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Essential oil of *Salvia officinalis* L. from Serbia and Montenegro

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ABSTRACT: The content and composition of essential oil in the leaves and flowers of 11 populations of *Salvia officinalis* L. native in Montenegro (nine populations) and Serbia (two populations) have been studied. The yield of oils was generally higher in the leaves than the flowers. The Serbian populations proved to be the richest in leaf oil (average content 1.66%). Montenegro speciments showed significant interpopulation variation and the yields averaged 1.41% for the leaves and 1.13% for the flowers. The main compounds in the leaves were oxygenated monoterpenes: α-thujone ($15.79 \pm 4.9\%$), β-thujone ($3.49 \pm 1.21\%$), 1.8-cineol ($12.09 \pm 3.5\%$), camphor ($11.49 \pm 7.69\%$), borneol ($4.17 \pm 2.23\%$) and bornyl acetate ($2.19 \pm 1.22\%$). Among the dominant sesquiterpenes were: α-humulene ($7.70 \pm 3.12\%$), viridiflorol ($13.19 \pm 5.17\%$) and manool ($7.67 \pm 2.98\%$). In the flowers, percentages of α-thujone and camphor were significantly lower than in the leaves and averaged $9.97 \pm 1.49\%$ and $5.82 \pm 5.6\%$, respectively, whereas the ratios of borneol ($6.35 \pm 2.47\%$) and sesquiterpenes, particularly manool ($13.48 \pm 3.56\%$), were higher. Great variation was found in the proportions of the major compounds between the populations examined. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: *Salvia officinalis* L; sage populations; essential oil composition; thujones; camphor; manool; sesquiterpenes

Introduction

Salvia officinalis L. (lamiaceae), garden or red sage, is a perennial hardy subshrub native to Mediterranean regions. The herb is found abundantly on the Dalmatian coast and adjacent areas of the Adriatic Sea, Croatia and Albania. In Serbia and Montenegro *S. officinalis* has extremely disjunctive aerial parts. It is distributed at coastal and sub-Mediterranean area of Montenegro and at limited continental area of south and south-east Serbia.[1,2] The plant grows under complex heterogeneous climatic conditions, mainly influenced by the Mediterranean.

Sage is one of the most popular medicinal and culinary herbs. Dalmatian sage oil composition was widely examined from the early 1960s until recent years.[3–6] In addition, data of sage oil from Morocco,[7] Egypt[8] and Italy[9,10] has been reported. However, there have been few attempts to examine sage growing wild in Montenegro and especially in the continental part of the Balkan peninsula.[11] Several papers have reported on the variation in essential oil composition induced by environmental,[12,13] physiological[13,14] and morphological factors.[15,16] The present study was carried out in order to provide more data on the infraspecific variation of essential oil composition among the authotonous populations of *S. officinalis* in Montenegro and Serbia.

Material and Methods

Plan Material

The aerial parts of *S. officinalis* L. were collected while in flower from 11 natural populations, two from Serbia and nine from Montenegro (Table 1). Voucher specimens were deposited in the Herbarium of the Department of Botany, Faculty of Pharmacy, University of Belgrade, and identified by the authors. For the further study, air-dried leaves and flowers were analysed separately.

Essential Oil Analysis

The essential oil was isolated by the hydrodistillation European Pharmacopoeia,[17] method, using *n*-hexane as collecting solvent. The obtained hexane extract was dried over anhydrous sodium sulphate and decanted.

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Table 1. Geographic area of *S. officinalis* study populations

Group	Geographic area	Climate	Samples
A	River Piva Gorge in the north-west of Montenegro	Submediterranean	A-1
B	Background of the Montenegran coast	Intermediate of perhumide submediterranean and continental perhumide	B-1, B-2, B-3, B-4, B-5, B-6
C	Coastal line of Montenegro	Mediterranean	C-11, C-2
D	Continental sites, gorges in the south-east Serbia	Intermediate of submediterranean and aegean-subcontinental climate	D-11, D-2

A-1, Piva; B-1, Nikšić-Viljusi; B-2, Rumija; B-3, Rumija Lake; B-4, Lijeva Rijeka (East; B-5, Lijeva Rijeka (north; B-6, Morača; C-1, Vrbanja; C-2, Miločer; D-1, Miljkovac; D-2, Sičevo.

