identified compounds accounted for 98.7% of the total oil. The main components of the oil were: germacrene D (18.6%), (*E*)-caryophyllene (11.2%), 2-methyloctane (9.5%), α-pinene (6.5%), bicyclogermacrene (5.0%) and (*E*)-β-ocimene (4.6%). The volatile profile of *H. perforatum* was characterized by a large content of sesqiuterpenoids (57.7%), especially sesquiterepene hydrocarbons (48.7%). Monoterpenoids (22.4%) also consisted mostly of hydrocarbons (21.4%). Non-terpenoid compounds amounted to 18.1% of the total oil.

Keywords: *Hypericum perforatum*, Hypericaceae, essential oil composition.

Introduction

More than 480 species of the genus *Hypericum* L. (Hypericaceae) naturally occur in, or have been introduced to every continent except in Antarctica [1]. The plants of the genus *Hypericum* have been used as traditional medicinal plants all over the world [2], especially *Hypericum perforatum* (St. John's wort). *Hypericum perforatum* is a perennial, rhizomatous herb. This species is characterized by a very wide ecological amplitude and can grow under different environmental conditions. It is found in various types of oak forest, in thickets, meadows and pastures, forest clearings, burnt areas, etc. Both extracts and essential oils of *Hypericum* species have been shown to possess significant antiviral, wound healing, antioxidant and antimicrobial activities [3-10]. Some of these actions were attributed to the presence of phloroglucinols (antibacterial, wound healing, antimalarial and antidepressant activity), naphthodianthrones (antiviral and antidepressant activity), xanthones (antimicrobial, antiviral and antidepressant activity), tannins (antiviral, antimicrobial and antioxidant activity), flavonoids (antifungal, antioxidant and antidepressant activity) and volatile oils (antimicrobial activity) [11-14].

There are many studies on *H. perforatum* essential oil composition and they show a significant variation in volatile profile of this species. Germacrene D, α-pinene, β-caryophyllene, 2-methyloctane and n-nonane were among major components in *H. perforatum* essential oils reported by many authors [15-35].

The aim of this study was to perform a detailed compositional analysis of *H. perforatum* volatiles and compare the obtained results with previously published essential oil profiles of the mentioned species.

Experimental

Plant material.

Above-ground parts of *H. perforatum* in the flowering phase were collected in the region of southeastern Serbia in July 2008. Voucher specimens were deposited in the Herbarium of the Faculty of Science and Mathematics, University of Niš, under the acquisition number 7292.

Essential oil isolation.

Fresh aerial parts (400 g) of *H. perforatum* were subjected to hydrodistillation for 2.5 h using the original Clevenger-type apparatus and yielded 0.08% (w/w) of the pale yellow essential oil. The obtained oil was separated, dried over anhydrous magnesium sulfate and immediately analyzed.

Essential oil analyses.

The chemical composition of the oil was investigated by GC and GC/MS. The GC/MS analyses (three repetitions) were carried out using a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column HP-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 °C and 300 °C, respectively. The oven temperature was raised 70° – 290 °C at a heating rate of 5 °C/min and then isothermally held for 10 min. As a carrier gas, helium at 1.0 mL/min was used. The sample, 1 μL of oil solution in diethyl ether (1 : 100), was injected in a pulsed

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split mode (the flow was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40 : 1). MS (electron impact) conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35-500, scan time 0.32 s. Oil constituents were identified by comparison of their linear retention indices (relative to n-alkanes [36] on the HP-5MS column) with literature values [37] and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils, and wherever possible, by co-injection with an authentic sample. GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition of the oil was computed from the GC peak areas without any corrections.

Results and Discussion

The results of the chemical analysis of *H. perforatum* essential oil by using GC and GC/MS methods are listed in Table 1. One hundred and thirtyfour components were identified, making 98.7% of total oil ingredients. The main components of *H. perforatum* oil were: germacrene D (18.6%), (*E*)-caryophyllene (11.2%), 2-methyloctane (9.5%), α-pinene (6.5%), bicyclogermacrene (5.0%) and (*E*)-β-ocimene (4.6%). The oil was characterized by the fraction of terpenoid compounds amounting to 80.1% (Table 1). Within this fraction, sesquiterpenoids (57.7%) were mostly made of hydrocarbons (48.7%), whereas oxygenated derivatives were present with 9.0%. Sesquiterpenoids were represented 2.5 times more than monoterpenoids (22.4%), the latter consisting mostly of hydrocarbons - 21.4%, while the oxygenated fraction was present only with 1.0%. Monoterpenoids were dominated by the pinane structure compound type (10.1%) and acyclic monoterpenes (7.2%), while sesquiterpenoids consisted mainly of germacrane (23.6%), caryophyllane (12.8%) and cadinane (12,6%) structure types. Non-terpenoid compounds amounted to 18.1% of the total oil. This compound class consisted mainly of alkanes (14.1%): isoalkanes (10.7%), n-alkanes (2.0%) and anteiso-alkanes (1,4%) and fatty acids and their derivatives (FAD) (3.8%).

