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Chemical Composition of the Essential Oils of *Salvia officinalis*, *S. fruticosa*, *Melissa officinalis*, and their Infusions

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Summary: Dried leaves of commercially available *Salvia officinalis*, *Salvia fruticosa*, and *Melissa officinalis* were divided into two parts; the first part was subjected to hydrodistillation and the second part was used for the preparation of the infusions. The essential oil and the infusion of each sample were subjected to analysis by means of GC-FID and GC-MS. The oxygenated monoterpenes 1,8-cineole (27.5%) and camphor (11.5%) appeared as the most important metabolites in *Salvia officinalis*, *a*- and *β*-thujone (16.5%, 16.4%), followed by 1,8-cineole (8.8%) were characterizing the essential oil of *Salvia fruticosa*, whereas in the oil of *Melissa officinalis* the sesquiterpene caryophyllene oxide (14.9%) was the most abundant constituent, followed by geranial (12.2%), neral (11.2%) and citronellal (6.7%). The infusions were characterized by the higher levels of the most important compounds found in the essential oil of the respective sample, as in the case of *S. officinalis* (1,8-cineol 53.6%, camphor 25.8%) and *S. fruticosa* (*a*- thujone 61.2%, *β*-thujone 24.3%, 1,8-cineole 14.5%), while the infusion of *M. officinalis* was dominated by palmitic and stearic acid (25.5%, 19.5%).
Key words: chemical composition, essential oils, infusions, *Melissa officinalis*, *Salvia fructicosa*, *Salvia officinalis*

Introduction

Many herbs with potentially beneficial effects which are attributed to their volatile constituents are used as herbal teas. The genus *Salvia* is one of the largest members of the family Lamiaceae (subfamily Nepetoideae), comprising more than 500 species (Hedge, 1972). In the European flora the genus is represented by 36 species (Hedge, 1972). Members of the genus *Salvia* have been shown to possess a significant spectrum of pharmacological properties (Newall et al. 1996; Weiss, 1998).

Melissa L. on the other hand, is a small genus, represented in Europe by *Melissa officinalis* L. (Fernandez, 1972). Lemon balm, *M. officinalis*, is a perennial herb, cultivated for lemon-scented leaves used as seasoning and in medicine (Mabberley, 1997). In folk medicine this aromatic plant is used against colds and in functional disorders of the circulation. In the Commission E monographs its use is recorded for nervous sleeping disorders and functional gastrointestinal complaints (Blumenthal, 2000).

The origin however of commercially available medicinal plant material is an important topic for debate since many pharmacies, along with various herbal stores provide raw material as plant drugs without certification and accurate botanical identification. Against this background, aim of the present study was the comparison of the essential oil of commercially available Common sage (*Salvia officinalis*), Greek sage (*Salvia fruticosa*), and Lemon balm (*Melissa officinalis*) to the literature data. Additionally, the volatile compounds of the infusions of these taxa was investigated, and compared to the chemical analysis of the respective essential oils.

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Materials and Methods

Plant material

Plant material included commercially available dried leaves of *Salvia officinalis* (SOF) and *S. fruticosa* (SFR), of Albanian and Greek (N. Pindos Mt., Metsovo) origin respectively and dried leaves of *Melissa officinalis* (M2) originating also from Greece (Crete). The analyzed plant material was purchased from various pharmacies in Athens.

Isolation of the essential oils

The plant material was subjected to hydrodistillation for 3 h using a modified Clevenger apparatus to obtain the essential oil. The essential oils were dried over anhydrous sodium sulfate and kept at -4°C until analysis.

Preparation of the infusions

2 g of each sample were added to 60 mL of boiling distilled water in a stainless steel pot, left at 25°C for 15 min and consequently filtered. The filtrate was further subjected to partitioning with n-heptane, and the organic layer was subject to chromatographic analysis by means of GC-FID and GC-MS.

GC-FID & GC-MS

GC-MS analysis was carried out using a Hewlett Packard 5973-6890 GC-MS system operating in the EI mode at 70 eV, equipped with a 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 was heated to 280°C with a 3°C/min rate (carrier gas He, flow rate 2 ml/min).