Hexane was evaporated under reduced pressure and the oil yield was measured. A suitable dilution of the oil in hexane (10 mg/ml) was then analyzed by GC–FID and GC–MS. The GC conditions used were: column, HP-5 fused silica capillary column, 30 m × 0.25 mm, film thickness 0.25 μm; column temperature, 50 °C for 5 min, then heated to 250 °C at a rate of 3 °C/ min on-Perkin-Elmer 8500 gas chromatograph; carrier gas, He; injector, 280 °C; FID detector, 280 °C. Mass spectra were obtained from a Hewlett-Packard 5973-6890 GC–MS system operating on EI mode at 70 eV, equipped with HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The initial temperature of the column was 60 °C and it then was heated to 280 °C at a rate of 3 °C/ min. The identification of individual compounds were made by comparison of their retention times and mass spectra with those obtained from authentic samples and/or the NIST/NBS, Wiley libraries spectra as well as with literature data.

Results and Discussion

The percentage yields of essential oil in the leaves and flowers of the sage populations examined are presented in Table 2. In the leaves, oil content varied from 1.16% (B-2) to 1.68% (B-5, C-2 and D-1). In the flowers, the oil contents were lower and varied from 0.74% (B-2) to over 1.31% (A-1). In the leaves of sage populations native in Montenegro, Ristić *et al.*[18] found a significantly higher oil yield (2.7–4.4%).

Table 2. The yield (%) of essential oil in examined populations of *S. officinalis*[1]

Sample	Oil content (%)	
	Leaf	Flower
A-1	1.23	1.31
B-1*	1.24	—
B-2	1.16	0.74
B-3	1.41	0.87
B-4	1.64	1.14
B-5	1.68	0.86
B-6	1.41	0.91
C-1	1.48	1.26
C-2	1.68	0.84
D-1	1.68	0.91
D-2*	1.64	—

[1] Averages from three replications are given.
*Essential oil was isolated and examined only in the leaves.

The composition of the essential oils from the leaves and flowers of populations studied are reported in Tables 3 and 4, respectively. Variation in the oil composition between different populations, as well as between distinct plant parts, was also found.

In the leaves, 55 compounds were identified. The results presented revealed that the oxygenated monoterpenes (1,8-cineol, α- and β-thujone, camphor, borneol and bornyl acetate) were the most abundant, but their quantities varied over a wide range. Thus, 1.8-cineol varied from 6.35% (D-2) to 18.54% (B-3); α-thujone from 8.47% (C-2) to 25.35% (B-1); β-thujone from 1.33% (C-2) to 6.04% (B-1); camphor from 0.15% (B-1) to over 24.8% (D-2); borneol from 0.20% (B-1) to 8.50% (C-2); and bornyl acetate from 0.05% (B-1) to 4.91% (D-1). In the leaf oil, considerable amounts of sesquiterpenes were also found. The most abundant were: α-humulene, which varied from 3.35% (B-2) to 12.49% (B-1); viridiflorol, 5.97% (D-2) to 26.10% (B-1); manool, 5.2% (D-2) to 15.92% (C-2); and *trans*-caryophyllene, 1.04% (D-2) to 9.41% (B-5).

In most of the populations examined, the inverse proportion between the ratio of total thujones and ratios of camphor and borneol was noticable. Thus, in sample B-1, which was collected from the inland of Montenegro, the highest content of both α- and β-thujone was found, but the lowest of camphor, borneol and bornyl acetate. In the same sample a high proportion of the sesquiterpenes viridiflorol and α-humulene was noteworthy. Populations C-1 and C-2 from the coastline of Montenegro showed a considerable low proportion of camphor. In sample C-2, the highest ratios of borneol (8.50%) and manool (15.92%) were also registered. By contrast, in population D-2, collected from the continental part of south-east Serbia (Sicevo Gorge), the highest proportion of camphor (24.8%) and significantly high thujone ratios were noteworthy. In the same sample, a low proportion of

