Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (6): 140-146
Received: 27-01-2014
Accepted: 10-02-2014

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Components of *Prunella vulgaris* L. Grown in Ukraine

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ABSTRACT

The composition of volatile components in the spikes, leaves, stems and roots of *Prunella vulgaris* L. (Lamiaceae) grown in Ukraine was studied using gas chromatography-mass spectrometry. The main compound of volatile components was found to be squalene, myristic acid, spathulenol, viridiflorol, germacrone. Different anatomical parts also showed altered volatile profiles.

Keywords: *Prunella Vulgaris* L., Common Self-Heal, GC-MS volatiles

1. Introduction

In recent years there is a tendency to research and create new and more effective herbal medicines. The actual problem is the systematic study of biologically active compounds in the plant material, as well as development and implementation in practice of modern methods for identification and quantification of natural substances. Special attention is assigned to plants that are widely used in folk medicine. Common self-heal (*Prunella vulgaris* L.) belongs to these plants and is a perennial herb found in Europe and West Asia (Lamiaceae) [1-3]. In medical practice common self-heal is used in a variety of dosage forms (capsules «AcNoMore», tablets «Influbiotic Rapid» and spray «Circiderm»), for which its content is not indicated. In Ukraine was registered only one multi-drug «Mammoleptin» (Herbapex, capsules) containing self-heal spikes along with 15 other components.

Fig 1: Common Self-heal (*Prunella vulgaris L.*)

It is important that common self-heal has a wide distribution area in the world, including Ukraine, sufficient raw materials and is easily introduced into the herbal culture.

Fig 2: Distribution of *Prunella vulgaris L.* in Ukraine

Therefore, a systematic study of *Prunella vulgaris* L. to develop new original drugs is an important area of researches, which has theoretical and practical significance. Previous phytochemical studies on the bioactive components of *P. vulgaris* L. have reported the isolation of essential oil from its herb [4-7]. But the yield of essential oil in raw material of self-heal is extremely small and there is no purpose to isolate this fraction.

The main compounds identified in this essential oil were α-camphor, germacrene D, α-pinene, β-elemene and β-caryophyllene. Generally not more than 30 components were revealed by previous studies.

2. Materials and Methods

2.1. Plant material

The plant material of *P. vulgaris* L. (PV) (inflorescences (spikes) (F), leaves (L), stems (S) and roots (R)) harvested during the plant's flowering was collected in July 2012 from Carpathian mountain (near Otyniya village, Ivano-Frankivskiy region, Ukraine). The plant material was allowed to air dry in a dark with no heat source. After two weeks, when the samples attained constant weight, the samples were then analyzed.

The voucher specimens were deposited at the Herbarium of the Faculty of Pharmaceutical Chemistry and Pharmacognosy of Medical University of Ukrainian Association of Popular Medicine.

All solvents and reagents were of analytical grade: HPLC-grade tridecane and pentane were purchased from Sigma–Aldrich, USA.

2.2. Plant sample extraction

To investigate the content and composition of volatile compounds in raw PV material a weighed powdered sample of material (0.5-5 g) was placed in a 20 mL vial and 10 mkl of tridecane was added (internal standard). To the sample 10 ml of distilled water was added and volatiles were extracted by distillation with steam for 2 hours. In the process of stripping the volatiles adsorbed on the inner surface of the reflux condenser. The adsorbed material was washed off after cooling, by addition of 3 ml of pentane in dry 10 ml vial. Flushings were concentrated in a stream of nitrogen to a residual volume of 10 ml which was collected by chromatographic syringe. Further concentration of the sample is carried in the syringe to a volume of 2 ml and was subjected to GC/MS analysis.

2.3. Gas chromatography-mass spectrometry analysis (GC/MS)

GC/MS analyses were performed on an Agilent Technologies 6890 capillary gas chromatograph directly coupled to the mass spectrometer system (model 5973).