Identification of components

The identification of the chemical constituents was based on comparison of their Kováts indices, relative retention times and mass spectra with those reported in the NIST/NBS and Wiley libraries and the literature (Adams, 2007).

Results and Discussion

Chemical composition of the essential oils

The chemical composition of the essential oils is presented in Table 1. Oxygenated monoterpenes were the most important class of compounds of all three samples (45.9%-56.1%).

Oxygen containing monoterpenes 1,8-cineole (27.5%) and camphor (11.5%) were the most abundant metabolites in *S. officinalis* essential oil, followed by the sesquiterpenes guaiol (7.7%) and (*E*)-caryophyllene (6.9%). Monoterpene hydrocarbons *a*-pinene and myrcene were also found in considerable amounts (6.2%, 5.0%).

Salvia fruticosa essential oil on the other hand, was dominated by the presence of *cis*- and *trans*-thujone (16.5%, 16.4%), while 1,8-cineole (8.8%) came third in order, followed by the sesquiterpene hydrocarbons *δ*-cadinene (8.1%), (*E*)-caryophyllene (6.2%) and *a*-cubebene (5.7%) along with the monoterpene hydrocarbon myrcene (5.5%). Worth of notice is the higher abundance of the sesquiterpene fraction in the oil of *S. fruticosa* reaching 37.2%, in comparison to *S. officinalis* essential oil (12.1%).

According to published data (Pitarokili et al., 2005), *a*-thujone (10-60%), *β*-thujone (4-36%), camphor (5-20%), 1,8-cineole (1-15%), *β*-pinene (0.5-17.9%), camphene (1.7-10.3%), as well as *a*-pinene (0.9-7.2%) are typical *S. officinalis* essential oil components. Furthermore, Cvetkovikj et al. (2015), who investigated the chemical diversity of southeast European populations of Dalmatian sage, identified four distinct chemotypes (A-D). Chemotype A was represented by the high content of α-thujone and camphor, along with the low levels of β-thujone, and characterized the majority of the studied material in the aforestated report. Chemotype B on the other hand, was characterized by the high levels of α-thujone and α-humulene, but camphor was detected only in low amounts. High concentration of both isomers of thujene, along with camphor defined chemotype C, whereas chemotype D was characterized by the high percentage of camphor and β-pinene, and by the low abundance of α- and β-thujone. In addition, Stešević et al. (2014), who studied the chemical variation among twelve populations of indigenous Dalmatian sage in Montenegro, identified the oxygen containing monoterpenes α-thujone (16.98-40.35%), camphor (12.75-35.37%), 1,8-cineole (6.40-12.06%), and β-thujone (1.50-10.35%), as well as the monoterpene hydrocarbon, camphene (2.26-9.97%), as the most abundant essential oil components of *S. officinalis*.

Hence, it is clear that the commercial sample of Albanian origin, declared as *S. officinalis*, and investigated in our study does not comply with either of the aforementioned chemotypes of Dalmatian sage, since both isomers of thujone are found in significantly lower amounts than expected, whereas the content in 1,8-cineole is strikingly higher. However, Miguel et al. (2011), who evaluated the effect of hydrodistillation time on Dalmatian sage essential oil composition of commercially available samples as well, identified also 1,8-cineole (64.3-67.1%) as the most dominant metabolite, followed by α-pinene (6.5-8.2%) and camphor (5.3-6.1%), while α-thujone (1.2-1.4%) and β-thujone (2.3-2.8%) were detected only in low amounts too, compared to our analysis. In our analysis the abundance of 1,8-cineole was also remarkably high (27.5%), and α-pinene, the second most abundant metabolite in the sample of Miguel et al. possessed also a considerable amount (6.2%). According to Asllani (2000), who investigated the variation of the chemical composition of wild grown *S. officinalis* essential oil from Albania, the abundance of these compounds can vary depending on time of harvest, and collection site. Variations in the chemical profile of *S. officinalis* are also attributed to genetic factors (Bazina et al. 2002; Bazina et al. 2015). Moreover, according to Asllani (2000), the levels of 1,8-cineole and camphor seem to be inversely related to the content of both *a*- and *β*-thujone.