Table 3. Percentage composition of the essential oil from leaves of *S. officinalis* populations

Constituents	KI	A-1	B-1	B-2	B-3	B-4	B-5	B-6	C-1	C-2	D-1	D-2	Average
(*Z*)-Salvene	858	0.34	0.30	0.17	t	t	0.13	t	0.23	—	0.18	t	
(*E*)-Salvene	868	t	t	t	t	t	t	t	t	t	t	t	
Tricyclene	829	t	t	t	t	t	0.19	t	t	t	t	t	
α-Pinene	942	3.0	3.11	2.82	2.93	4.27	2.78	3.69	3.46	4.58	3.2	3.02	3.35 ± 0.60
Camphene	956	1.66	0.29	3.39	2.30	3.83	2.76	4.27	2.04	3.47	2.41	5.28	2.88 ± 1.37
β-Pinene	983	1.39	1.69	0.63	0.8	0.82	0.58	0.77	0.87	1.34	0.81	0.52	0.93 ± 0.38
β-Myreene	994	0.71	0.49	0.73	0.61	0.63	0.70	0.67	0.47	0.36	0.70	0.75	0.62 ± 0.13
α-Phellandrene	1008	t	t	t	t	t	t	t	t	t	t	t	
α-Terpinene	1021	t	0.34	t	t	t	0.24	t	t	t	t	t	
p-Cymene	1026	t	1.19	t	t	t	t	t	t	0.58	t	1.89	
I,8-Cincole	1036	12.07	6.9	12.82	18.54	11.91	11.35	11.01	13.45	12.00	16.66	6.35	12.09 ± 3.5
(*Z*)-β-Ocimene	1043	1.82	0.84	0.41	t	0.82	1.07	0.65	0.88	0.18	—	t	
Benzene	1046	0.40	t	t	t	t	t	t	t	t	t	—	
Acetaldehyde													
(*E*)-β-Ocimene	1053	0.74	0.12	t	t	0.19	0.32	0.16	0.20	—	t	—	
γ-Terpinene	1066	0.59	0.59	0.47	0.43	0.30	0.38	0.38	0.41	0.42	0.32	t	
Terpinolene	1092	t	t	0.34	t	0.32	0.29	0.38	t	t	0.27	t	
α-Thujone	1106	16.82	25.35	17.07	16.87	11.35	11.36	10.87	15.82	8.47	19.54	19.90	15.79 ± 4.9
β-Thujone	1118	3.70	6.04	4.34	3.44	2.32	2.98	2.71	4.12	1.33	3.79	3.49	3.49 ± 1.21
Camphor	1147	4.43	0.15	16.54	8.89	17.83	11.05	21.12	4.24	7.62	9.74	24.8	11.49 ± 7.69
trans-Pinocanphone	1163	0.34	—	t	t	t	t	0.14	t	—	t	t	
Borneol	1169	3.41	0.20	2.66	3.12	4.35	7.19	3.63	3.71	8.50	3.72	5.40	4.17 ± 2.23
cis-Pinocamphone	1176	0.34	—	t	t	t	t	0.14	t	—	t	t	
Terpin-4-ol	1181	0.51	0.34	0.34	0.33	0.30	0.39	0.36	0.30	0.33	0.35	0.46	0.36 ± 0.07
α-Terpineol	1193	0.45	0.14	0.21	t	0.51	0.22	0.19	t	0.19	0.17	t	
Myrtenol	1198	0.30	t	t	t	t	t	0.22	t	t	t	t	
trans-Carveol	1221	—	—	t	t	t	—	t	t	—	—	t	
Bornyl acetate	1289	0.99	0.05	2.26	1.62	2.66	2.57	1.95	1.94	2.23	2.92	4.91	2.19 ± 1.22

(*continued overleaf*)

Table 3. (*Continued*)