Table 1. Percentage composition of *H. perforatum* essential oil

RI	Compound	Content [%]	Identification method
764	(*Z*)-2-Penten-1-ol	0.1	a,b
765	3-Methyl-2-buten-1-ol (syn. prenol)	0.1	a,b
778	3-Methyl-2-butenal (syn. prenal)	tr	a,b
800	Octane	tr	a,b,c
832	2-Methylbutanoic acid	tr	a,b,c
847	(*E*)-Hex-3-en-1-ol	tr	a,b
851	(*Z*)-Hex-3-en-1-ol	1.2	a,b
859	2-Methyloctane	9.5	a,b
862	Hexan-1-ol	tr	a,b
867	3-Methyloctane	tr	a,b
900	Nonane	1.5	a,b,c
924	α-Thujene	0.7	a,b
932	α-Pinene	6.5	a,b,c
952	Camphene	tr	a,b
954	2-Methylnonane	tr	a,b
959	Benzaldehyde	tr	a,b,c
965	3-Methylnonane	1.4	a,b
972	Sabinene	0.9	a,b
974	β-Pinene	3.6	a,b,c
981	6-Methyl-5-hepten-2-on	tr	a,b
988	β-Myrcene	1.1	a,b,c
1000	Decane	tr	a,b,c
1001	(*E*)-Hex-3-enyl acetate	tr	a,b
1005	α-Phellandrene	0.1	a,b,c
1116	α-Terpinene	0.4	a,b
1023	*p*-Cymene	0.1	a,b,c
1027	Limonene	0.4	a,b,c
1027	β-Phellandrene	0.4	a,b
1030	1,8-Cineole	tr	a,b
1032	(*Z*)-β-Ocimene	1.5	a,b
1044	(*E*)-β-Ocimene	4.6	a,b
1056	γ-Terpinene	0.9	a,b,c
1057	2-Methyldecane	1.0	a,b
1063	3-Methyldecane	tr	a,b
1068	*cis*-Linalool oxide (furanoid)	tr	a,b
1087	Terpinolene	0.2	a,b
1100	Undecane	0.5	a,b,c
1102	Linalool	tr	a,b,c
1102	Nonanal	0.1	a,b
1103	2-Methylbutyl-3-methylbutanoate	tr	a,b
1135	*cis*-*p*-Ment-2-en-1-ol	tr	a,b
1137	*trans*-*p*-Ment-2-en-1-ol	0.1	a,b
1145	Camfor	tr	a,b,c
1165	(*E*)-2-Nonen-1-ol	0.2	a,b
1177	Terpinen-4-ol	0.6	a,b,c
1181	(*E*)-3-Hexenylbutanoate	tr	a,b
1183	*p*-Cymen-8-ol	tr	a,b
1191	α-Terpineole	0.3	a,b,c
1203	Decanal	0.1	a,b,c
1229	(*Z*)-Hex-3-enyl-2-methylbutanoate	tr	a,b
1232	(*Z*)-Hex-3-enyl-3-methylbutanoate	0.1	a,b
1239	Thymol methyl ether (syn. Methyl-thymol)	tr	a,b
1263	2-Methyldodecane	0.2	a,b
1278	(*E*)-2-Decen-1-ol	tr	a,b
1288	Undecan-2-one	tr	a,b
1300	Tridecane	tr	a,b,c
1327	Isobutyl benzoate	tr	a,b,c
1336	Bicycloelemene	0.1	a,b
1350	α-Cubebene	0.1	a,b
1353	α-Longipinene	tr	a,b
1361	(*E*)-2-Undecenal	tr	a,b
1367	(*E*)-2-Undecen-1-ol	0.1	a,b
1373	α-Ylangene	0.1	a,b
1374	α-Copaene	0.3	a,b
1381	2-*epi*-α-Funebrene	tr	a,b
1384	*Unidentified*	0.1	
1387	β-Bourbonene	0.2	a,b
1391	β-Cubebene	0.2	a,b
1396	β-Elemene	0.1	a,b
1397	(*Z*)-Jasmone	tr	a,b
1406	Dodecanal	tr	a,b
1411	α-Gurjunene	tr	a,b
1414	2-*epi*-β-Funebrene	1.0	a,b
1422	(*E*)-Caryophyllene	11.2	a,b,c
1424	β-Cedrene	tr	a,b
1431	β-Copaene	0.4	a,b
1435	Isoamyl benzoate	0.3	a,b,c
1437	Aromadendrene	0.3	a,b
1440	(*Z*)-β-Farnesene	tr	a,b

