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Essential oil composition of wild growing Sage from R. Macedonia

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Abstract

The main objective of this study was to analyze and identify the essential oil composition of *S. officinalis* populations growing in Republic of Macedonia and to evaluate these data according to different standards' requirements for, commercially most utilized, Dalmatian sage. The essential oil yield, obtained after hydrodestilation from leaves, of three different populations of *Salvia officinalis* L. from Republic of Macedonia was determined, varying from 1.40 to 3.46%. The GC/FID/MS analysis of the composition of the essential oils revealed 63, 57 and 51 components in Galicica Mtn., Jablanica Mtn. and Karaorman Mtn. sage populations, respectively. The main components of the oil, in all three samples, were the terpene hydrocarbons, encompassing the monoterpenes: camphor (13.15 - 25.91%), α-thujone (19.25 - 26.33%), β-thujone (2.03 - 5.28%), 1,8-cineole (6.51 – 13.60%), α-pinene (0.93 – 1.47%), borneol (1.07 – 4.67%), then sesquiterpenes: *trans* (E)-caryophyllene (1.72 – 5.33%), α-humulene (2.89 – 7.99%), viridiflorol (4.27 – 7.99%), and the diterpene manool (2.13 - 3.79%).

Thus, our results for the essential oil composition of sage complied with the reference values specified in the DAC 86 monograph for *Salvia* essential oil.

Key words: *Salvia officinalis*, essential oil composition, GC/FID/MS, R. Macedonia.

Introduction

Most of the species from genus *Salvia* have medicinal and horticultural importance as they produce many useful natural constituents including terpenes and flavonoids (Kelen and Tepe, 2008). They are counted as one of the largest members of the Lamiaceae family that includes around 900 species and has an almost cosmopolitan distribution (Mediterranean, Asia Minor, Central Europe and America etc).

"Sage", the dialect name of the genus *Salvia* is attributed to different species that are widely used in the food, drug and fragrance industry. The high diversity in secondary metabolites (essential oils and the phenolic derivatives) isolated from sage plants, possess excellent antimicrobial activity as well as antioxidant capacity and some are used as anticancer agents or have hypoglycemic effect (Kintzios, 2000; Miladinovic and Miladinovic, 2000; Khalil and Li, 2011). Of the many existing *Salvia* species, *Salvia officinalis* also known as "Dalmatian sage" or "Garden sage", has economic importance and can be used for preparation of various extracts and herbal remedies with antiseptic and antibacterial properties which are attributed to the rich chemical content of the essential oil and proven by the modern medical science (Velickovic et.al., 2003; Avato et.al., 2005; Delamare et.al., 2005; Bernotiene et.al., 2007). Although sage is an ancient spice and remedy, its importance today is quite limited to the Mediterranean countries (starting from Italy till Greece) (Mockute et.al., 2003; Hager, 2006). Some of the *Salvia* species, including *S. officinalis* (*S. officinalis folium*) and *S. triloba* (*S. triloba folium*) can be found in many pharmacopeias (Flamini et.al, 2005; Eur.Ph.7.0, 2010). Pharmacopoeias monographs of *S. officinalis* essential oil (Salviae aetheroleum - Helv VII;

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Salviae officinalis aetheroleum - DAC 86) are available, as well. The essential oil can be obtained by steam distillation of the leaves of *S. officinalis* (Helv VII), or from aboveground parts of the sage thus obtaining essential oil rich in thujone (DAC 86).

The well studied Dalmatian sage oil show differences in the total oil yield varying from 1.20 to 3.60% (with maximum in July) and gives characteristic sequence of the major constituents: α + β-thujone> camphor> cineole. The principal components in the sage essential oil are the volatile monoterpenes, present in the following average distribution: 8.4 to 24.0% camphor, cineol (8.4 to 24.0%), α-thujone from 22.2 to 36.8%, β-thujone from 4.0 to 27.5%, borneol (2.1%), bornyl acetate (1.6%), camphene (4.4%), β-caryophyllene (3%), α-humulene (4.4%), α-pinene (3.5%), β-pinene (2.2%) and viridiflorol (6%). The content and composition of the oil is subjected to periodic daily variations and fluctuations as well as to other ecological factors (climatic and soil conditions) (Hager, 2006). The relative amounts of major constituents in the sage essential oil are regulated with the German Drug Codex and the ISO 9909 standard (Table 3).

The Macedonian flora includes 37 species of genus *Salvia*. Up to present there are no evident data about essential oil composition of *S. officinalis* populations growing in Republic of Macedonia. The importance of *S. officinalis* drew our attention to examine and identify the active ingredients of the essential oils obtained by hydrodistillation of sage leaves collected from R. Macedonia.