Constituents	KI	A-1	B-1	B-2	B-3	B-4	B-5	B-6	C-1	C-2	D-1	D-2	Average
Thymol	1293	t	t	—	t	t	t	t	t	t	t	t	
trans-Sabinyl acetate	1294	t	t	t	t	t	t	t	t	t	0.26	0.57	
Carvacrol	1301	t	t	t	t	t	t	t	t	t	t	0.22	
Cugenol	1359	t	t	t	t	t	t	t	t	t	t	t	
Neryl acetate	1368	t	t	t	t	t	t	t	t	t	t	t	
α-Copaene	1379	t	t	t	0.34	t	t	t	0.50	t	t	t	
Geranyl acetate	1386	t	t	t	—	t	t	t	—	t	t		
cis-Caryophyllene	1407	t	t	t	t	t	t	t	t	t	t	t	
trans-Caryophyllene	1421	8.78	1.09	5.28	8.44	6.22	9.41	5.18	5.04	6.60	3.73	1.04	5.53 ± 2.81
Aromadendrene	1442	0/72	—	—	—	—	—	—	t	t	0.49	—	
α-Humulene	1457	12.28	12.49	3.35	5.74	6.42	8.58	7.31	11.07	5.94	7.59	3.97	7.70 ± 3.12
allo-Aromandrene	1464	0.16	0.23	t	t	t	0.12	t	t	t	t	t	
γ-Muurolene	1480	t	t	0.26	0.58	t	t	t	0.77	t	09.28	t	
β-Sclinene	1488	t	—	—	—	t	t	t	t	—	—	—	
Viridiflorene	1496	0.33	0.18	t	t	t	0.12	t	0.37	t	t	—	
γ-Cadinene	1516	t	t	t	0.26	t	t	t	0.35	t	t	t	
δ-Cadinene	1527	t	t	0.42	1.01	t	t	t	1.33	0.27	0.44	0.26	
α-Calacorene	1545	—	—	—	t	—	—	—	0.22	t	t	t	
Caryophyllenol	1571	t	—	t	t	t	t	t	t	t	t	t	
Caryophyllene oxide	1584	1.01	t	0.37	0.49	0.51	0.81	0.43	t	0.47	0.69	0.21	
Viridiflorol	1595	9.19	26.10	11.67	12.74	14.03	10.81	14.06	15.61	15.32	9.59	5.97	13.19 ± 5.17
Humulene epoxide II	1609	1.82	1.14	0.60	0.64	0.83	0.89	1.04	1.13	0.75	1.63	1.48	1.09 ± 0.41
Caryophylla-4(12), 8(13)-Dien-5β-ol	1640	0.30	t	0.19	0.26	0.26	0.35	0.21	t	t	0.24	t	
β-Cudesmol	1651	t	t	—	t	t	t	—	t	t	—	t	
14-Hydroxy-9-cpi-(*E*)-caryophyllene	1662	0.77	0.77	0.51	0.65	0.35	0.85	0.31	t	t	0.31	0.23	
α-Cudesmol	1655	t	—	—	t	—	—	—	—	—	—	t	
Manool	2060	6.96	8.37	7.23	7.01	6.85	6.71	5.28	9.14	15.92	5.71	5.20	7.67 ± 2.98
trans-Ferrugional	2329	t	t	t	t	t	t	t	t	0.39	t	t	

* Constituents are listed in order of their elution from HP-5MS column.
t, traces $< 0.05\%$.

Table 4. Percentage composition of the essential oil from flowers of *S. officinalis* populations

