Macedonian Journal of Chemistry and Chemical Engineering, Vol. **34**, No. 2, pp. 333–341 (2015)

MJCCA9 – 691

ISSN 1857-5552
e-ISSN 1857-5625

Received: October 6, 2014
Accepted: December 16, 2014

UDC: 547.979.8:544.723.3
Original scientific paper

ESSENTIAL OIL COMPOSITION OF INDIGENOUS POPULATIONS OF *HYPERICUM PERFORATUM* L. FROM SOUTHERN ALBANIA

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The aim of this study was to investigate the yield and chemical composition of the essential oil (EO) isolated from over-ground parts of different populations of *Hypericum perforatum* L. (Hypericaceae) (HP) from southern Albania. The EO yield of 11 specimens of indigenous populations of HP ranged from 2.50 ml/kg to 11.00 ml/kg. GC/FID/MS analyses of the EOs revealed a total of 126 identified compounds representing 77.35–88.29% of the oils. Based on the prevalence of principal components, two types of EO were distinguished: pinene-type, which included seven populations with EO rich in α-pinene, and caryophyllene-type, which included four populations with EO rich in *trans*-(*E*)-caryophyllene and caryophyllene oxide. The information obtained can help to assess the potential of the studied Albanian populations for further sustainable wild exploitation to take it into a consideration as a resource of valuable genetic material or for further cultivation and breeding.

Keywords: Saint John's wort; GC/FID/MS; wild populations; types of oil

СОСТАВ НА ЕТЕРИЧНО МАСЛО ОД ДИВОРАСТЕЧКИ ПОПУЛАЦИИ НА *HYPERICUM PERFORATUM* L. ОД ЈУЖНА АЛБАНИЈА

Целта на оваа студија беше да се испита приносот и хемискиот состав на етеричните масла (ЕО) изолирани од надземниот дел на различни популации на растението *Hypericum perforatum* L. (Hypericaceae) (HP) од јужна Албанија. Содржината на маслото од 11 диворастечки популации на HP се движи од 2,50 ml/kg до 11,00 ml/kg. Со GC/FID/MS беше извршена анализа на соодветните етерични масла, беа идентификувани вкупно 126 компоненти, што претставува 77,35% до 88,29% од вкупната содржина на маслата. Врз основа на застапеноста на главните компоненти, се разликуваат два типа масла: пиненски тип, во кој спаѓаат седум популации чијашто главна индивидуална компонента е α-пинен, и кариофиленски тип, во кој спаѓаат четири популации богати со *trans*-(*E*)-кариофилен и кариофилен оксид. Добиените резултати можат да придонесат за процена на потенцијалот на албанските диворастечки популации за понатамошна нивна експлоатација, но и нивно користење како вреден извор на генетски материјал или за нивно култивирање и размножување.

Клучни зборови: кантарион; GC/FID/MS; диворастечки популации; типови на етерично масло

1. INTRODUCTION

The *Hypericum* species are perennial plants distributed predominantly in the temperate regions of the world with approximately 470 species in total [1]. *Hypericum perforatum* L. (Hypericaceae) (Saint John's wort), the most thoroughly studied taxon of the genus, is well known for its traditional and contemporary use as a medicinal plant, with a complex mixture of secondary metabolites. Due to the large range of secondary metabolites that are active principals of phytopharmaceuticals, *Hypericum perforatum* (HP*)* possess a plethora of pharmacological activities including antiviral, antimicrobial, anti-inflammatory, antioxidant, antidepressive, hepatoprotective and anti-tumoral [2–6]. An infused oil of the flowers (*Hyperici oleum*), which is prepared by macerating fresh flowers in olive or sunflower oil and exposing the mixture to sunlight for two to three weeks, has a history of traditional use in Europe for the treatment of burns and ulcers [7, 8]. Monographs for the crude drug and extracts of HP, prepared from the aerial flowering parts of the plant, have been fully integrated in the European Pharmacopoeia (since Ph. Eur. 6) [2]. *Hyperici oleum* was included in the German Commission E and the Swiss Pharmacopoeia monographs [7, 9].

The bioactive compounds found in HP comprise the naphthodianthrone derivatives (hypericin and pseudohypericin), acylated phloroglucinol derivatives (hyperforin and adhyperforin), flavonoids (quercetin, quercitrin, hyperoside, rutin, kaempferol, biapigenin and amentoflavone), phenolic acids (chlorogenic acid) and xanthones (mangiferin) [4, 5]. A number of studies refer to the composition of the essential oil (EO) of HP [10–16]. Typical HP EO constituents for the material collected in southern Europe (France, Italy, Turkey and Serbia) include the monoterpenes α- and β-pinene, limonene and myrcene, the sesquiterpenes *trans*-(*E*)-caryophyllene and caryophyllene oxide [17–20] and hydrocarbons such as *n*-decane, C_{16} and C_{29} alkanes and C_{24}, C_{26} and C_{28}-alkanols [21]. *Hypericum* species are generally classed as essential oil-poor plants (oil yield <1.00 %, w/w) [22, 23]. The content of EO in HP is highest during the full-bloom stage versus the pre-bloom or fruiting stage (0.35% versus 0.12% and 0.18%, respectively) [23–25]. Despite the poor content, the steam-distilled EO of HP has recently become available on a broader scale on the market and is largely produced by small companies in Europe (Poland, France), especially in some South East European countries (Serbia, Croatia and Bulgaria) [26]. Despite the abundant literature data about the EO composition of HP growing in southern Europe, to date, there are no data about chemical investigations performed on samples from Albania. Therefore, the aim of this study was to investigate the yield and the chemical composition of the EO isolated from over-ground parts of different populations of HP from southern Albania, estimating the chemical potential of wild exploitation of this plant for HP EO isolation.