Materials and methods

Plant material

The plant material from three different populations was harvest from Galicica Mtn., Jablanica Mtn., and Karaorman Mtn., located in the western part of Macedonia, nearby the Albanian border, during June year 2009 and 2010. The leaves were air dried and stored in a cool and dark place until distillation. The herb was authenticated as *Salvia officinalis* L. (Lamiacecae) by Dr. Gjoshe Stefkov, and voucher specimens (No. So-MKD 13/10; So-MKD 14/10; So-MKD 15/10) were deposited at the Herbarium at the Department of Pharmaceutical Botany, Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, Macedonia.

Essential oil isolation

Essential oil isolation from sage leaves was performed by hydrodistillation in all-glass Clevenger apparatus following the procedure from European Pharmacopeia (Ph. Eur.7.0, 2010).

Analysis of essential oils' chemical composition

Each sample of essential oil was dissolved in xylene (1:1000 v/v) and further analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C mass spectrometer. HP-5ms 5% phenyl 95% dimethylpolysiloxane bonded phase capillary column (30 m x 0.25 mm, film thickness 0.25 μm) was used. Analytical conditions were: oven temperature at 60 °C for 5 min, then increased to 80 °C at rate of 1 °C/min and held 2 min and at the end increased to 280 °C at rate of 5 °C/min and held 5 min; helium as carrier gas at a flow rate of 1ml/min; temperature of the injector 260 °C and that of the FID detector 270 °C; the GC split ratio 1:1. 1μl of each sample was injected per GC run. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50 - 500 Da. The MS was operated in scan mode.

The compounds were identified on the basis of literature and estimated Kovat′s (retention) indices that were determined using mixture of homologous series of normal alkanes (C_9-C_{25}) analyzed under Automated Mass Spectral Deconvolution and Identification System (AMDIS)' conditions. Confirmation was done by comparing the mass spectra obtained from AMDIS with the reference spectra from Nist, Wiley and Adams mass spectra libraries.

Quantification of the essential oils components was performed using the normalization method of the GC/FID peak areas.

Results and discussion

The essential oil yields, obtained with hydrodistillation of the upper leaves from each sage population from Macedonia were as followed: So-MKD 13/10 = 1.8%, So-MKD 14/10 = 1.4% and So-MKD 15/10 = 3.46%. The amount of oils from Galicica and Jablanica populations (1.8% and 1.4%, respectively) were in accordance with literature references 1-2.5% (ESCOP Monographs) or 1-3% (Eur.Ph.7), while the isolate from Karaorman Mtn. (essential oil, 3.46%) was above these values, and close to the upper limit for the Dalmatian essential oil.

By the means of GC-MS, total of sixty nine components, in all three samples of *S. officinalis* were identified and presented in Table 1.

The GC-MS analysis revealed sixty three components (37 monoterpenes, 17 sesquiterpenes, 5 diterpenes and 4 other components) in the essential oil from Galicica sage population, fifty seven (34 monoterpenes, 12 sesquiterpenes, 5 diterpenes and 6 other constituents) from Jablanica sage population and fifty one from Karaorman sage population (27 monoterpenes, 13 sesquiterpenes, 5 diterpenes and 6 other components - aromatic and aliphatic hydrocarbons, esters and etc). The quantities of the constitutive chemical groups of the essential oils of *Salvia officinalis* populations are shown in Fig. 1.

The most abundant constituents, in all three samples, were the terpene hydrocarbons, encompassing the volatile monoterpenes: camphor (13.15 - 25.91%), α-thujone (19.25 - 26.33%), β-thujone (2.03 - 5.28%), 1,8-cineole

Table 1. Chemical composition of essential oils isolated from three different populations of *S. officinalis* from R. Macedonia