Constituents	K1	A-1	B-2	B-3	B-4	B-5	B-6	C-1	C-2	D-1	Average
(*Z*)-Salvene	856	t	t	t	t	t	t	t	t	0.19	
(*E*)-Salvene	866	t	t	T	t	t	t	t	t	t	
Tricyclene	927	t	t	t	t	t	T	t	t	t	
α-Pinene	940	7.36	3.65	2.69	3.68	5.47	4.01	5.60	3.81	4.07	4.48 ± 1.41
Camphene	954	2.09	2.20	1.28	2.70	2.91	5.52	1.92	4.49	2.41	2.84 ± 1.34
β-Pinene	981	3.16	1.03	1.13	1.02	1.99	6.23	1.93	0.77	2.10	2.15 ± 1.7
β-Myrcene	992	0.65	0.39	0.35	0.36	0.56	0.46	0.38	0.60	0.40	0.46 ± 0.11
α-Phellandrene	1006	t	t	t	t	t	t	t	t	t	
α-Terpinene	1019	0.42	t	t	t	t	t	t	t	t	
p-Cymene	1024	t	t	0.57	0.70	t	t	t	t	0.58	
1,8-Cincole	1033	16.48	11.79	15.19	9.47	15.57	11.71	16.04	13.26	15.68	13.91 ± 2.45
(*Z*)-β-Ocimene	1041	0.43	—	0.22	—	0.56	0.61	0.26	—	—	
(*E*)-β-Ocimene	1051	t	—	—	t	0.13	t	—	t	—	
γ-Terpinene	1063	0.60	0.28	0.31	t	0.45	0.24	0.35	0.31	0.22	
Terpinolene	1089	0.32	t	t	t	0.28	t	t	t	t	
α-Thujone	1103	9.63	9.06	10.10	8.96	8.60	8.71	10.23	13.27	11.19	9.97 ± 1.49
β-Thujone	1115	2.23	1.89	2.31	1.45	2.01	1.52	2.99	2.21	1.96	2.06 ± 0.46
Camphor	1144	1.21	3.98	2.93	8.85	2.33	11.16	1.64	17.69	2.62	5.82 ± 5.6
Borncol	1166	4.70	6.19	6.40	5.59	12.45	4.80	4.45	5.19	7.37	6.35 ± 2.47
Terpin-4-ol	1178	0.46	0.29	0.30	0.28	0.47	0.32	t	0.35	0.31	0.36 ± 0.07
α-Terpincol	1190	0.69	0.28	0.33	0.51	0.39	0.27	t	t	0.21	

(*continued overleaf*)

Table 4. (*Continued*)

Constituents	K1	A-1	B-2	B-3	B-4	B-5	B-6	C-1	C-2	D-1	Average
Myrtenol	1195	—	t	t	t	t	t	t	—	t	
trans-Carvcol	1218	—	—	—	—	—	t	t	t	—	
Bornyl acetate	1286	0.34	1.28	0.76	2.01	1.24	1.37	0.50	3.71	1.09	1.37 ± 1.01
Thymol	1291	t	t	t	t	t	t	t	t	t	
Carvacrol	1299	t	t	t	t	t	t	t	t	t	
α-Copaene	1377	t	0.30	0.38	t	t	t	0.50	t	0.20	
cis-Caryophyllene	1405	t	t	t	t	t	t	t	t	t	
trans-Caryophyllene	1419	11.02	8.95	10.83	9.16	9.69	7.41	5.95	5.68	6.95	8.40 ± 1.99
α-Humulene	1455	7.84	4.26	4.15	5.74	4.99	6.99	6.87	8.11	5.60	6.06 ± 1.47
allo-Aromandrene	1462	0.31	0.23	t	t	t	t	t	t	0.24	
γ-Muurolene	1478	t	0.49	0.64	t	—	t	0.76	t	0.31	
β-Sclinene	1486	t	t	t	t	t	t	t	t	t	
Viridiflorene	1494	0.66	0.32	t	t	t	t	0.44	t	0.41	
α-Numurolene	1500	t	t	t	t	—	t	t	t	t	
γ-Cadinene	1514	t	t	t	t	t	t	t	t	t	
δ-Cadinene	1525	t	0.84	1.10	t	t	t	t	t	0.51	
Caryophyllene oxide	1582	0.55	0.46	0.70	0.63	0.65	0.61	t	t	0.61	
Viridiflorol	1592	13.52	18.72	19.05	17.01	13.58	17.69	18.25	10.66	13.41	15.76 ± 3.00
Humulene epoxide II	1607	—	0.48	0.42	0.83	t	—	0.78	0.96	—	
Manool	2057	10.47	17.53	14.60	16.26	10.60	12.19	17.25	7.15	15.08	13.48 ± 3.56
trans-Ferruginol	2327	0.38	0.45	0.50	0.35	0.20	t	t	t	0.21	

Constituents are listed in order of their elution from HP-5MS column.
t, traces <0.05%.

sesquiterpenes was also registered. However, in the other continental population, D-2, the camphor ratio was lower but a high ratio of 1,8-cineole (16.66%) was registered. An equal ratio of thujone and camphor was noticed in the sample B-2.