2. EXPERIMENTAL SECTION

Plant material. Aerial flowering parts of *Hypericum perforatum* populations from 11 different locations from southern Albania were collected. The plant material was air dried, packed in paper bags and kept in a dark and cold place until analysis. Plant identity was verified and voucher specimens were deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, R. Macedonia (Table 1).

Essential oil isolation. The EO was isolated from dried and minced plant material by hydrodistillation in all-glass Clevenger apparatus for 2 hours according to Ph. Eur. The obtained oil was dried with anhydrous Na_2SO_4 and dissolved in hexane for further analyses.

Analysis of essential oils' chemical composition. EO samples in hexane (1:1000) were analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C mass spectrometer. For that purpose, HP-5 ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm) was used. Analytical conditions were as follows: oven temperature at 60 °C (0 min), 3 °C/min to 240 °C (1 min) and at the end increased to 280 °C at a rate of 10 °C/min (1 min); helium as carrier gas at a flow rate of 1 ml/min; injector temperature 220 °C and that of the FID detector 270 °C. One μl of each sample was injected at a split ratio of 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C, and mass range from 50–550 Da. The MS was operated in scan mode.

Identification of the components. The compounds were identified on the basis of literature [27] and estimated Kovat′s (retention) indices that were determined using a mixture of homologous series of normal alkanes (C9-C25) analyzed under Automated Mass Spectral Deconvolution and Iden-

tification System (AMDIS)' conditions [28]. Confirmation was made by comparing the mass spectra of the components present in the EOs with the reference spectra obtained from Nist, Wiley and Adams mass spectra libraries. Quantification of the EOs components was performed using the normalization method of the GC/FID peak areas without any correction factors.

Statistical analysis. The Principal Component Analysis (PCA) based on seven main essential oil constituents was performed using SIMCA 13 [29].

T a b l e 1

Sampling localities and essential oil (EO) yields of 11 Hypericum perforatum L. populations from southern Albania

Population	(Albania) Town	Latitude (N)	Longitude (E)	Height above the sea level (m)	Date of getting the sample	EO yield ml/kg
HP01	Kolonja	40°18'42.75"	20°39'55.42"	1095	12.7.2011	5.00
HP02	Korca	40°36'50.90"	20°46'39.81"	875	12.7.2011	4.00
HP03	Pogradec	40°54'01.83"	20°39'50.12"	742	12.7.2011	4.00
HP04	Elbasan	41°07'01.68"	20°05'32.97"	284	13.7.2011	3.00
HP05	Tirana	41°19'57.88"	19°49'53.23"	163	15.7.2011	2.50
HP06	Berat	50°42'31.19"	19°56'37.30"	234	13.7.2011	10.00
HP07	Lushnje	40°56'00.08"	19°42'00.11"	226	13.7.2011	4.00
HP08	Fier	40°43'48.47"	19°34'23.41"	215	13.7.2011	11.00
HP09	Tepelene	40°18'00.03"	20°00'59.96"	674	14.7.2011	4.00
HP10	Gjirokastra	40°04'45.11"	20°08'53.53"	318	14.7.2011	11.00
HP11	Saranda	39°52'36.42"	20°00'00.68"	243	14.7.2011	9.00

3. RESULTS AND DISCUSSION

Essential oil yield. The EO yield of 11 specimens of indigenous populations of HP from southern Albania ranged from 2.50 ml/kg to 11.00 ml/kg (0.25–1.10 %) (Table 1). The highest yield was recorded in two indigenous populations from the regions of Fier and Gjirokastra (HP08 and HP10, respectively) while the lowest was found in the population located in Tirana region (HP05). Differences in the EO content were evident when comparing populations from the vicinity of the Saranda, Gjirokastra, Fier and Berat, which are cities located closer to the Ionic See, to those from Tirana and Lushnje, located more northern, and Podgradec, Kolonja, Korcha and Elbasan, located more eastern. According to literature data, large variation in EO content (0.04–1.93%) was also reported for plants from six different localities in Serbia [30]. Additionally, the yields of EOs from HP from Kosovo differed greatly depending on the population and ranged from 0.04 to 0.26% based on dry weight [31].

EO composition. Data analysis of the EO chemical composition revealed six different classes of components: monoterpene hydrocarbons (MH), oxygen-containing monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygen-containing sesquiterpenes (OS), diterpenes (D) and non-terpene components (NT). Generally, MH and SH were dominant fractions in all tested samples. In this order, six populations contained EO rich in SH, while the EOs isolated from five other populations showed the MH fraction to be dominant. Additionally, oxygen-containing derivatives were present in lower amounts. Nine of the 11 populations encompassed EO that contained more OS, while only two EO populations had OM as the main fraction. Regarding the content of diterpenes (D), it is important to emphasize that manool is the only diterpene compound that was identified in only one EO sample. Beside terpene compounds, these EOs contained high levels of other non-terpene components (NT) which mainly consisted of aliphatic chains (Table 2).