No.	KIL	KIE	Component	So-MKD 13/10 % (m/m)	So-MKD 14/10 % (m/m)	So-MKD 15/10 % (m/m)
1	/	921.0	4-heptanol	/	0.26	0.42
2	926	924.7	tricyclene	/	0.08	0.08
3	931	927.9	alpha-thujene	0.05	/	/
4	939	935.8	alpha-pinene	1.20	0.93	1.47
5	953	951.2	camphene	2.91	1.94	1.96
6	978	976.7	1-octen-3-ol	0.07	0.07	0.05
7	980	980.0	beta-pinene	0.78	0.22	0.46
8	991	991.2	myrcene	0.80	0.50	0.81
9	994	969.1	mesitylene	0.09	0.06	0.10
10	1000	999.9	n-decane	0.05	0.04	0.06
11	1005	1007.1	alpha-phellandrene	0.09	0.06	0.03
12	1018	1018.6	alpha-terpinene	0.16	0.15	0.10
13	1026	1026.1	p-cymene	0.28	0.41	0.79
14	1031	1030.4	limonene	2.15	1.84	1.63
15	1033	1033.5	1,8-cineole	6.51	9.92	13.60
16	1062	1059.8	gamma-terpinene	0.29	0.19	0.54
17	1065	1069.0	cis-sabinene hydrate	0.05	/	/
18	1088	1090.6	terpinolene	0.51	0.26	0.26
19	1098	1101.3	linalool	0.60	0.40	0.30
20	1110	1110.4	cis-thujone	19.98	19.25	26.33
21	1111	1119.4	trans-thujone	2.03	3.26	5.28
22	1125	1128.6	alpha-campholenal	0.06	0.03	0.03
23	1133	1136.9	iso-3-thujanol	0.07	0.06	/
24	1139	1143.7	trans-sabinol	0.10	0.07	0.10
25	1143	1150.1	camphor	25.91	23.79	13.15
26	1156	1160.0	isoborneol	0.07	0.06	/
27	1160	1163.9	trans-pinocamphone	/	0.06	0.16
28	1165	1168.9	borneol	4.26	4.67	1.07
29	1177	1179.3	terpinen-4-ol	0.39	0.43	0.44
30	1183	1186.3	p-cymene-8-ol	0.06	0.09	0.04
31	1189	1192.6	alpha-terpineol	0.20	0.17	0.11
32	1194	1199.2	myrtenol	0.04	0.26	0.26
33	1217	1220.1	trans-carveol	0.07	0.17	0.04
34	1226	1225.0	cis-carveol	0.03	/	/
35	1233	1230.0	isobornyl formate	0.03	0.05	/
36	1235	1237.2	neral	0.02	0.04	/
37	1237	1241.6	pulegone	0.03	/	/
38	1255	1254.0	geraniol	0.02	/	/
39	1267	1269.0	iso-3-thujanol acetate	0.03	/	/
40	1273	1276.0	neo-3-thujanol acetate	0.06	0.08	/
41	1285	1288.4	bornyl acetate	3.86	3.53	0.43
42	1290	1293.8	trans-sabinyl acetate	0.31	0.33	0.16
43	1298	1301.2	carvacrol	0.05	0.12	0.10
44	/	1327.5	myrtenyl acetate	/		0.05
45	1337	1338.4	trans-carvyl acetate	0.03	0.07	/
46	1342	1344.2	piperitenone	/	0.06	/
47	/	1381.7	6,9-guaiadiene	0.11	/	/
48	1386	1390.4	alpha-isocomene	0.18	/	0.14

No.	KIL	KIE	Component	So-MKD 13/10 % (m/m)	So-MKD 14/10 % (m/m)	So-MKD 15/10 % (m/m)
49	1409	1418.6	alpha-gurjunene	0.05	/	/
50	1418	1424.2	(E)-caryophyllene	1.716	4.21	5.33
51	/	1454.8	trans-cadina-1(6),4 -diene	1.69	1.32	/
52	1453	1458.5	alpha-humulene	2.89	5.21	7.34
53	1467	1465.5	9-epi-(E)-carryophyllene	0.33	0.44	0.34
54	1478	1479.0	gamma-muurolene	0.44	/	/
55	1493	1499.5	viridiflorene	2.67	2.77	2.04
56	1528	1510.9	zonarene	0.07	0.07	0.04
57	1524	1527.1	delta-cadinene	0.15	0.09	0.04
58	1581	1588.2	caryophyllene oxide	0.25	0.40	0.35
59	1590	1597.3	viridiflorol	4.27	/	7.99
60	1593	1600.0	humulene epoxide I	0.16	/	/
61	1606	1614.4	humulene epoxide II	0.49	0.75	0.81
62	1630	1637.0	muurola-4,10(14)-dien-1-b-ol	0.45	1.21	1.19
63	1664	1674.8	14-hydroxy-(Z)-caryophyllene	0.17	0.77	0.46
64	/	1810.2	alpha-bisabolene	/	0.16	0.11
65	1894	1901.8	rimuene	0.08	0.08	0.05
66	1950	1917.4	isopimara-9(11)-15-dien	0.19	0.21	0.16
67	1960	1668.8	sandaracopimara- 8(14), 15- diene	0.25	0.21	0.13
68	/	1977.7	labd-7,13-dien-15-ol	0.11	0.09	0.06
69	2056	2061.2	manool	2.13	3.79	3.06
			Total:	93.13	95.73	100.00

*No. - Ordinal number according to the elution order of the components; KIL - Kovat's Index Literature; KIE - Kovat's Index Estimated, So-MKD 13/10 – *Salvia officinalis* population from Galicica Mtn.; So-MKD 14/10 – *Salvia officinalis* population from Jablanica Mtn.; So-MKD 15/10 – *Salvia officinalis* population from Karaorman Mtn.

