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Full Length Research Paper

Variability of essential oil composition in Albanian accessions of *Satureja montana* L.

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***Satureja montana* L. is an important aromatic and medicinal plant. The present study is aimed to contribute to a better knowledge of chemical biodiversity features of *S. montana* in Albania. The authors studied the variation in the chemical composition of its essential oil in six populations collected from agro-climatically diverse sites. Dried leaves were hydrodistillated and the essential oil was analyzed by a combination of GC and GC/MS. The concentration of essential oil ranged for the different populations from 0.22 - 1.61%. The oil was characterized to have 38 to 58 components, where the main compound was carvacrol (2.21 - 55.95%).**

Key words: *Satureja montana*, essential oil, Albania.

INTRODUCTION

Albania is one of the most important exporters of medicinal and aromatic plants in Europe (Asllani, 2004). *Satureja montana* L. (winter savory, is an important medicinal and aromatic plants in Albania. It is a perennial shrub which grows wild throughout Albania (Paparisto et al., 1996) and as a medicinal and aromatic plant plays an important role in everyday life. It is consumed fresh and dried as seasonings, stews, meat dishes, poultry, sausages, vegetables phytomedicines, herbal tea, etc. (Paparisto and Balza, 2003). Its flowers are known to attract honeybee and the honey is a famous folk remedy for bronchitis (Paparisto and Balza, 2003). In addition, *S. montana* is being used as a stimulant, stomachic, carminative, expectorant, anti-diarrheic, and aphrodisiac in Albanian folkloric medicine (Asllani, 2004).

The plant of *S. montana* contains various biologically active constituents such as essential oil and triterpenes (Escudero et al., 1985), flavonoids, and rosmarinic acid (Reschke, 1983). The tea and extracts of this plant among many groups of natural compounds can contain free and glycoconjugated aroma compounds. The essential oil is high in carvacrol and thymol (Lawrence, 1979). Further, the content of thymol and carvacrol is variable and depends on the origin and vegetative stage of the plant (Kustrak et al., 1996). The essential oil is used in the food industry as a flavoring agent in liqueurs and perfumery.

According to Ciani et al. (2000), this oil has high and diffused antimicrobial activity and it could be used to control potential pathogenic and spoilage yeasts (Ciani et al., 2000). The variation in the essential oils and pharmaceutical characters has been attributed to genetic and agro-climatic factors in *S. montana* (Hay and Waterman, 1993) as well as in *S. hortensis* (Hadian et al., 2008). The dominant components in the oil of *S. montana* were reported to be caryophyllene and geraniol (Sevarda et al., 1986) and carvacrol (Palić and Gisic, 1993; Chalchat et al., 1999). In spite of *S. montana* being an important medicinal and aromatic plant in Albania and its being included in Albanian National Plants Red Data Book, there is little information on the variation among

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Table 1. Basic characterization of the sites from which six *S. montana* populations were collected.

Population	Collection site	Altitude (masl)[a]	Latitude (N)	Longitude (E)	Exposition	Climatic subzone
P_1	Kakariq, Lezhë	4	41° 91'	19°50'	Plain field on the West of a North-South hill	Mediterranean
P_2	Zogaj, Shkodër	15	42°70'	19°41'	Eastern slope of a North-South hill	Mediterranean
P_3	Dajt, Tiranë	1100	41°35'	20°09'	Eastern slope of a North-South mountain	Mediterranean
P_4	Qafë Mali, Pukë	800	42°11'	20°11'	Eastern slope of a North-South mountain	Mediterranean
P_5	Qafë Kerrabë, Elbasan	750	41°12'	19°59'	Western slope of a North-South mountain	Mediterranean
P_6	Xibrakë, Librazhd	207	41°98'	20°15'	Western slope of a North-South hill	Mediterranean

[a]Altitude in meters above sea level.

populations growing in agro-climatically diverse regions. The authors collected six populations from different sites in Albania and studied the variability for concentration of essential oil and for the compounds of the oil.