T a b l e 2

Chemical composition (%) of the essential oils from 11 Hypericum perfratum L. populations, HP01-HP11

No.	Components	KIL[a)]	KIE[b)]	HP01	HP02	HP03	HP04	HP05	HP06	HP07	HP08	HP09	HP10	HP11
1	Cumene	926	929	2.67	2.66	3.71	1.78	-	0.86	0.90	0.37	0.66	-	-
2	α-Pinene	939	941	13.37	14.03	12.65	2.03	20.61	7.03	29.48	1.15	15.31	36.74	26.52
3	Camphene	953	957	0.09	-	-	-	-	-	-	-	-	-	-
4	Propylbenzene	960	964	0.10	0.06	0.11	0.10	-	0.04	-	0.06	0.08	-	-
5	3-Methyl-nonane	971	974	0.63	0.77	0.73	0.76	1.55	0.98	1.82	0.54	1.56	2.76	2.41
6	Sabinene	976	984	-	-	-	-	-	0.04	-	-	-	-	-
7	β-Pinene	980	985	1.88	5.55	3.99	0.36	6.81	0.62	2.98	0.30	6.89	6.70	1.95
8	β-Myrcene	991	996	0.23	0.33	0.33	0.15	0.93	0.31	0.90	0.10	0.66	0.39	0.60
9	*n*-Decane	999	999	0.06	-	-	0.06	0.16	0.08	0.20	0.03	0.14	0.18	0.23
10	α-Phellandrene	1005	1010	-	-	-	-	0.05	0.03	0.04	-	-	0.08	0.06
11	α-Terpinene	1018	1023	-	-	-	-	0.07		0.04	-	-	0.06	0.06
12	o-Cymene	1022	1029	-	0.05	0.05	0.06	0.17	0.12	0.16	-	0.29	0.91	0.37
13	Limonene	1024	1031	-	0.28	0.28	0.12	0.86	0.34	0.60	0.07	0.70	2.03	0.86
14	1,8-Cineole	1026	1034	-	0.05	0.04	-	-	-	-	-	-	-	0.29
15	β-*Z*-Ocymene	1032	1035	-	-	-	0.06	0.12	0.05	-	0.06	-	-	-
16	β-*E*-Ocymene	1044	1039	0.18	0.24	0.22	0.24	0.38	0.18	0.35	0.26	0.29	0.06	0.14
17	γ-Terpinene	1062	1069	-	0.06	0.08	-	0.21	-	0.15	-	-	0.24	0.21
18	2-Methyl-decane	1064	1070	1.05	1.54	1.65	1.02	2.28	1.67	2.73	0.82	2.95	3.14	2.67
19	*cis*-Linalol oxide	1067	1073	0.13	0.12	-	-	-	0.09	-	0.04	-	-	-
20	*trans*-Linalool oxide	1088	1090	-	-	0.09	-	-	-	0.57	-	-	-	-
21	α-Terpinolene	1088	1091	-	-	-	0.07	-	-	-	-	0.25	-	-
22	*n*-Undecane	1100	1099	0.63	0.98	1.01	0.65	1.24	0.81	1.47	0.49	1.34	1.37	1.03
23	Nonanal	1098	1103	0.09	0.08	0.08	0.05	0.04	0.07	-	0.04	0.13	-	-
24	*cis*-Thujone	1101	1111	-	-	-	-	-	-	-	-	-	0.13	0.14
25	*endo*-Fenchol	1121	1127	-	-	-	-	0.07	-	0.04	-	-	0.41	0.04
26	α-Campholenal	1125	1130	0.19	0.26	0.26	0.05	0.49	0.28	0.75	0.07	0.46	3.17	1.14
27	*trans*-Pinocarveol	1135	1139	0.16	0.18	0.20	0.07	0.27	0.14	0.37	0.09	-	1.72	-
28	*cis*-Verbenol	1137	1142	-	-	-	-	-	-	-	-	-	-	0.13
29	*trans*-Verbenol	1140	1145	0.27	-	0.38	0.07	0.39	-	0.40	0.12	-	2.17	-
30	Camphor	1141	1149	-	-	-	-	-	-	-	-	-	-	1.10
31	*trans*-Pinocamphone	1160	1166	-	-	-	-	-	-	-	-	-	-	0.04
32	Pinocarvone	1162	1167	tr.	-	-	-	0.07	-	0.09	-	-	-	0.09
33	Borneol	1165	1171	0.34	0.32	0.30	-	-	-	0.33	-	-	-	-
34	*p*-Mentha-1,5-dien-8-ol	1172	1177	-	-	-	-	-	-	-	0.13	-	1.82	0.64
35	Terpinene-4-ol	1177	1181	-	0.09	0.10	0.06	0.12	0.09	0.07	0.08	-	0.21	0.11
36	*p*-Cymene-8-ol	1183	1187	-	-	0.09	0.05	-	-	0.08	-	-	0.12	0.15
37	α-Terpineol	1189	1196	0.15	0.23	0.20	0.08	0.27	0.10	0.17	0.07	0.19	0.74	0.21
38	Myrtenol	1194	1200	0.11	0.14	0.16	0.06	0.20	0.11	0.19	0.07	-	-	0.31
39	Methyl chavicol	1195	1201	-	-	-	-	-	-	-	-	0.40	-	-
40	Myrtenal	1195	1202	-	-	-	-	-	-	-	-	-	0.51	-
41	Verbenone	1204	1207	0.23	0.22	0.31	0.07	0.28	0.11	0.20	0.13	0.33	2.41	0.57
42	*trans*-Carveol	1215	1219	0.12	0.13	0.11	0.02	0.16	0.13	0.19	0.05	0.17	1.12	0.49
43	*trans*-Chrys-anthemyl acetate	1235	1240	-	0.09	0.12	-	-	-	-	-	-	-	-
44	Carvacrol methyl ether	1240	1246	-	-	-	-	-	-	-	-	-	-	0.24
45	Carvone	1243	1247	-	-	-	-	-	-	-	-	-	0.12	-

T a b l e 2 (cont.)