1446	*cis*-Muurola-3,5-diene	0.2	a,b
1450	Geranyl acetone	tr	a,b
1452	*trans*-Muurola-3,5-diene	0.1	a,b
1456	α-Humulene	tr	a,b,c
1457	(*E*)-β-Farnesene	1.5	a,b
1463	*allo*-Aromadendrene	tr	a,b
1469	β-Acoradiene	0.2	a,b
1471	Dodecan-1-ol	0.8	a,b
1472	*cis*-Muurola-4(14),5-diene	0.6	a,b
1475	*trans*-Cadina-1(6),4-diene	0.2	a,b
1481	γ-Muurolene	1.4	a,b
1484	Germacrene D	18.6	a,b
1492	(*Z*,*E*)-α-Farnesene	1.2	a,b
1494	*cis*-Cadina-1,4-diene	0.6	a,b
1497	*trans*-Muurola-4(14),5-dien	tr	a,b
1501	Bicyclogermacrene	5.0	a,b
1501	α-Muurolene	tr	a,b
1506	(*E*,*E*)-α-Farnesene	0.9	a,b
1508	δ-Amorphene	0.3	a,b
1509	β-Curcumene	tr	a,b
1516	γ-Cadinene	1.0	a,b
1526	δ-Cadinene	2.3	a,b
1528	Zonarene	0.1	a,b
1534	*trans*-Cadina-1,4-diene	0.2	a,b
1539	α-Cadinene	0.3	a,b
1545	α-Calacorene	tr	a,b
1567	(*E*)-Nerolidol	0.6	a,b
1569	(*Z*)-Hex-3-enyl benzoate	0.5	a,b
1580	Spathulenol	0.3	a,b,c
1586	Caryophyllene oxide	1.6	a,b,c
1604	Viridiflorol	0.4	a,b
1604	Rosifoliol	0.2	a,b
1610	Ledol	tr	a,b
1617	1,10-di-*epi*-Cubenol	0.1	a,b
1619	Humulene epoxide II	0.1	a,b
1622	Junenol	0.2	a,b
1630	1-*epi*-Cubenol	0.2	a,b
1633	*cis*-Cadin-4-en-7-ol	1.5	a,b
1643	*epi*-α-Cadinol (τ-Cadinol)	0.5	a,b
1644	*epi*-α-Muurolol (syn. τ-Muurolol)	1.1	a,b
1648	α-Muurolol (syn. Torreyol)	0.4	a,b
1652	β-Eudesmol	tr	a,b
1657	α-Cadinol	1.5	a,b
1670	Tetradecan-1-ol	0.6	a,b
1681	α-Bisabolol	0.3	a,b
1742	Mint sulfide	tr	a,b
1762	Benzyl benzoate	0.1	a,b
1841	Hexahydrofarnesyl acetone	0.1	a,b
1876	Hexadecan-1-ol	tr	a,b
1900	Nonadecane	tr	a,b,c
2100	Heneicosane	tr	a,b,c
2300	Tricosane	tr	a,b,c
2500	Pentacosane	tr	a,b,c
2700	Heptacosane	tr	a,b,c
2900	Nonacosane	tr	a,b,c
	Total	**98.7**	
	Terpenoids	**80.5**	
	Hemiterpenoids	**0.4**	
	Monoterpenoids	**22.4**	
	Monoterpene hydrocarbons	21.4	
	Oxygenated monoterpenes	1.0	
	Sesquiterpenoids	**57.7**	
	Sesquiterpene hydrocarbons	48.7	
	Oxygenated sesquiterpenes	9.0	
	Diterpenoids	**-**	
	Non-terpenoids	**18.1**	
	Unidentified	0.1	

RI - experimentally determined retention indices on an HP-5MS column; tr - less than 0.05%; syn. - synonym; *a* - retention indices matching with literature data; *b* - mass spectra matching; *c* - co-injection with an authentic sample.