MATERIALS AND METHODS

Plant material

The material comprised of six naturally growing populations of *S. montana* at six agro-climatically diverse sites in Albania (Table 1). The plant materials, 500 g of fresh leaves of 30 plants in each population, were collected at the full blooming period, end of August. The material was air-dried in a room (under shade) for 22 days. The voucher specimens are deposited at the Crop Production Department, Faculty for Agriculture and Environment, Agricultural University of Tirana, Albania.

Isolation of the essential oil

The essential oil was isolated from 100 g of dried leaf material by hydro-distillation for 3 h using a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulphate and stored at 4°C. The oil solution (1%) in ethanol was used for chromatographic analysis. Preliminary GC–MS analysis showed the absence of 1-octanol as potential algycone of *S. montana*. The oil yield was determined by gravimetry. The component percentages were calculated as the mean value on column HP-20M and HP-101 for duplicate analyses.

Analytical gas chromatography (GC/FID)

The GC/FID analysis of the oils was carried out on a Hewlett-Packard HP-5890 Series II GC apparatus equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (25 m 0.32 mm, 0.52 µm film thickness) and fitted to flame ionization detector (FID). Carrier gas (hydrogen) flow rate was 1 ml/min, split ratio was 1:30, injector temperature was 250°C, and detector temperature was 300°C, while column temperature was linearly programmed from 40 -260°C (a t rate of 4°/min

Solutions of essential oil samples in ethanol (~1%) were consecutively injected by ALS (1 µl, split mode). Area percent reports, obtained as a result of standard processing of chromatograms, were used as base for the quantification purposes.

Gas chromatography/mass spectrometry (GC/MS)

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with column HP-5MS (30 m 0.25 mm, 0.25 µm film thickness), using Hewlett-Packard HP G 1800C Series II GCD system. Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV); in m/z range 40 - 450. Sample solutions in ethanol (~1 %) were injected by ALS (200 nl, split mode).

Identification of components

The components of the oil were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1), compared to those from available literature (Adams, 2007), and used as additional tool to approve MS findings.

RESULTS AND DISCUSSION

The oil isolated by hydro-distillation from the leaves collected at the same stages of development of six population of *S. montana* growing naturally in agro-climatically diverse sites, was found to be yellow liquid. The yield obtained ranged from 0.22 - 1.61% (w/w) based on dry weights (Table 2). The highest oil yield content was found in P_6-Xibrakë, Librazhd (1.61%) collected from the East part of Albania and the lowest in P_4-Qafë Mali, Pukë (0.22%) collected from North part of Albania. In the study by Milos et al. (2001), the yield of essential oils of *S. montana* ranged from 0.7 - 1.9%.

Table 2. The concentration and composition of the essential oil in six *S. montana* populations (P_1 to P_6) collected from six agro-climatically diverse sites.