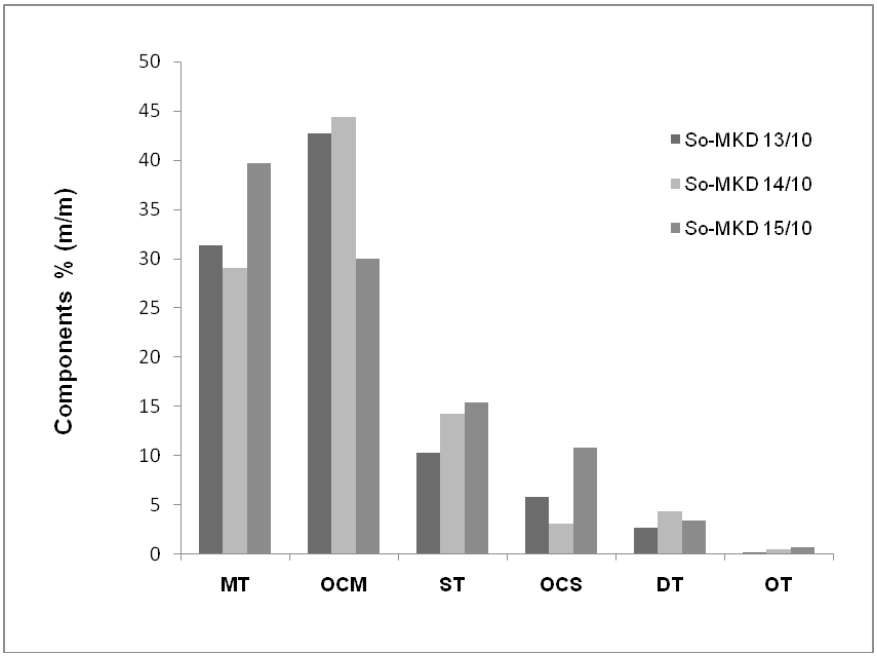

Fig. 1. Presentation of different classes of terpenes and their abundance in the essential oils of Macedonian sage plants
*MT - monoterpene; OCM - oxygen containing monoterpene; ST - sequiterpene; OCS - oxygen containing sesquterpene; DT - diterpene; OT - other type (aliphatic and aromatic hydrocarbons, aromatic esters etc.); So-MKD 13/10 – *Salvia officinalis* population from Galicica Mtn.; So-MKD 14/10 – *Salvia officinalis* population from Jablanica Mtn.; So-MKD 15/10 – *Salvia officinalis* population from Karaorman Mtn.

(6.51 – 13.60%), α-pinene (0.93 – 1.47%), borneol (1.07 – 4.67%), the sesquiterpenes: *trans* (E)-caryophyllene (1.72 – 5.33%), α-humulene (2.89 – 7.99%), viridiflorol (4.27 – 7.99%), and the diterpene manool (2.13 - 3.79%).

Our results for the essential oil composition of sage, thus, comply with the reference values specified in the German Drug Codex monograph for *Salvia officinalis* essential oil. The composition analysis of the volatile constituents of the three Macedonian sage populations showed that only the essential oil from Karaorman Mtn. population belongs to the thujone-rich oils, while essential oils isolated from Galicica Mtn. and Jablanica Mtn. populations had camphor as major component. Typically, according to some authors (Perry et.al., 1999; Walch et.al., 2011), three are three Dalmatian Sage chemotypes with low (9%), medium (22-28%), and high (39-44%) thujone contents. Concerning the latter, essential oils from *S. officinalis* samples from Galicica Mtn. and Jablanica Mtn. populations, belong to the Sage group containing medium thujone amounts and cannot meet with the requirements for the sage essential oil chemical composition reported in other available scientific literature where the demand of thujone content is stated as 35-60% (e.g. Radulescu et.al., 2004; Maksimovic et.al., 2007). Due to the camphor predominance in essential oil isolated from Galicica Mtn. population, this essential oil cannot meet the ISO 9909 standard (Table 2).

This inter-specific comparison of the essential oils isolated from three *Salvia officinalis* populations from Macedonia showed differences in the amount of the principle components especially in the oils obtained from Galicica and Jablanica populations what emphasize the role of the environmental factors (light, soil, water, time of harvest, drying, etc.) on the yield and chemical composition of the essential oil even when they are from same region.