A fused silica capillary column 5 % phenyl-poly-dimethyl-siloxane (DB-5 capillary column (30m * 0.25 mm inner diameter, with 0.25 mm film thickness) was used under the following conditions: oven temperature program from 50 °C (3 min hold) to 320 °C at 4 °C/min; injector temperature 250 °C; carrier gas He, flow rate 1.2 mL/min; the volume of injected sample was 1.5 µl.

Injection was carried out in a chromatographic column mode splitless, i.e. this mode allows to introduce the sample without loss division and significantly (10-20 fold) increase sensitivity of chromatography [8, 9].

Individual components were identified by comparison of their mass spectra using both "NIST-MS Library 05" and "Wiley GC-MS Library 2007" [10, 11].

Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatograms. The volatiles content was defined as the signal/concentration ratio between volatile compound and the internal standard.

2.4. Statistical analysis

Values were expressed as means ± standard deviation. Differences at $P < 0.05$ were considered statistically significant.

3. Results and discussion

The volatiles of PV different parts were investigated using GC/MS. The components identified for each part of PV, their percentages and their RT are listed in Table 1 along with their calculated concentrations and Kovats indices on a DB-5 column.

As shown in Table 1, 104 components were identified in flowers, leaves, stems and roots of *Prunella vulgaris* L.

Volatiles identified in the self-heal were 26 hydrocarbons, 13 aldehydes, 10 aromatic compounds, 9 sesquiterpenes, 8 ketones, 7 monoterpenes, 6 acids and esters, and 18 miscellaneous compounds.

Table 1: Volatile composition of flowers, leaves, stems and roots of *P. vulgaris* L.

No.	t_R, min	Compounds	RI	Amount, mg / kg			
				Flowers	Leaves	Stems	Roots
Monoterpene hydrocarbons							
8	8.5	Limonene	1023	-	-	-	2.9
Oxygenated monoterpenes							
16	10.61	*trans*-Linalool oxide (Furanoid)	1109	-	-	-	2.2
18	11.36	*cis*- Linalool oxide	1144	-	-	-	2.3
19	11.48	Linalool	1165	-	-	-	2.4
37	17.31	β-Cyclocitrale	1283	-	4.5	-	-
58	22.31	γ-Nonalactone (Prunolide)	1585	-	-	0.31	-
59	22.53	Geranyl acetone	1586	-	-	0.26	-
Sesquiterpene hydrocarbons							
47	19.98	β-Bourbonene	1444	-	3.2	-	-
55	21.56	β-Farnesene	1557	-	-	-	9.5