No.	Components	KIL[a)]	KIE[b)]	HP01	HP02	HP03	HP04	HP05	HP06	HP07	HP08	HP09	HP10	HP11
46	Carvotanacetone	1246	1251	-	-	-	-	-	-	-	-	-	0.15	0.05
47	Gearniol	1249	1255	-	0.17	0.16	0.05	-	-	0.17	-	0.21	-	-
48	*cis*-Geraniol	1255	1259	-	-	-	-	0.14	-	-	-	-	-	-
49	2-Methyl dodecane	1268	1273	1.12	2.06	2.24	0.78	1.26	-	1.51	-	2.11	1.30	0.85
50	*E*-Anethole	1282	1286	-	-	-	-	-	-	-	-	5.79	0.54	0.43
51	Bornyl acetate	1285	1291	-	-	0.10	0.06	0.05	0.06	0.06	0.07	-	-	-
52	Thymol	1290	1299	0.13	0.15	0.12	0.06	0.11	0.08	0.05	0.08	-	0.22	1.43
53	Tridecane	1294	1301	0.25	0.51	0.58	0.23	0.25	0.19	0.33	0.24	0.55	0.29	-
54	Carvacrol	1298	1353	0.68	0.30	0.28	0.10	0.31	0.22	0.21	0.14	0.32	0.87	5.60
55	α-Longipinene	1350	/	-	0.34	0.56	-	-	-	-	-	0.37	0.54	-
56	α-Cubebene	1351	/	-	-	-	0.19	0.05	0.11	0.06	0.18	-	-	-
57	α-Ylangene	1373	1381	-	0.31	0.38	0.24	0.22	0.18	0.19	0.17	0.28	0.08	0.09
58	α-Copaene	1376	1389	0.37	0.52	0.66	0.46	0.45	0.42	0.55	0.28	0.61	0.21	0.52
59	β-Bourbonene	1384	1397	0.14	0.16	0.18	0.16	0.17	0.24	0.17	0.18	0.35	-	-
60	β-Elemene	1391	1410	tr.	-	-	-	-	0.46	0.11	0.63	-	-	0.20
61	Italicene	1405	1415	-	0.11	0.16	-	-	-	-	-	-	-	-
62	2-*epi*-β-Funebrene	1411	1422	0.26	0.24	-	-	-	-	-	-	-	-	-
63	β-Cedrene	1418	1424	-	-	-	-	0.18	-	-	-	-	-	-
64	*trans*-(*E*)-Caryophyllene	1418	1432	6.22	7.61	4.88	19.27	4.40	10.24	4.24	11.69	5.74	0.50	1.22
65	β-Copaene	1430	1446	0.19	0.34	0.46	-	0.20	-	0.16	-	0.20	-	-
66	Aromadendrene	1439	1452	0.11	0.25	0.38	0.30	0.17	0.11	0.09	0.23	0.23	-	0.11
67	α-Himachalene	1449	1455	0.16	0.17	-	-	-	-	-	-	0.18	0.26	-
68	α-Hummulene	1452	1460	-	-	-	-	-	1.88	-	-	-	-	-
69	β-(*Z*)-Farnesene	1454	1465	2.45	2.98	3.76	2.47	1.67	-	1.01	4.40	2.39	0.19	0.94
70	Sesquisabinene	1457	1471	-	-	-	2.48	-	-	-	-	-	-	-
71	4,5-di-*epi*-Aristolochene	1467	1476	-	-	-	-	-	-	-	0.86	-	-	0.52
72	Dodecanol	1473	1482	5.22	1.71	1.55	6.69		4.76	2.00	-	-	-	1.02
73	γ-Muurolene	1477	1486	-	-	2.20	1.45	2.82	-	-	4.05	1.49	0.29	0.42
74	Germacrene D	1480	1489	-	1.82	1.05	-	-	-	-	-	0.76	-	-
75	β-Selinene	1485	1499	7.26	3.21	1.34	6.10	7.05	11.38	6.42	13.86	1.87	3.73	10.26
76	α-Selinene	1494	1501	6.16	3.61	1.93	7.01	6.15	9.89	4.48	10.63	2.04	0.41	5.99
77	α-Muurolene	1495	1512	-	-	0.35	-	-	-	-	-	-	-	-
78	α-Farnesene	1505	1516	-	-	0.23	-	-	-	-	-	-	-	-
79	β-Bisabolene	1505	1525	0.27	-	-		-	-	-	-	-	-	-
80	γ-Cadinene	1513	1528	0.51	0.78	1.06	1.08	0.61	0.60	0.44	0.87	0.74	0.18	0.38
81	7-*epi*-α-Selinene	1520	1537	0.21	0.10	-	-	0.25	0.36	0.17	0.46	-	-	0.26
82	δ-Cadinene	1524	1541	1.10	1.51	2.12	2.03	1.18	1.13	0.90	1.56	1.44	0.18	0.70
83	*trans*-Cadina-1,4-diene	1532	1551	-	0.38	0.21	0.26	0.16	0.17	0.12	0.29	0.18	-	-
84	α-Cadinene	1538	1569	-	-	0.37	0.31	0.24	0.22	0.18	0.28	0.27	-	0.70
85	α-Calacorene	1547	1573	0.20	0.26	0.34	0.26	0.36	0.17	0.15	0.26	0.25	0.10	0.22
86	*E*-Nerolidol	1564	1580	1.04	1.08	1.59	0.83	0.63	0.88	0.56	1.26	1.03	0.32	0.42
87	Dodecanoic acid	1568	1584	-	-	-	-	-	-	-	-	-	0.32	-
88	Spathulenol	1576	1594	-	1.56	1.62	6.29	0.16	-	3.30	5.18	1.75	0.39	1.25
89	Caryophyllene oxide	1581	1596	11.32	5.38	4.17	3.50	4.32	12.35	3.01	2.93	5.11	1.15	1.70
90	Viridiflorol	1590	1607	-	-	0.54	-	-	-	-	-	-	-	-
91	Longiborneol	1592	1611	-	-	0.38	-	-	-	-	-	-	-	-
92	Ledol	1602	1622	-	0.40	-	-	0.41	-	-	0.87	-	-	-
93	Humulene epoxide II	1606	1631	-	0.49	0.87	-	-	-	0.38	-	-	-	-