Conclusion

The essential oils' yields obtained after hydrodestilation of leaves from three different populations of sage from Macedonia varied from 1.40 to 3.46%. The essential oil composition of all three sage populations complies with the reference values specified in the DAC 86 monograph for *Salvia* essential oil. Yet, the requirements of the ISO 9909 standard have not been met. Nevertheless, the set of components identified in the three samples of the Macedonian sage oil match up with the essential oil composition of the Dalmatian sage, though the set differs in relative content of the principle components.

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DAC 86 – Deutsher Arzneimittel Codex (German Drug Formulary) 86.

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Table 2. Comparison of the dominant constituents of the essential oils obtained after hydrodestilation of the three *Salvia officinalis* populations from R. Macedonia with referent literature

Constituents % (m/m)	So-MKD 13/10	So-MKD 14/10	So-MKD 15/10	DAC 86[1]	ISO 9909[2]	Serbia[3]	Romania[4]	Croatia[5]
1,8-cineole	6.51	9.92	13.61	6.00-16.00	5.50-13.00	9.79	6.72	0.90
cis-thujone	19.98	19.25	26.33	>20.00	18.00-43.00	24.88	21.85	57.00
trans-thujone	2.03	3.26	5.28		3.00-8.50	/	5.51	15.00
camphor	25.91	23.79	13.15	14.00-37.00	4.50-24.50	16.03	11.25	3.30
borneol	4.26	4.67	1.07	≤5.00	/	/	2.58	/
bornyl acetate	3.86	3.53	0.43	≤5.00	<2.50	/	3.22	/
α-pinene	1.20	0.93	1.47	/	1.00-6.50	/	/	/
α-humulene	2.89	5.21	7.34	/	0-12.00	/	4.51	/
camphene	2.91	1.94	1.96	/	1.50-7.00	/	1.66	/
limonene	2.15	1.84	1.63	/	0.50-3.00	/	/	/
viridiflorol	4.27	/	7.99	/	/	/	11.71	14.20
manool	2.13	3.79	3.06	/	/	/	9.15	/

[1]Monograph for *Salvia officinalis* essential oil in DAC 86 (German Drug Formulary 86); [2]ISO 9909 standard for medicinal uses regulates the amounts of nine constituents in the sage essential oil, Ref: Mockute *et.al.,* 2003; Bernotiene *et.al.,* 2007; [3]*Salvia officinalis* essential oil from Serbia, Ref.: Miladinovic and Miladinovic, 2000; [4]*Salvia officinalis* essential oil from Romania, Ref.: Radulescu *et.al.,* 2004; [5]*Salvia officinalis* essential oil from Croatia, Ref.: Maksimovic *et.al.,* 2007; So-MKD 13/10 – *Salvia officinalis* population from Galicica Mtn.; So-MKD 14/10 – *Salvia officinalis* population from Jablanica Mtn.; So-MKD 15/10 – *Salvia officinalis* population from Karaorman Mtn.

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Резиме

Состав на етерично масло од диво растечки жалфии од Р. Македонија

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Клучни зборови: *Salvia officinalis*, состав на етерично масло, GC/FID/MS, Р. Македонија.

Главна цел на студијата беше анализа и идентификација на составните компоненти на етеричното масло изолирано од популациите на *S. officinalis* кои растат во Република Македонија и нивна споредба со далматинска жалфија. Приносот на етерично масло добиено со хидродестилација на листовите од трите различни популации на *Salvia officinalis* L. од Република Македонија се движи од 1.46 до 3.46%. Со GC/FID/MS анализата на етеричните масла идентификувани се 63, 57 и 51 компонента кај популациите од Галичица, Јабланица и Караорман, соодветно. Како главни компоненти на маслата во сите три примероци се терпенските јаглеводороди, вклучувајќи ги монотерпените: камфор (13.15 - 25.91%), α-тујон (19.25 - 26.33%), β-тујон (2.03 - 5.28%), 1,8-цинеол (6.51 – 13.60%), α-пинен (0.93 – 1.47%), борнеол (1.07 – 4.67%), по што следат сескитерпените: транс (E)-кариофилен (1.72 – 5.33%), α-хумулен (2.89 – 7.99%), виридифлорол (4.27 – 7.99%) и дитерпенот манол (2.13 - 3.79%). Следствено, составот на етеричното масло од испитуваните жалфии од Р. Македонија одговара на референтните вредности утврдени во монографијата за етерично масло на жалфија во Германскиот кодекс за лекови (DAC 86).