57	22.01	Humulene	1579	-	-	-	15.9
60	22.66	Germacrene D	1598	-	5.7	-	-
Oxygenated sesquiterpenes							
69	25.23	Spathulenol	1714	20.2	7.7	-	-
70	25.3	Viridiflorol	1742	-	-	-	33.5
71	25.77	β-Elemenone	1755	-	-	-	9.4
79	27.55	Germacrone	1843	-	-	-	22.0
81	27.94	Hexahydro farnesyl acetone	1979	10.4	-	0.66	-
Aliphatic hydrocarbons							
6	6.14	Decane	999	16.1	7.4	0.97	15.7
10	9.04	Undecane	1051	7.6	3.7	0.51	9.8
24	12.33	Dodecane	1202	13.1	-	0.84	12.4
31	14.31	2,7,10- Trimethyl dodecane	1258	7.0	3.3	0.37	-
40	17.8	2,7,10- Trimethyl tridecane	1310	5.5	2.7	-	-
43	18.85	Tetradecane	1394	26.5	12.6	3.60	18.9
48	20.06	Methyltetradecane	1456	-	6.2	-	10.4
54	21.24	Pentadecane	1498	20.7	10.7	0.75	11.1
61	23.18	Hexadecane	1600	18.5	8.7	1.01	16.6
66	24.59	2,7,10- Trimethyl hexadecane	1680	13.0	5.6	0.50	10.0
68	24.84	Heptadecane	1696	10.2	6.4	-	10.0
72	26.18	Methyl heptadecane	1757	-	-	-	12.9
74	26.19	2,6,10,14- Tetramethyl hexadecane	1777	11.7	4.8	-	-
75	26.35	Octadecane	1800	12.2	5.2	0.47	6.9
80	27.74	Nonadecane	1900	8.5	-	0.34	-
84	29.04	Eicosane	2000	17.8	4.8	0.46	6.3
88	30.28	Heneicosane	2103	19.3	7.0	0.70	11.2
92	32.58	Tricosane	2300	9.3	-	-	-
93	33.66	Tetracosane	2400	9.3	3.0	0.31	5.0
94	34.69	Pentacosane	2500	22.7	4.0	0.65	6.1
95	35.69	Hexacosane	2600	9.7	2.9	0.31	4.3
96	36.66	Heptacosane	2700	27.8	3.8	0.58	7.0
98	37.59	Octacosane	2800	6.9	-	-	-
99	38.51	Nonacosane	2901	44.7	11.8	1.05	19.3
102	40.21	Untriacontane	2301	13.0	8.9	-	-
104	41.83	Tritriacontane	2330	19.0	11.9	-	-
Aliphatic alcohols							
3	4.71	*cis*-3-Hexen-1-ol	818	1.1	-	-	-
Aliphatic aldehydes							
1	3.72	Hexanal	803	-	-	0.13	-
4	5.18	*trans*-2-Hexenal	827	-	23.1	-	-
7	7.98	trans-2-Heptenal	1011	-	-	0.25	-
12	9.66	*cis*-2,4-Heptadienal	1086	-	3.3	0.21	-
13	10.38	*trans*-2,4-Heptadienal	1095	-	6.6	0.27	-
17	11.21	*trans*-2-Octenal	1135	-	-	0.71	-
21	12.07	Nonanal	1174	-	2.4	-	-
34	14.76	2,6-Nonadienal	1270	-	2.5	-	-
35	15.49	Decanal	1275	1.8	1.3	0.23	-
41	17.96	*trans*-2-Decenal	1374	-	-	1.86	-

49	20.07	2,4-Decadienale	1461	-	-	0.81	-
50	20.07	2,7,10,14- Tetramethyl tridecane	1478	10.5	-	-	-
51	20.21	*cis*-2-Dodecenale	1479	-	-	0.43	-
Aliphatic esters							
52	20.57	Ethyl caprinate	1481	-	-	-	26.5
33	14.7	Ethyl caprilate	1265	-	-	-	7.9
53	20.63	*trans*-2-Dodecenale	1485	-	-	1.78	-
62	23.29	Methyl-9-oxononanoate	1612	-	-	0.43	-
65	24.34	Ethyl laurinate	1647	-	-	-	19.4
91	32.52	Ethyl linolenate	2292	8.8	-	-	-
Aliphatic ketones							
2	3.93	Nononane	805	-	-	0.23	-
9	8.59	6-Methyl-5-Hepten-2-one	1031	-	1.2	-	-
14	10.5	3-Octene-2-one	1097	-	-	0.30	-
20	11.64	2-Nonanone	1171	-	-	-	4.8
22	12.09	*cis*-3,5-Octadiene-2-one	1176	-	-	0.41	-
25	13.02	*trans*-3,5-Octadiene-2-one	1212	-	-	0.32	-
28	13.55	6-Methyl-3,5-heptadien-2-one	1241	-	-	0.23	2.4
42	18.32	Undecanone-2	1385	-	-	-	11.4
Aliphatic acids							
29	14.13	Caprylic acid	1249	-	7.2	-	-
77	27.15	Myristic acid	1823	-	11.6	0.74	9.0
82	28.51	Pentadecanoic acid	1983	-	-	0.37	10.1
85	29.77	Palmitoleic acid	2047	-	-	-	9.5
86	29.84	Palmitic acid	2075	-	8.7	-	-
90	32.15	Oleic acid	2278	-	5.4	-	7.0
Aromatic compounds							
11	9.28	Benzaldehyde	1085	-	1.9	0.55	-
23	12.31	Phenyl acetaldehyde	1179	-	9.3	-	-
26	13.04	Acetophenone	1230	-	-	0.21	-
36	16.95	Methyl-2,6,6-trimethyl-1-cyclohexenoate-1	1280	-	3.1	-	-
44	19.28	Benzothiazole	1409	-	-	0.55	-
45	19.77	Anethol	1415	-	1.2	-	-
78	27.46	Benzophenone	1833	-	-	0.34	-
87	29.89	Phtalate	2089	34.1	-	5.60	65.8
89	31.25	Phtalate	2207	38.6	10.5	2.18	22.1
97	37.05	Phtalate	2734	31.2	5.7	1.31	21.1
Miscellaneous compounds							
5	5.53	Furfural	828	-	-	0.23	2.2
15	10.61	Unknown*	1102	-	1.3	-	-
27	13.15	Unknown*	1236	-	5.0	0.35	-
30	14.3	Unknown*	1250	-	-	-	5.3
32	14.59	Unknown*	1262	-	-	0.23	-
38	17.46	Unknown*	1291	-	-	-	5.1
39	17.79	Unknown*	1299	-	-	-	4.4
46	19.85	Unknown*	1418	-	1.3	-	-
56	21.93	5-pentyl-2(5H)-Furanone	1536	-	-	0.47	-
63	23.73	Unknown*	1625	-	4.8	0.36	-