T a b l e 2 (cont.)

No.	Components	KIL[a)]	KIE[b)]	HP01	HP02	HP03	HP04	HP05	HP06	HP07	HP08	HP09	HP10	HP11
94	1,10-di-*epi*-Cubenol	1618	1640	-	-	-	-	-	-	-	-	0.33	-	-
95	1-*epi*-Cubenol	1627	1645	-	-	0.40	0.24	-	-	0.22	0.35	-	-	-
96	*cis*-Cadin-4-en-7-ol	1635	1649	-	-	-	-	-	-	-	-	-	0.51	-
97	*epi*-α-Murrolol+*epi*-α-Cadinol	1641	1655	-	-	5.16	-	-	-	-	-	-	-	-
98	α-Muurolol (Torreyol)	1645	1659	-	1.94	-	-	-	-	-	-	-	-	-
99	Vulgarone B	1649	1661	-	-	0.37	-	-	-	-	-	-	0.14	-
100	α-Cadinol	1653	1675	-	-	0.70	1.79	-	-	-	2.96	0.65	-	-
101	*neo*-Intermedeol	1656	1681	2.51	-	-	-	1.63	2.38	1.59	-	-	-	1.68
102	*n*-Tetradecanol	1671	1730	7.63	7.10	-	-	1.83	-	2.57	2.28	-	1.60	-
103	α-Cadalene	1675	1764	-	-	7.77	-	4.35	-	-	-	-	-	-
104	Amorpha-4,9-dien-2-ol	1700	1765	-	-	-	-	-	-	3.00	-	-	-	-
105	Methyl tetradecanoate	1726	1778	0.25	0.22	0.32	-	0.33	0.25	0.21	0.33	0.42	0.07	0.18
106	Cyclocolorenone	1759	1780	-	0.10	-	-	-	-	-	0.23	-	-	-
107	Benzyl benzoate	1759	1839	0.23	0.15	0.32	-	-	0.16	0.14	0.37	0.37	-	0.21
108	*n*-Pentadecanol	1773	1854	-	-	-	6.92	0.11	-	-	-	-	0.07	-
109	*epi*-Cyclocolorenone	1774	1886	-	-	-	-	0.15	-	-	-	-	-	-
110	2-Pentadecanone	1835	1888	0.72	-	1.66	-	-	0.64	0.53	0.86	1.09	-	0.43
111	6,10,14-trimethyl 2-Pentadecanone	1848	1899	-	1.32	-	0.81	0.67	-	-	-	-	0.43	-
112	*n*-Hexadecanol	1879	1933	0.85	2.48	1.04	1.73	-	1.05	0.88	-	0.71	0.23	0.15
113	Cyclohexadecane	1881	1975	-	-	-	-	2.24	-	-	1.78	-	-	-
114	Nonadecane	1900	2001	0.39	0.61	0.91	0.23	0.17	0.18	0.18	0.35	0.68	0.12	0.15
115	Methyl hexadecanoate	1927	2062	0.38	0.70	0.69	0.54	-	0.53	0.49	-	0.70	-	0.29
116	Hexadecanoic acid	1970	2099	0.55	0.11	0.96	0.52	0.81	0.53	0.41	1.29	0.48	0.22	0.28
117	Eicosane	2000	2101	0.17	0.27	0.26	0.09	0.09	0.09	0.09	0.20	0.23	0.04	0.06
118	Manool	2056	2129	-	-	-	-	-	-	-	-	-	-	0.07
119	Methyl linoleate	2095	2145	-	0.20	0.19	0.18	0.24	0.12	-	0.13	0.18	0.07	0.06
120	Heneicosane	2100	2199	0.99	2.18	2.16	0.94	0.97	0.76	0.84	1.36	1.58	0.44	0.60
121	Methyl octadecanoate	2124	2300	-	-	-	0.04	0.06	-	-	0.04	-	-	-
122	(Z)-9-Octadecenoic acid	2137	2401	-	-	-	-	-	-	-	0.12	-	-	-
123	Docosane	2200	2501	0.10	0.20	0.26	0.09	0.07	0.07	0.08	0.10	0.20	0.05	0.05
124	Tricosane	2300	1730	0.24	0.59	0.75	0.27	0.23	0.21	0.25	0.29	0.55	0.38	0.28
125	Tetracosane	2400	1764	-	-	0.08	0.03	-	-	0.02	0.03	-	-	-
126	Pentacosane	2500	1765	-	0.37	0.22	0.22	0.08	-	0.03	0.16	0.14	0.04	0.30

