SUPPLEMENTARY MATERIAL

Phytochemical analysis of *Saponaria officinalis* L. shoots and flowers essential oils

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Abstract: Phytochemical analysis by GC and GC/MS of the essential oil samples obtained from fresh shoots and flowers of *Saponaria officinalis* L. allowed the identification of 96 components in total, comprising 94.7% and 86.0% of the total oils compositions, respectively. Regarding the shoots essential oil, the major of 87 identified volatile compounds were phytol (14.1%), tricosane-6,8-dione (13.4%), patchouli alcohol (7.9%) and tricosane (7.2%), whereas patchouli alcohol (20.0%), heneicosane (11.5%) and tricosane (8.4%) were dominant among the 66 volatiles in the flower oil. Nonterpenoid compounds had the highest contribution in *Saponaria officinalis* shoots essential oil (53.7%) while in the flower oil, constituents were almost evenly distributed between the oxygenated sesquiterpenoid (41.2%) and nonterpenoid compounds (39.5%).

Keywords: *Saponaria officinalis* L., essential oil, chemical composition, GC/MS.

Experimental

Plant material and essential oil preparation

Aerial parts of wild *S. officinalis* population, about 46 individuals, were collected in the vicinity of the urban area of the city of Niš, South Serbia, during Jun 2016. The plant was identified by us and a voucher specimen was deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, under the acquisition number 13104. The ground plant

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material of the fresh shoots (585 g) and flowers (95 g) were hydrodistilled for 3 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (1988). The oils were obtained in a yield of 0.006% and 0.009% (w/w) for shoots and flowers essential oils, respectively, trapped in *n*-hexane and dried over anhydrous sodium sulfate. Samples were stored at 4 °C in the dark until analyzed.

GC and GC/MS analysis

GC/MS analyses were performed on an Agilent 7890 gas chromatograph with 7000B GC/MS/MS triple quadrupole system, operating in MS1 scan mode, and equipped with a fused-silica capillary column Agilent HP-5 MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The chromatographic analyses were carried out in the following conditions: He as carrier gas at a flow rate of 1.0 mL/min, GC oven temperature was kept at 70 °C for 2.25 min and programmed to 300 °C at a rate of 5 °C/min and hold for 10 min at that temperature, split ratio was adjusted at 10:1, injection volume 1 μL. Post run: back flash for 1.89 min, at 280 °C, with helium pressure of 50 psi. The injector temperature was set at 300 °C. Ionization mode was electronic impact at 70 eV. Mass range was set from 40 to 560 Da.

For GC/FID analyses, the same column and chromatographic conditions were applied as described for GC/MS. FID detector temperature was 300 °C. The percentage amounts of the separated compounds were calculated from the GC peak areas using the normalization method without correction factors.

The data are reported as mean value of three sample injections.

Identification of volatile compounds

Components were identified by comparison of their mass spectra with those of Wiley 6, Adams 2007, NIST 11 and Essentialoils libraries, applied on Agilent Mass Hunter Workstation (B.06.00) and AMDIS (2.1, *DTRA/*NIST, 2011) software and confirmed by comparing of their relative retention indexes (relative to C8-C32 *n*-alkanes) with authentic standards and components of known oils or values from the literature.

Table S1. Chemical compositions of the *Saponaria officinalis* shoots and flowers essential oils obtained by GC and GC/MS

No	RI	RA	Compound	Content %		Class
				Shoots	Flowers	
1	956	952	Benzaldehyde	/	tr	O
2	959	959	1-Heptanol	0.1	/	O
3	1042	1036	Benzene acetaldehyde	0.1	0.3	O
4	1064	1063	1-Octanol	0.1	tr	O
5	1097	1095	Linalool	0.1	/	MO
6	1167	1165	1-Nonanol	tr	0.2	O
7	1191	1186	α-Terpineol	tr	tr	MO
8	1198	1195	Estragole	tr	/	O
9	1203	1201	Decanal	tr	tr	O
10	1222	1217	β-Cyclocitral	0.1	/	MO
11	1252	1249	Geraniol	tr	0.1	MO
12	1261	1260	(*E*)-2-Decenal	0.1	0.1	O
13	1300	1294	1-nitro-2-Phenylethane	/	0.1	O
14	1315	1315	(*E*,*E*)-2,4-Decadienal	0.1	/	O
15	1322	1319	(*Z*)-3-Hexenyl tiglate	0.2	/	O
16	1386	1383	(*E*)-β-Damascenone	0.1	/	MO
17	1389	1386	(*Z*)-β-Damascone	0.3	tr	MO
18	1392	1390	(*E*)-Jasmone	tr	0.2	MO
19	1399	1392	(*Z*)-Jasmone	1.0	2.1	MO
20	1406	*	Osmorhizol	0.2	0.2	O
21	1416	1413	(*E*)-β-Damascone	0.8	0.2	MO
22	1425	1417	(*E*)-Caryophyllene	tr	0.2	SH
23	1467	1464	9-epi-(*E*)-Caryophyllene	0.3	0.3	SH
24	1470	1469	*n*-Dodecanol	0.1	/	O
25	1478		Unidentified 1	/	1.5	U
26	1480	1478	γ-Muurolene	0.1	0.2	SH
27	1483		Unidentified 2	/	1.0	U
28	1487		Unidentified 3	0.2	0.8	U
29	1495	1495	2-Tridecanone	tr	0.1	O
30	1499	1493	epi-Cubebol	0.8	1.1	SO
31	1507	1509	Tridecanal	0.1	0.4	O
32	1520	1514	Cubebol	0.8	1.7	SO
33	1527	1522	δ-Cadinene	0.2	0.6	SH
34	1536	*	Dihydroactinidiolide	0.1	/	MO
35	1563	1561	(*E*)-Nerolidol	0.1	0.2	SO