64	24.25	Unknown*	1636	-	-	-	7.0
67	24.84	Unknown*	1687	-	-	0.48	-
73	26.19	Unknown*	1766	-	-	0.55	-
76	26.95	Unknown*	1805	-	-	-	11.4
83	28.75	Unknown*	1998	7.6	-	-	-
100	38.7	Squalene	2802	164.3	26.6	7.34	156.5
101	40.0	Unknown*	2845	13.3	5.4	0.56	-
103	41.35	Unknown*	2204	-	-	0.53	7.4
Identified from total area				715.1	305.9	46.2	715.1
Monoterpene hydrocarbons				0	0	0	2.9
Monoterpenoids				0	4.5	0.57	6.9
Sesquiterpene hydrocarbons				0	15.1	0	35.8
Sesquiterpenoids				30.6	7.7	0.66	64.9
Others				681.4	278.6	44.97	599.8

The class of aliphatic compounds displayed the highest amount, followed by aromatic compounds, among the above classes.
The chemical composition of volatiles varies depending on the plant parts.
The major constituent was squalene (164.3 mg/kg in flowers and 156.5 mg/kg in roots).
The main monoterpene components were β-cyclocitrale (4.5 mg/kg in flowers) and linalool (2.4 mg/kg in roots).
Viridiflorol (33.5 mg/kg) and germacrone (22.0 mg/kg) were the main sesquiterpenes in the roots of PV.
To the best of our knowledge, the volatiles of *P. vulgaris* L. has not been the subject previously studied.