Concentration/Compounds of the essential oil		Retention index[a]	Population					
			P1	P2	P3	P4	P5	P6
Concentration of essential oil (% V/m)			1.26	1.00	1.24	0.22	1.35	1.61
	Compounds							
1	Trans-2-hexenal	873.8	-	-	-	0.13	-	-
2	Tricyclene	914.5	t[b]	t	t	0.21	0.08	-
3	α-Thujene	919.5	0.56	0.54	0.97	0.19	0.81	
4	α-Pinene	926.8	1.05	0.74	0.90	1.56	1.35	1.02
5	Camphene	939.1	1.25	0.79	1.07	1.44	2.07	1.11
6	Thuja-2,4(10)-diene	955.6	-	-	-	0.03	-	1.26
7	Sabinene	969.3	-	-	-	3.06	-	-
8	β-Pinene	978.0	-	-	-	0.94	-	-
9	1-Octen-3-ol	982.8	0.81	0.97	0.60	-	1.12	-
10	β-Myrcene	990.7	0.71	0.70	0.98	7.81	0.82	0.95
11	3-Octanol	998.5	0.11	0.09	0.14	0.04	0.11	0.92
12	α-Phellandrene	1002.5	0.06	0.04	0.06	0.36	0.05	0.13
13	α-Terpinene	1010.4	0.80	1.26	1.35	0.04	1.03	0.06
14	p-Cymene	1019.3	14.79	15.31	13.34	1.13	16.22	1.16
15	β-Phellandrene	1026.6	0.55	0.56	0.55	6.09	0.70	17.40
16	1,8-Cineole	1028.6	0.66	0.49	0.70	0.12	1.07	0.78
17	Cis-β-ocimene	1044.8	-	-	-	2.38	-	0.60
18	Trans- β-ocimene	1052.0	-	-	-	0.76	-	-
19	γ-Terpinene	1056.0	4.80	4.87	8.86	0.31	5.25	-
20	Cis-sabinene hydrate	1065.9	0.46	3.20	0.47	0.16	0.46	5.16
21	α-Terpinolen	1082.6	0.19	0.41	0.14	0.21	0.18	6.17
22	Linalool	1099.2	2.45	3.63	0.35	1.63	0.42	0.19
23	1-Octen-3-yl acetate	1114.0	-	-	-	0.68	-	0.79
24	Cis-p-menth-2-en-1-ol	1116.9	0.07	0.25	t	0.18	0.06	-
25	Trans-pinocarveol	1134.7	0.12	0.24	0.09	0.28	0.14	0.10
26	Camphor	1137.8	0.14	0.22	0.11	6.86	0.20	0.18
27	Borneol	1160.6	4.27	2.96	3.82	9.64	6.68	4.12
28	Terpinen-4-ol	1173.2	1.13	3.42	0.95	0.80	1.34	1.35
29	p-Cymen-8-ol	1180.7	0.20	0.32	0.20	0.08	0.25	0.16
30	α-Terpineol	1185.4	0.54	0.54	0.33	0.36	0.55	0.53
31	Cis-dihydrocarvone	1191.2	0.12	0.18	0.09	0.36	0.15	t
32	Trans-dihydrocarvone	1198.0	t	0.15	t	0.07	t	t
33	Carvacrol methyl ether	1237.1	t	0.22		0.15	0.07	0.18
34	Carvone	1256.1	0.32	0.97	4.65	0.27	1.75	5.24
35	Geraniol	1267.4	0.12	4.64	t	-	t	t
36	Isobornyl acetate	1278.8	t	0.19	t	0.36	t	t
37	Neryl formate	1284.2	0.08	0.08	0.07	-	0.07	t
38	Thymol	1289.3	0.38	2.97	12.43	1.46	27.29	0.08
39	Carvacrol	1313.6	55.95	39.53	37.53	2.21	21.07	40.51
40	Eugenol	1324.2	0.43	0.39	0.14	-	0.17	1.45
41	α-Ylangene	1364.3	t	t	t	-	0.13	t
42	α-Copaene	1367.5	t	0.09	0.13	0.27	t	0.14
43	Carvacrol acetate	1369.5	t	0.14	t	-	t	t
44	Geranyl acetate	1380.6	t	0.07	0.17	-	0.19	0.14
45	β-Bourbonene	1381.6	-	-	-	1.68	-	2.53
46	Trans-β-caryophyllene	1410.8	3.37	3.53	3.46	10.79	2.86	-
47	β-Gurjunene (calarene)	1419.0	t	0.07	0.10	0.41	0.12	0.11

Table 2. Contd.