Chemical classes	HP01	HP02	HP03	HP04	HP05	HP06	HP07	HP08	HP09	HP10	HP11
Monoterpene hydrocarbons (MH)	15.75	20.54	17.60	3.09	30.21	8.72	34.70	1.94	24.39	47.21	30.77
Oxygen-containing monoterpenes (OM)	2.51	2.45	3.02	0.80	2.93	1.41	3.94	1.14	1.68	15.89	12.77
Sesquiterpene hydrocarbons (SH)	25. 61	24.70	22.62	44.07	26.33	37.56	19.44	50.88	19.39	6.67	22.53
Oxygen-containing sesquiterpenes (OS)	14.87	10.95	15.80	12.65	7.30	15.61	12.06	13.78	8.87	2.51	5.05
Diterpenes (D)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
Non-terpene components (NT)	24.32	26.87	29.25	24.73	19.03	14.05	17.68	12.28	23.04	13.66	11.68
Total (%)	**83.06**	**85.51**	**88.29**	**85.34**	**85.80**	**77.35**	**87.82**	**80.02**	**77.37**	**85.94**	**82.87**

[a)] KIL - Kovat's Index Literature [27]; [b)] KIE - Kovat's Index Estimated (AMDIS) [28]; tr. = traces (< 0.02%).

In several Lithuanian HP samples, the sesquiterpene hydrocarbons and oxygenated sesquiterpenes made up 62.00–81.80% of the EO. The aliphatic compounds (2-methyl octane, *n*-nonane, *n*-decane, *n*-undecane, *n*-tetradecanol, *etc*.) varied from 1.70 to 19.60% [29]. Terpenoid constituents, particularly sesquiterpenes, were described as primary components in Serbian HP EOs [30]. In Sardinia the sesquiterpene hydrocarbons had the highest contribution (36.80%). This fraction was dominated by germacrene D (17.60%). The monoterpene hydrocarbons represented 28.40% of the total EO with α-pinene (15.80%) as the major compound. The oxygenated sesquiterpenoid fraction was relatively poor. It represented 4.60% of the total EO with *cis*-cadin-4-en-7-ol (1.70%) as the major constituent. The oxygenated monoterpenes constituted only 1.00%. Compared to these results, our tested EOs contained 6.67–50.88% of sesquiterpene hydrocarbons with *trans*-(*E*)-caryophyllene (0.50–19.27%), and α- and β-selinene (0.41–10.63% and 1.34–13.86%, respectively) as the dominant components and 1.94–47.21% of monoterpene hydrocarbons with α-pinene as the dominant component (1.15–36.74%). Furthermore, the content of oxygen-containing derivatives, both mono- and sesquiterpenes, was the opposite, as our tested samples comprised 0.80–15.89% of oxygen-containing mono- and 2.51–15.80% of oxygen-containing sesquiterpenes.

GC/FID/MS analyses of the isolated EOs revealed a total of 126 compounds representing 77.35–88.29% of the oils (Table 2). Twenty five components were detected in all analyzed EOs and seven of these compounds were found in amounts higher than 3.00%. These components were considered principal components of HP EOs: α-pinene (2.03–36.74%), β-pinene (0.36–6.89%), 2-methyldecane (0.82–3.14%), carvacrol (0.14–5.60%), *trans*-(*E*)-caryophyllene (0.5–19.27%), β-selinene (1.34–13.86%) and caryophyllene oxide (1.15–12.35%). Although chemical diversity of the HP EOs was high, two main types of HP EOs can generally be distinguished: pinene-type (populations HP02, HP03, HP05, HP07, HP09, HP10 and HP11 with EO reach in α-pinene) and caryophyllene-type (populations HP01, HP04, HP06 and HP08 with EO rich in *trans*-(*E*)-caryophyllene and caryophyllene oxide), which was confirmed by PCA analysis (Fig. 1).

Concerning the dominant components in the EOs from HP growing wild in Lithuania, three different chemotypes (*trans*-(*E*)-caryophyllene, caryophyllene oxide and germacrene D) have been reported [32].

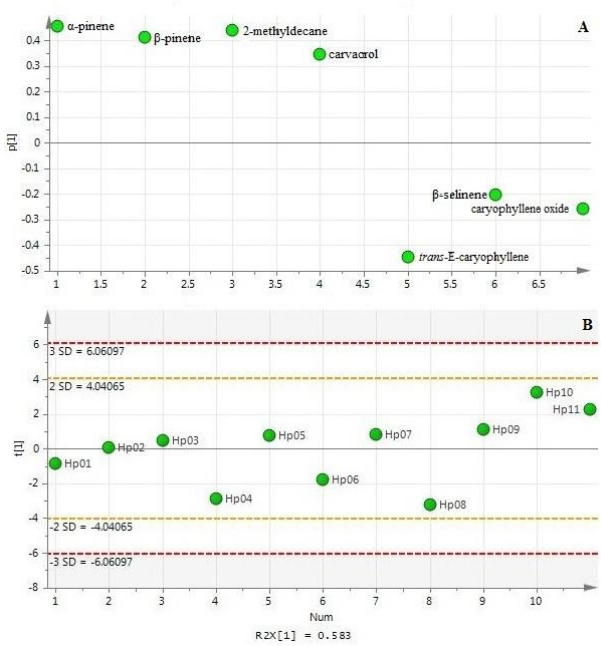

Fig. 1. PCA analysis of the seven main EO components and their influence (**A**) on the distinguishing two types of populations (pinene-type, above baseline and caryophyllene-type, below baseline) (**B**)