In the flowers, 42 compounds were identified, in which α- and β-pinene, camphene, 1,8-cineole, α- and β-thujone, camphor, borneol, bornyl acetate, *trans*-caryophyllene, α-humulene, viridiflorol and manool were dominant. The greatest variation was found in the content of β-pinene, which ranged from 0.77% (C-2) to 6.23% (B-6). The highest ratios of α-thujone and camphor were found in C-2, 13.27% and 17.69%, respectively. In A-1 the highest value of 1,8-cineol (16.48%) was registered, whereas B-5 contained the highest percentage of borneol (12.45%). Among the sesquiterpenes, the highest viridiflorol content was found in B-3 (19.05%) and of manool in B-2 (17.53%) and C-1 (17.25%), respectively. The results revealed differences in the oil compositions between different plant parts. Thus, in the flowers, the ratios of borneol and sesquiterpenes, especially manool, were higher, whereas the ratios of α-thujone and camphor were significantly lower than in the leaves. It is interesting that in the leaf oil of C-2 the lowest, but in the flower oil the highest, content of α-thujone was registered.

Most of the available literature revealed the essential oil obtained from sage leaves. Kuštrak *et al*.[19,20] and Pitarević *et al*.[21] reported on great variability in genuine Dalmatian sage oil. They also found an inverse proportion in the ratio of thujones and camphor in most samples. In sage populations from the Dalmatian coastline and islands, α-thujone varied from 7.20% (Hvar Island) to 40% (Pelješac Peninsula), whereas the camphor content ranged from 0.69% (Pelješac Peninsula) to 20.5% (Hvar Island).[20] The earlier investigations on sage oil in the continental part of the former Yugoslavia was carried out by Ivanić *et al*.[11,22] In contrast to our results, they reported a very low camphor content in the samples analysed. Thus, in the sage population collected from Sićevo Gorge they found only 0.9% camphor and 28.08% α-thujone. The same authors found very low sesquiterpene contents. Among sesquterpenes they identified only β-caryophyllene (3.3%).[11]

Rhyu[23] reported on the oil composition in sages originated from Croatia, Albania and Yugoslavia. He emphasized the better quality of Albanian and Croatian oil regarding the content of thujones and camphor. However, he reported a very high portion of linalool (11.9–35.0%) in all three samples. These results were not afterwards confirmed by Vermin and Metzger.[24] who performed a similar study but did not find a significant percentage of linalool. Recently, Ristić *et al*.[18] analysed the essential oil of eight populations of *S. officinalis* native to Montenegro and reported on the relations of oil composition and geographic origin. They noticed that the thujone content decreased from the northern (34.85%) to southern localities (13.50%), whereas the percentage of camphene, borneol and caryophyllene increased in the same direction. The results presented here are not in agreement with their findings. We found considerable variation within the samples collected from a very narrow geographical area. Furthermore, in the populations studied, we found a considerably lower camphor content (0.15–24.8%) compared with the results previously reported (20.16–29.01%). The main differences between the composition of the essential oil of sage cited previously and the sage here studied was in the ratio of sesquiterpenes, especially in viridiflorol, α-humulene and manool, which were higher in our studied population. The latter compound was not detected in the previous study.[18] High manool (14.74%) and viridiflorol (13.46%) was reported only in cultivated sage from Cuba.[25]

Regarding the proportion of thujones and camphor, sage collected from the continental part of Serbia, namely Sićevo Gorge (D-2), has an oil with high values. However, sample B-1, with high percentage of camphor but a very low thujone content, should be of interest for pharmaceutical uses, particularly since it is well known that thujone application could provoke considerable toxic effects, such as tachycardia, hot flushes, convulsions and dizziness.[26] Finally, the observed interpopulation variation in the essential oil composition of sage might be the result of various complex factors, both endogenous and exogenous, such as geographical and climatic conditions.

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