48	Aromadendrene	1431.2	0.33	0.38	0.33	0.33	0.60	0.65
49	α-Humulene	1443.1	0.12	0.13	0.14	0.77	0.11	0.10
50	Cis-cadina-1(6),4-diene	1460.5	-	-	-	0.20	-	0.26
51	γ-Muurolene	1469.7	0.11	0.10	0.26	0.32	0.29	-
52	Germacrene D	1473.7	t	0.08	0.50	10.44	0.09	0.12
53	Viridiflorene (ledene)	1487.5	0.16	0.24	-	-	0.50	0.60
54	Bicyclogermacrene	1499.2	-	-	-	4.09	-	0.94
55	β-Bisabolene	1501.9	0.57	0.37	1.68	-	1.06	-
56	γ-Cadinene	1506.4	t	0.10	0.20	0.89	0.18	0.16
57	δ-Cadinene	1516.0	0.16	0.18	0.39	1.60	0.38	0.33
58	α-Calacorene	1540.0	-	-	-	0.59	-	0.78
59	Elemol	1550.6	-	-	-	0.40	-	-
60	Spathulenol	1572.0	0.15	0.57	0.48	3.47	0.56	-
61	Caryophyllene oxide	1577.2	1.92	2.67	1.18	5.29	1.40	1.30
62	Salvial-4(14)-en-1-one	1595.7	-	-	-	0.49	-	t
63	Humulene epoxide II	1600.1	t	0.07	-	0.73	t	-
64	Caryophylla-4(12),8(13)-dien-5-ol	1628.3	t	0.15	0.10	-	t	0.14
65	γ-Eudesmol	1629.4	-	-	-	1.93	-	0.13
66	α-Cadinol	1654.4	-	-	-	1.76	-	-
67	14-Hydroxy-9-epi-trans-caryophyllene	1663.8	t	0.17	-	0.43	t	-
68	Germacra-4(15),5,10(14)-trien-1-α-ol	1687.2	-	-	-	0.76	-	1.02
Total number of compounds			38	50	41	58	44	42

[a]RI = retention index experimentally determined (AMDIS). [b]t = trace less than 0.05%.

The compounds of the essential oil of *S. montana* are listed in order of their elution on the DB-5 column (Table 2). The oil composition of these populations, with respect to the number of compounds, their relative concentration as well as the relative concentration of the main components (Figure 1), showed large variation. A total of 38 - 58 compounds were identified, representing 100% (area percent) of the total oil. All 58 compounds were identified in P_4 -Qafë Mali Pukë, and 38 compounds, the lowest number; were identified in P_1-Kakariq Lezhë. The most abundant compound in the essential oil across all six populations in the present study was carvacrol (2.21 - 55.95%), along with thymol (0.38 - 40.51%), p-cymene (1.13 - 17.40%), borneol (1.35- 9.64%), y-terpinene (0.31 - 8.86%), and trans-β-caryophyllene (0.11 -10.79%). Carvacrol was the first major compound of the essential oil in P_1, P_2, and P_3, trans-β-caryophyllene in P_4, thymol in P_5, P_6. The lowest concentration of carvacrol and trans-β-caryophyllene was found in P_6 and that of thymol in P_1. The highest concentration of p-cymene was observed in P_6 and the lowest in P_4. The opposite was true for borneol. The concentration of γ-terpinene was in the highest in P_3 and the lowest in P_4. Caryophyllene oxide was present in the oil of all populations except of P_6. Some minor or trace components, such as trans-2-hexenal, thuja-2, 4(10)-diene, sabinene, β-pinene, cis-β-ocimene, trans-β-ocimene, 1-octen-3-yl acetate, cis-cadina-1(6), 4-diene and salvial-4(14)-en-1-one were found in essential oil of only P_4.