In general, the components β-pinene, α-pinene, 2-methyloctane, spathulenol, caryophyllene oxide, germacrene D and *trans*-(*E*)-caryophyllene were the most frequently reported constituents in *Hypericum* EOs [33]. For instance, the EO of HP from southeastern France was rich in α-pinene and the EO of HP ssp. *angustifolium*, also from France, was characterized by spathulenol (21.10%) and tetradecanol (9.10%) [19, 20, 34]. On the other hand, 2-methyloctane (21.10%), germacrene-D (17.60%) and α-pinene (15.80%) were the major compounds in the same subspecies in Italy [35].

Germacrene D was the main component found in the hydrodistilled EOs from wild-grown (22.80%) and cultivated plants (16.90%) from several regions of Greece, followed by 2-methyloctane (10.80–17.80%), *trans*-(*E*)-caryophyllene (6.60–10.30%), α-pinene (5.20–10.10%) and bicyclogermacrene (4.10–4.80%) [36]. In the same way, α- and β-pinene were found to be the main components in another study of Greek HP [37].

HP collected from the Barelić region in Serbia contained 8.60% of α-pinene, but it was absent from plant material from the Rujan region [30]. In the same study, *trans*-(*E*)-caryophyllene and caryophyllene oxide were reported to be principal components. In the region of Kosovo, HP were characterized by the following main constituents: α-pinene (3.70-36.50%), 2-methyl-octane (1.10–15.50%), *trans*-(*E*)-caryophyllene (1.20–12.40%),

caryophyllene oxide (3.30–17.70%) and *n*-tetradecanol (3.60–10.40%) [31].

According to these data, our EOs have the largest similarity to the HP EOs from the neighboring regions of Greece, Serbia and Kosovo, as α-pinene, *trans*-(*E*)-caryophyllene and caryophyllene oxide were found to be the predominant components.

According to some authors, qualitative and quantitative variation in the content of secondary metabolites in HP is influenced by genetic, ecological and environmental factors [38, 39]; thus, it is very difficult to compare data about EO with scarce information on the status of plant material (fresh/dry) used for hydrodistillation, as well as the plant development status or the environmental variables of collection sites. Also, EOs from HP wild populations and cultivated in uniform conditions are very likely to be genetically determined because no considerable differences were found in the composition of the volatile constituents [18]. However, obtained information about EO yield and composition of the studied Albanian HP can help to assess the potential of these populations for further sustainable wild exploitation and can also be taken into a consideration as a resource of valuable genetic material for further cultivation and breeding.

REFERENCES

[1] N. K. B. Robson, *Hypericum Botany, Hypericum: The Genus Hypericum.* Taylor and Francis, New York, USA, 2003.

[2] European Pharmacopoeia, 6th Ed. Council of Europe, Strasbourg, France, 2008.

[3] R. Berghöfer, *Analytik und Isolierung phenolischer Inhaltsstoffe von Hypericum perforatum L. aus Anbau und Wildvorkommen und Vergleich mit anderen heimischen Hypericum-Arten*. Cramer, Stuttgart, Berlin, Germany, 1987.

[4] K. Linde, G. Ramirez, C. D. Mulrow, A. Pauls, W. Weidenhammer, D. Melchart, St. John's wort for depression: an overview and meta-analysis of randomized clinical trials. *Br. Med. J*., **313**, 253–258 (1996).

[5] S. L. Crockett, N. K. B. Robson, Taxonomy and chemotaxonomy of the genus *Hypericum*. *Medicinal and Aromatic Plant Sci. Biotech*. (in press).

[6] W. E. Müller, *St. John's wort and its active principles in depression and anxiety*, Birkhäuser Verlag, Basel, Switzerland, 2005.

[7] American Botanical Council, *St. John's Wort*. The Complete German Commission E monographs: Therapeutic guide to herbal medicines, Austin, Texas, 1998, pp. 214–215.

[8] European Medicines Agency, *Assessment report on Hypericum perforatum L., herba*, London, UK, 2008.

[9] Commission Suisse de Pharmacopée. *Pharmacopoea Helvetica*. 8th ed. Département fédéral de l'intérieur*;* Berne, Switzerland, 1997.

[10] J. Reichling, A. Weselerand, R. Saller, A current review of the antimicrobial activity of *Hypericum perforatum* L., *Pharmacopsychiatry*, **34**, 116–118 (2001).

[11] S. Kasper, *Hypericum perforatum*–A review of clinical studies, *Pharmacopsychiatr.y*, **34**, 51–55 (2001).

[12] I. Lopez-Bazzocchi, J. B. Hudson, G. H. Towers, Antiviral activity of the photoactive plant pigment hypericin, *Photochem. Photobiol*., **54**, 95–98 (1991).

[13] D. Meruelo, G. Lavie, D. Lavie, Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin, *Proc. Natl. Acad. Sci. USA*, **85**, 5230–5234 (1988).

[14] P. Miskovsky, Hypericin - a new antiviral and antitumor photosensitizer: mechanism of action and interaction with biological macromolecules, *Curr. Drug Targets*, **3**, 55–84 (2002).