According to the literature data, *a*- and *β*-thujone, most abundant compounds of our sample, were generally found in lower amounts in cultivated *S. fruticosa*, which points to the fact that the chemical profile of cultivated plants also deviate (Pitarokoili et al. 2005). Furthermore, 1,8-cineole, and camphor, are usually identified as the main constituents of *S. fruticosa* essential oil (Bazina et al. 2002, Länger et al. 1996, Bellomaria et al. 1992). However, according to Papageorgiou et al. 2008, who investigated the seasonal variation of chemical composition of *S. fruticosa* collected in Greece, 1,8-cineole (46.0-58.9%) was

dominating in the essential oil composition regardless the time of harvest, while camphor was detected in significantly lower amounts (0.7-5.8%). Significant variation of our experimental data in comparison to the literature data could be of genetic background.

Despite the fact that the dominant group of metabolites are oxygenated monoterpenes (56.1%) in the oil of *Melissa officinalis*, the major compound was the sesquiterpene hydrocarbon (*E*)-caryophyllene (14.9%), followed by citral isomers, geranial (12.2%) and neral (11.2%), and citronellal (6.7%). Obtained data were generally in accordance to the literature (Teuscher et al. 2003, Shakeri et al. 2016).

Table 1. Chemical composition of the essential oils

No.	Constituents	RI	SOF	SFR	M2
1	(*Z*)-salvene	847	-	0.2	-
2	tricyclene	921	0.3	-	-
3	*a*-thujene	924	-	0.6	-
4	*a*-pinene	932	6.2	1.0	-
5	camphene	946	4.2	0.8	-
6	sabinene	969	tr	0.5	-
7	*β*-pinene	974	2.6	1.3	-
8	6-methyl-5-heptene	981	-	-	0.4
9	myrcene	988	5.0	5.5	-
10	2*E*,4*E*-heptadienal	1005	-	-	0.4
11	*a*-terpinene	1014	0.6	0.3	-
12	*p*-cymene	1020	tr	tr	tr
13	limonene	1024	-	tr	0.5
14	1,8-cineole	1026	27.5	8.8	0.4
15	benzene acetaldehyde	1036	-	-	0.4
16	(*E*)-*β*-ocimene	1044	-	0.4	-
17	*γ*-terpinene	1054	0.6	tr	tr
18	terpinolene	1086	0.2	tr	-
19	linalool	1095	0.4	-	-
20	*a*-thujone	1101	1.1	16.5	-
21	*cis*-rose oxide	1106	-	-	1.0
22	*β*-thujone	1112	1.2	16.4	-
23	*trans*-rose oxide	1126	-	-	0.5
24	camphor	1141	11.5	0.6	-
25	neoiso-3-thujanol	1147	-	tr	-
26	citronellal	1149	-	-	6.7
27	iso-menthone	1159	-	-	0.5
28	borneol	1165	tr	0.9	-
29	neo-iso-pulegol	1166	-	-	1.7
30	menthol	1167	-	-	2.0
31	terpinen-4-ol	1174	0.8	0.3	-
32	*a*-terpineol	1186	4.2	0.2	-
33	methyl chavicol	1195	-	-	3.1
34	citronellol	1223	-	-	1.2
35	neral	1235	-	-	11.2
36	carvone	1239	-	-	5.6
37	geraniol	1249	-	-	0.9
38	geranial	1264	-	-	12.2
39	*trans*-anethole	1282	-	-	4.3
40	isobornyl acetate	1283	0.7	1.1	-
41	thymol	1289	tr	tr	tr
42	carvacrol	1298	0.8	0.5	3.2
43	methyl geranate	1322	-	-	0.7
44	*a*-cubebene	1345	-	5.7	-
45	*a*-terpinyl acetate	1346	0.7	-	-