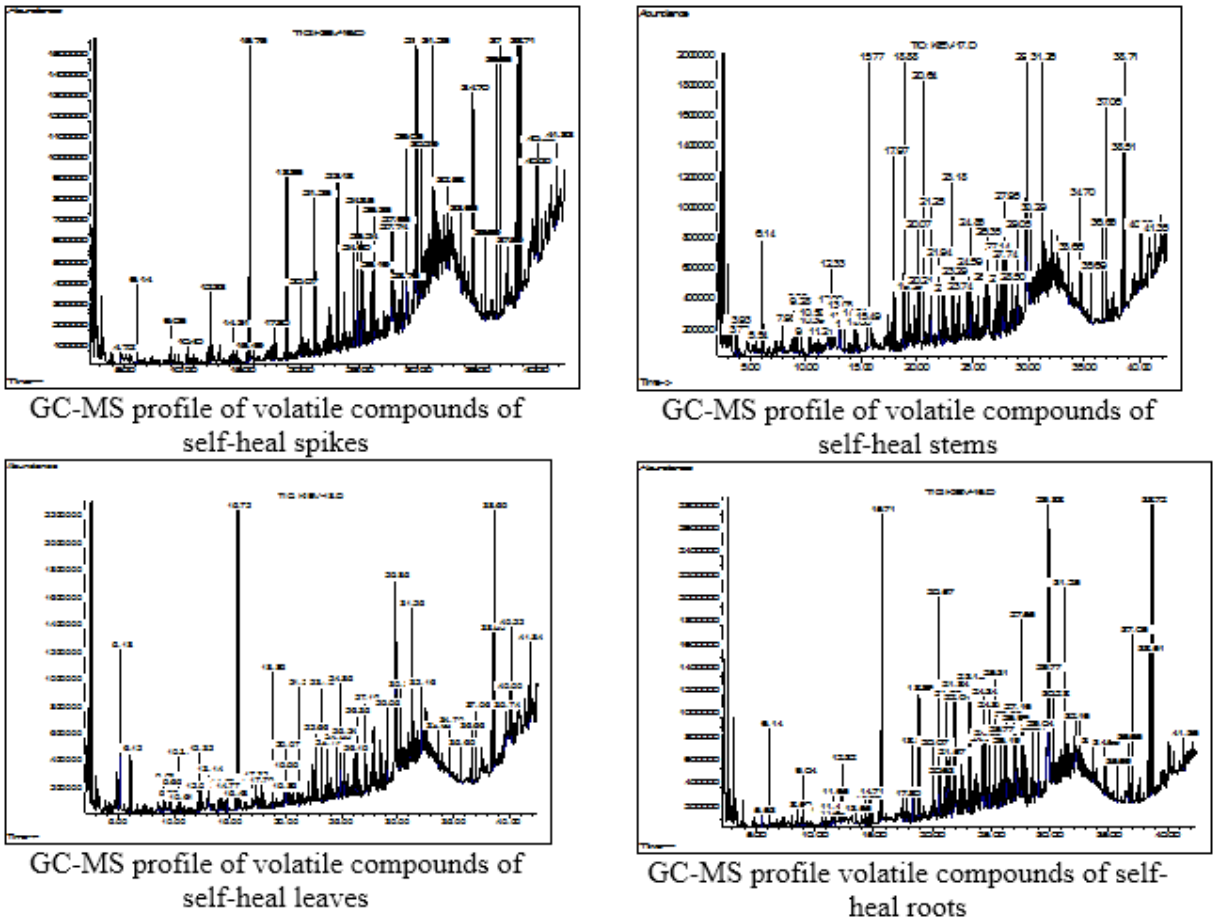

Fig 3: Fingerprint gas chromatograms of the volatile fraction determined by means of GC-MS or the flowers, leaves, stems and roots of *P. vulgaris* L.

As a part of the volatile components of self-heal among aromatic compounds was found benzaldehyde (1.9 mg/kg), which has almond flavor, phenylacetaldehyde (9.3 mg/kg) with the smell of hyacinths, pelargonic aldehyde (2.4 mg/kg) with the smell of roses and anethole (1.2 mg/kg) that induces apoptosis in cancer cells and inhibits herpes virus replication.

Relative content of some volatile compounds and group composition in different organs of PV shown in Figure 4 and Figure 5.