Previous studies on the chemical composition of *H. perforatum* essential oil indicated high variability of the composition [15-35, 38-40]. It is dificult to compare the published results, having in mind the fact that authors analyzed the plant material from various regions, characterized by their specific enviromental and ecological factors, and in many cases phenological phase of the analyzed plant material was not given. Some authors stated different chemical compositions of *H. perforatum* essential oils depending on the plant organs examined (leaves, flowers, stems) [28, 41]. Moreover, the differences in the composition of essential oils may originate from different varieties within the same species [27], and as in most papers this taxonomic category was not given, the comparison of essential oils becomes more difficult. Generally speaking, there are *H. perforatum* essential oils with sesquiterpenoids as dominant compound class [20-21, 27, 39], but there are also oil samples where alkanes [23, 29] or monoterpene hydrocarbons [33, 40] were the major classes of compounds. Concerning the major components identified in *H. perforatum* oil, most papers cited the following ones: germacrene D, (*E*)-caryophyllene, caryophyllene oxide, α-pinene and/or 2-methyloctane. Roughly, we can distinguish two chemotypes - two *H. perforatum* oil groups. The essential oils containing germacrene D, (*E*)-caryophyllene and caryophyllene oxide as main components [15-22, 24-25, 27] belong to the first group, while the second group includes the oils where α-pinene and/or 2-methyloctane dominate [26-28, 30-34, 42].

Bearing in mind the above mentioned, it is interesting to compare *H. perforatum* essential oil analyzed in this study with other oils originating from Serbia. So, Šmelcerović et al. [28] identified 2-methyloctane (20.5%), α-pinene (13.8%), spatulenol (9.8%) and hexadecanoic acid (4.0%) as the main components of *H. perforatum* essential oil; this oil was characterized by a high amount of non-terpenoid compounds (44.1%). In contrast to this, in oils of the same plant species other authors have found the following compounds as dominant: β-caryophyllene (14.2%) and 2-methyloctane (13.1%) [23], caryophyllene oxide [22], whereas Rančić et al. [29] identified n-nonane (63,8%), *p*-cymene (4.8%) and 3-methylnonane (4.5%) as main components - which made this oil sample completely different from others in the region (and beyond). Chatzopoulou et al. [26] ascertained that aliphatic compounds (43.83%) and sesquiterpenes (39.73%) prevailed in their oil of *H. perforatum* while Mimica-Dukić et al. [38] found that non-terpene components such as 1-tetradecanol, 10-methyl-1-undecene and cyclodecane dominated in oils of *H. perforatum* populations collected in lowland regions, whereas sesquiterpenes were predominant in oils of the plant material collected in highland regions.

On the basis of the above presented, it can be concluded that *H. perforatum* oil analyzed in this study di-ffers from others in the region considering the main oil component - germacrene D, but the other compo-

nents present in a significant amount (β-caryophyllene, 2-methyloctane and α-pinene) make it comparable to oil samples in the region. The results found also confirm the existence of a significant variability regarding the composition of *H. perforatum* essential oil.

Conclusion

A detailed investigation of the volatile constituents of *H. perforatum* essential oil resulted in the identification of 134 components. The oil was characterized by a sesquiterepene- (48.7%) and monoterpene hydrocarbons (21.4%). *Hypericum perforatum* essential oil under study is in agreement with previous reports concerning this species. Further investigations need to be conducted in order to provide better understanding of taxonomically complex relationships among *Hypericum* species.

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Izvod

HEMIJSKI SASTAV ETARSKOG ULJA BILJNE VRSTE *Hypericum perforatum* L.

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Etarsko ulje izolovano iz svežih nadzemnih delova biljne vrste *Hypericum perforatum* L. analizirano je metodama gasne hromatografije (GC) i kombinacijom gasne hromatografije i masene spektrometrije (GC/MS). Identifikovano je 134 komponenti, koje čine 98,7% ukupnog sastava ulja. Glavne komponente ulja *H. perforatum* bile su: germakren D (18,6%), (*E*)-kariofilen (11,2%), 2-metil-oktan (9,5%), α-pinen (6,5%), biciklogermakren (5,0%) i (*E*)-β-ocimen (4,6%). Ispitivano ulje *H. perforatum* bilo je okarakterisano velikom količinom seskviterpenoida (57,7%), naročito seskviterpenskim ugljovodonicima (48,7%). Monoterpenoide su takođe mahom činili ugljovodonici (21,4%). Količina ne-terpenoidnih jedinjenja iznosila je 18,1% ukupnog ulja.

Ključne riječi: *Hypericum perforatum*, Hypericaceae, sastav etarskog ulja.