36	1572	1565	(3*Z*)-Hexenyl benzoate	0.6	0.2	O
37	1591	*	Torilenol	0.5	1.2	SO
38	1612	*	Copaborneol	1.0	1.3	SO
39	1624	1618	1,10-di-epi-Cubenol	0.3	1.4	SO
40	1630	*	epi-α-Cadinol	/	0.3	SO
41	1634	1627	1-epi-Cubenol	0.5	1.0	SO
42	1647	1640	epi-α-Muurolol	1.2	2.4	SO
43	1652	1644	α-Muurolol	0.3	1.1	SO
44	1661	1652	α-Cadinol	1.4	2.9	SO
45	1665	1660	*cis*-Calamenen-10-ol	0.1	tr	SO
46	1671	1656	Patchouli alcohol	7.9	20.0	SO
47	1677	1676	Mustakone	0.5	0.4	SO
48	1689	1685	Germacra-4(15),5,10(14)-trien-1-α-ol	0.7	0.7	SO
49	1701		Unidentified 4	/	4.8	U
50	1715	1708	Thujopsenal	0.5	1.5	SO
51	1726		Unidentified 5	0.1	0.9	U
52	1745	1739	Oplopanone	/	tr	SO
53	1768	1759	Benzyl benzoate	5.5	4.2	O
54	1773	1766	Drimenol	2.0	2.7	SO
55	1800	1800	Octadecane	0.1	/	O
56	1808	1807	2-Ethylhexyl salicylate	0.2	0.5	O
57	1812	*	Hexadecanal	0.1	/	O
58	1824	1828	Isopropyl myristate	0.3	0.5	O
59	1834	*	Neophytadiene	0.1	/	DH
60	1841	*	Hexahydrofarnesyl acetone	2.6	1.1	SO
61	1857	*	Galaxolide	0.1	0.2	SO
62	1859	*	Phenylethyl benzoate	0.1	0.2	O
63	1873	1864	Benzyl salicylate	0.7	0.1	O
64	1900	1900	Nonadecane	0.4	1.1	O
65	1904	*	2-Heptadecanone	0.4	0.2	O
66	1916	1913	(5*E*,9*E*)-Farnesyl acetone	0.2	/	SO
67	1922	1921	Methyl hexadecanoate	0.2	0.2	O
68	1945	1942	Isophytol	0.2	/	DO
69	1988	1992	Ethyl hexadecanoate	0.2	/	O
70	2000	2000	Eicosane	0.4	0.6	O
71	2021	2024	Isopropyl palmitate	0.2	0.2	O
72	2027	2026	(*E*,*E*)-Geranyl linalool	0.1	/	DO
73	2053	2042	Kaurene	1.0	0.8	DH
74	2078	*	1-Octadecanol	0.1	/	O
75	2100	2100	Heneicosane	5.1	11.5	O

76	2111	*	Phytol	14.1	0.6	DO
77	2163	*	Ethyl oleate	0.1	/	O
78	2200	2200	Docosane	0.7	0.7	O
79	2255	*	2-Methyldocosane	0.7	0.6	O
80	2261	*	Citroflex A	0.2	0.1	O
81	2267	*	(*Z*)-9-Tricosene	0.2	/	O
82	2300	2300	Tricosane	7.2	8.4	O
83	2351	*	4,8,12,16-Tetramethylheptadecan-4-olide	0.2	/	DO
84	2400	2400	Tetracosane	0.8	0.6	O
85	2454	*	2-Methyltetracosane	0.4	tr	O
86	2466	*	7-Pentacosene	0.4	tr	O
87	2500	2500	Pentacosane	6.2	3.1	O
88	2576	*	Tricosane-6,8-dione	13.4	3.1	O
89	2600	2600	Hexacosane	0.5	0.3	O
90	2700	2700	Heptacosane	2.4	0.8	O
91	2722	*	Tetracosanoic acid, methyl ester	0.2	/	O
92	2800	2800	Octacosane	0.2	/	O
93	2823	*	Squalene	0.7	/	TH
94	2900	2900	Nonacosane	2.4	0.9	O
95	3000	3000	Triacontane	0.1	/	O
96	3100	3100	Hentriacontane	1.9	/	O
Total identified				**94.7**	**86.0**	
Monoterpenoids (MO)				2.5	2.6	
Sesquiterpenes (SH)				0.6	1.3	
Sesquiterpenoids (SO)				21.5	41.2	
Diterpenes (DH)				1.1	0.8	
Diterpenoids (DO)				14.6	0.6	
Triterpenes (TH)				0.7		
Others				53.7	39.5	
Unidentified (U)				0.3	9.0	

Compounds listed in order of elution on a HP-5 MS column. **RI**: experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes C_8-C_{32}; **RA**: Adams retention indices; *****: identified by comparison of the mass spectra with those of the commercial libraries Wiley 6, Nist 2.0 and Essentialoils-23b, as well as with those retention indices; **tr**: trace <0.05%); /: not detected. Unidentified (EI-MS: 70 eV, 230º, in m/z(rel %)): RI 1478: 59(25), 60(26), 69(46), 70(100), 95(17), 96(59), 97(83), 128(24), 129(57), 189(50); RI 1483: 70(9), 85(9), 96(12), 106(10), 108(13), 140(100), 141(12), 150(17), 154(8), 183(29); RI 1487: 140(100), 123(58), 183(25), 150(17), 108(15), 177(12), 106(12), 141(11), 107(10), 85(9); RI 1701: 70(10), 80(7), 96(100), 97(7), 108(21), 121(33), 140(38), 150(7), 183(11), 210(13); RI 1726: 70(9), 80(8), 96(100), 97(9), 108(22), 121(34), 140(35), 150(6), 183(10), 210(13).

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