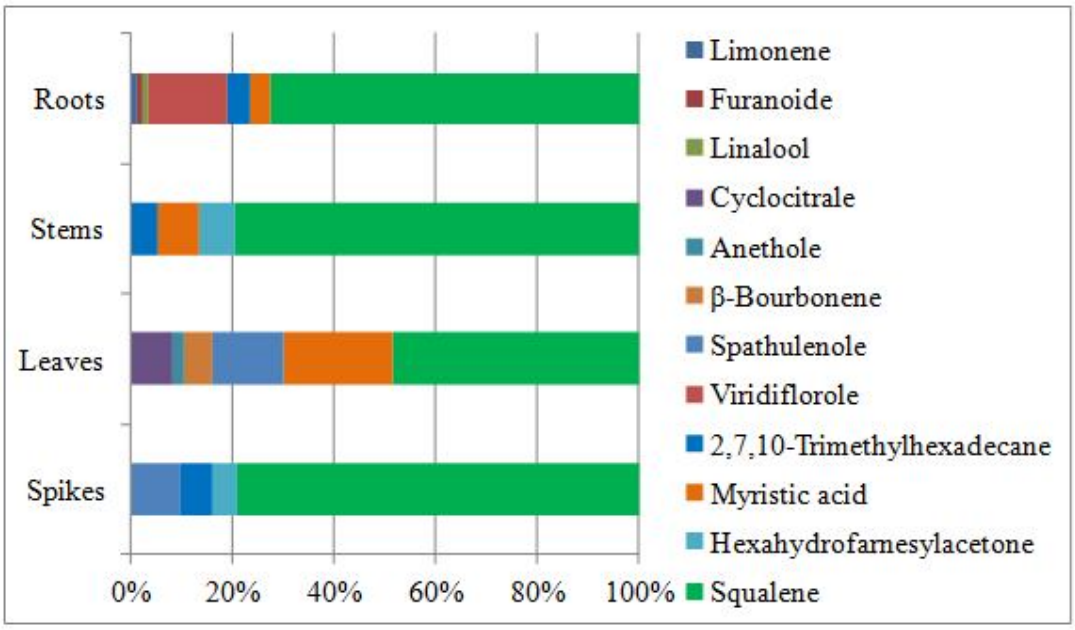

Fig 4: Relative content of some volatile compounds in different organs of PV

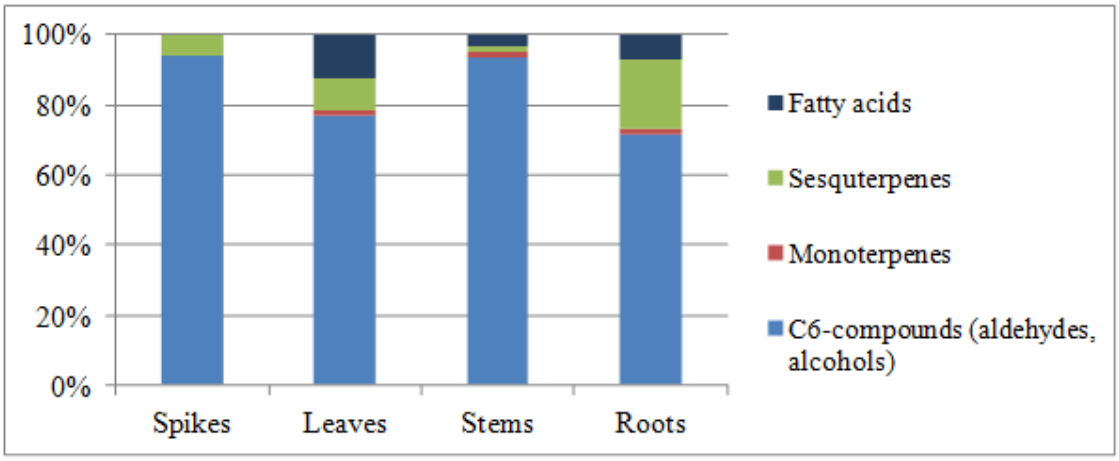

Fig 5: Relative content of group composition of volatile compounds in various organs of PV

Sesquiterpene compounds are presented by mainly β-burbonene (3.2 mg/kg) germacrene D (5.7 mg/kg), spathulenol (7.7 mg/kg). Triterpene hydrocarbon squalene in an amount 26 mg/kg of raw material domine in self-heal inflorescences and has antitumor, antiviral and antioxidant activities.

In all herbal parts C_6-compounds are predominant, amounting to 496.2 mg/kg.

4. Conclusions

The differences in composition and concentrations of volatiles in different parts of *P. vulgaris* L. were compared and analyzed by using GC-MS. This is the first application of GC-MS on the volatiles of *P. vulgaris* L. grown in Ukraine. One hundred four compounds were identified in different parts of common self-heal. The dominant compound squalene present in all parts of investigated plant. The total identified compounds accounted for about 715 mg/kg of raw material.

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