[15] A. I. Gurevich, V. N. Dobrynin, M. N. Kolosov, S. A. Popravko, I. D. Riabova, Antibiotic hyperforin from *Hypericum perforatum* L., *Antibiotiki*, **16**, 510–513 (1971).

[16] P. Maisenbacher, K. A. Kovar, Analysis and stability of *Hyperici oleum*, *Planta Med.*, **58**, 351–354 (1992).

[17] R. Melzer, U. Fricke, J. Holzl, Vasoactive properties of procyanidins from *Hypericum perforatum* L. in isolated porcine coronary arteries, *Arzneimittel-Forschung*, **41**, 481–483 (1991).

[18] P. A. De Smet, W. A. Nolen, St John's wort as an antidepressant, *Br. Med. J.*, **313**, 253–258 (1996).

[19] I. Schwob, J. M. Bessiere, J. Viano, Composition of the essential oils of *Hypericum perforatum* L. from southeastern France, *C. R. Biologies*, **325**, 781–785 (2002).

[20] C. Mathis, G. Ourisson, Chemotaxonomic study of the genus *Hypericum*. IV. Distribution of sesquiterpenes, monoterpene alcohols, and saturated aldehydes in the essential oils of *Hypericum*. *Phytochemistry*, **3**, 377–378 (1964).

[21] A. Nahrstedt, V. Butterweck, Biologically active and other chemical constituents of the herb of *Hypericum perforatum* L., *Pharmacopsychiatry*, **30** (Suppl.), 129–134 (1997).

[22] L. Stjepanović, M. Ćorović, R. Nikolić, S. Palović, P. Živanović, Essential oil content and number of oil vesicles in *Hypericum* species from various

habitats of Tara mountain, *Arhiv za farmaciju*, **15**, 177–188 (1965).

[23] L. Roth, *Hypericum, hypericin: Botanik, Inhaltstoffe, Wirkung*. Ecomed Verlagsgesellschaft GmbH, Landsberg, Germany, 1990.

[24] R. Omidbaigi, M. Azizi, Effect of time of harvest on hypericin and essential oil content of *Hypericum perforatum* L. from Iran, *Iran Agric. Res.*, **19**, 155–164 (2000).

[25] R. Lutz, The essential oils of different St. John's worts, *Ätherische Öle, Riechstoffe, Parfümerien Essenzen und Aromen*, **2**, 137–139 (1952).

[26] Personal communications: Aura Cacia (Urbana, Iowa, USA) and Mountain Rose Herbs (Eugene, Oregon, USA).

[27] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, Allured Publishing Corporation, IL, USA, 2007.

[28] Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1.), National Institute of Standards and Technology (NIST), Standard Reference Data Program, Gaithersburg, MD (USA).

[29] SIMCA 13®, Umetrics AB, Sweden.

[30] B. Gudzić, S. Đordević, R. Palić, G. Stojanović, Essential oils *Hypericum olympicum* L. and *Hypericum perforatum* L., *Flavour Frag. J.*, **16**, 201–203 (2001).

[31] A. Hajdari, B. Mustafa, D. Nebija, A. Kashtanjeva, J. Widelski, K. Glowniak, J. Novak, Essential oil composition and variability of *Hypericum perforatum* L. from wild population in Kosovo, *Curr. Issues Pharm. Med. Sci.*, **27**, 51–54 (2014).

[32] D. Mockute, G. Bernotiene, A. Judzentiene, Volatile compounds of the aerial parts of wild St. John's wort (*Hypericum perforatum* L.) plants, *Chemija (Vilnius)*, **14**, 108–111 (2003).

[33] S. L. Crockett, Essential Oil and Volatile Components of the Genus *Hypericum* (Hypericaceae), *Nat. Prod. Commun.*, **5**, 1493–1506 (2010).

[34] I. Schindler, J.-M. Bessiere, J. Viano, Composition of the essential oils of *Hypericum perforatum* L. from southeastern France, *C. R. boil.*, **325**, 781–785 (2002).

[35] G. Pintore, M. Chessa, G. Boatto, R. Cerri, M. Usai, B. Tirillini, Essential oil composition of *Hypericum perforatum* L. var. *angustifolium* DC Growing Wild in Sardinia (Italy), *J. Essent. Oil Res.*, **17**, 533–535 (2005).

[36] P. S. Chatzopoulou, T. V. Koutsos, S. T. Katsiotis, Chemical Composition of the Essential Oils from Cultivated and Wild Grown St. John's wort (*Hypericum perforatum*), *J. Essent. Oil Res*., 2006, **18**, 643 (2006).

[37] P. V. Petrakis, M. Couladis, V. Roussis, A method for detecting the biosystematic significance of the essential oil composition: The case of five Hellenic *Hypericum* L. species, *Biochem. Syst. Ecol.*, **33**, 873–898 (2005).

[38] E. Miraldi, M. Biagi, D. Giachetti, Chemical constituents and effects of topical application of Oleum Hyperici on skin sensitivity to stimulated sun exposure, *Nat. Prod. Commun*., **1**, 209–213 (2006).

[39] G. Zdunić, D. Godevac, M. Milenković, D. Vucićević, K. Savikin, N. Menkovic, S. Petrovic, Evaluation of *Hypericum perforatum* oil extracts for an anti-inflammatory and gastroprotective activity in rats, *Phytother. Res.*, **23**, 1559–1564 (2009).