Large variations in the relative concentration of the major components in the oil of *S. montana* have been reported by previous workers. Casin et al. (1985) reported the concentration of carvacrol and linalool being in the range of 1 - 69%, and these were followed by terpinene and *p*-cymene (1 - 31%). Further, Slavkovska et al. (2001**)** reported *trans*-sabinen hydrate, *ρ*-cymene, and linalool (23 - 27%), and borneol (3%); and Radinic and Milos (2003) reported thymol (45%), and *p*-cymene, γ-terpinene carvacrol, geraniol, and borneol (4 - 6%) to be the major compounds. On the other hand Sevarda et al. (1986) recorded that caryophyllene and geraniol were the dominant components. Carvacrol was observed to be the most important compound of *S. montana* in our study and by Slavkosvka et al. (2001) (40.8%), Milos et al. (2001) (52.4%), and Ćavar et al. (2008) (69%), has also been reported as the major compound of the oil of *S. boissieri* (40.8%, Kurcuoglu et al., 2001), *S. mutica* (30.9%, Sefidkon and Jamzad, 2005), *S. hortensis* (48.1%, Sefidkon et al., 2006), *S. hortensis* (67%, Krestev et al., 2009), *S. thymbra* (66.5%, Karousou et al., 2005). The Albanian *S. montana* has high concentration of carvacrol and is widely used for medicinal purpose.

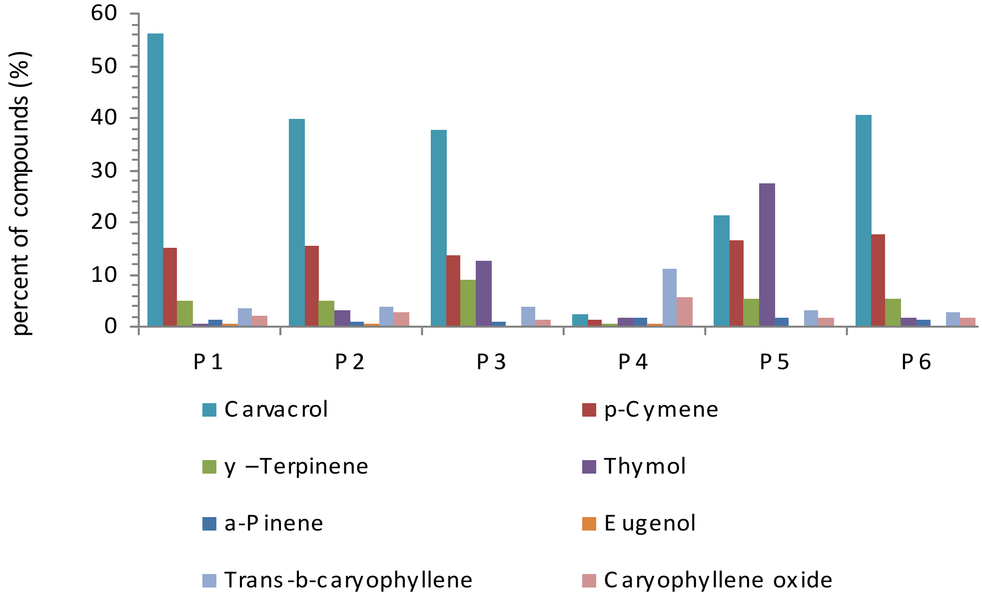

Figure 1. Comparative concentration of the important compounds in the essential oil of six populations of *S. montana* (P_1 - P_6).

Therefore, it seems that carvacrol has important medicinal properties. Further, antioxidant and antibacterial activities of thymol and *p*-cymene have also been reported (Helander et al., 1998; Mann et al., 2000; Ćavar et al., 2008). Albanian *S. montana* has high concentration of both thymol and *p*-cymene also. Due to antioxidant and antibacterial activities of thymol and *p*-cymene and their high concentration, there is a great opportunity to use Albanian *S. montana* as a source of flavouring agent in food, as natural food preservatives and also in the pharmaceutical industry.

The results of our study indicate the presence of a large variation both for concentration of essential oil as well as for the compounds of the oil in Albanian populations of *S. montana*. However, in order to improve our understanding of the genetic component of the observed variation, we suggest performing common garden experiments with *S. montana* populations collected in the wild.

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