No.	Constituents	RI	SOF	SFR	M2
46	eugenol	1356	-	-	-
47	*a*-ylangene	1373	-	0.3	-
48	*a*-copaene	1375	tr	2.7	0.4
49	(*E*)-*β*-damascenone	1383	-	-	1.9
50	*β*-bourbonene	1387	-	0.3	tr
51	(*E*)-caryophyllene	1417	6.9	6.2	5.8
52	*β*-gurjunene	1431	tr	-	-
53	neryl acetone	1434	-	-	0.7
54	*a*-guaiene	1437	-	0.1	-
55	aromadendrene	1439	1.1	0.2	-
56	myltayl-4(12)-ene	1445	0.1	-	-
57	*trans*-muurola-3,5-diene	1451	-	0.4	-
58	*a*-humulene	1452	1.6	1.3	0.4
59	*allo*-aromadendrene	1458	0.2	-	-
60	*cis*-cadina-1(6),4-diene	1461	-	0.2	-
61	*trans*- cadina-1(6),4-diene	1475	-	0.4	-
62	*γ*-muurolene	1478	0.2	1.5	-
63	ar-curcumene	1479	-	-	0.8
64	*a*-amorphene	1483	-	0.5	-
65	germacrene D	1484	-	0.4	-
66	(*E*)-β-ionone	1487	-	-	1.4
67	*β*-selinene	1489	-	0.3	-
68	*cis*-β-guaiene	1492	0.1	-	-
69	*trans*-muurola-4(14),5-diene	1493	-	0.9	-
70	*γ*-amorphene	1495	-	3.0	-
71	viridiflorene	1469	0.5	-	-
72	epizonarene	1501	0.2	-	-
73	*δ*-amorphene	1511	-	0.5	-
74	*γ*-cadinene	1513	0.2	0.5	tr
75	myristicin	1517	-	-	0.3
76	*δ*-cadinene	1522	tr	8.1	-
77	*cis*-calamenene	1528	0.6	0.4	-
78	*trans*-cadina-1,4-diene	1533	-	0.6	-
79	selina-3,7(11)-diene	1545	-	1.1	-
80	*β*-vetiverene	1554	0.4	-	-
81	germacrene B	1559	-	1.6	-
82	spathulenol	1577	-	0.2	-
83	caryophyllene oxide	1582	0.9	-	14.9
84	guaiol	1600	7.7	-	-
85	humulene epoxide II	1608	0.3	tr	0.7
86	1-epi-cubenol	1627	-	0.7	-
87	14-hydroxy-(*Z*)-caryophyllene	1666	1.2	-	1.3
88	(*E*)-14-hydroxy-9-*epi*-caryophyllene	1668	0.6	-	-
89	hexadecanoic acid	1959	-	-	2.1
90	manool	2056	2.7	-	-
	Grouped identified components				
	monoterpene hydrocarbons		19.7	10.0	0.5
	oxygenated monoterpenes		51.5	45.9	56.1
	sesquiterpene hydrocarbons		12.1	37.2	9.3
	oxygenated sesquiterpenes		10.7	0.9	19.3
	other compounds		2.7	-	2.5
	Total		96.7	94.0	87.7
	oil yield % w/v		2.08	1.53	0.03

Constituents listed in order of elution from an HP-5 MS column.
RI, retention indices on the HP-5 MS column relative to C_9-C_{23} *n*-alkanes.
Identification based on the following scales: I; retention index, MS, mass spectrum obtained from the libraries of the gas chromatography-mass spectrometry system and from the literature.
tr, trace (<0.1%)

Table 2. Chemical analysis of the infusion volatiles

No.	Constituents	RI	SOF	SFR	M2
1	*a*-pinene	932	tr	-	-
2	camphene	946	tr	-	-
3	myrcene	988	tr	tr	-
4	*p*-cymene	1020	tr	-	-
5	1,8-cineole	1026	53.6	14.5	-
6	linalool	1095	tr	-	-
7	*a*-thujone	1101	1.1	61.2	-
8	*β*-thujone	1112	1.1	24.3	-
9	camphor	1141	25.8	-	-
10	*δ*-terpineol	1162	1.4	-	-
11	borneol	1165	1.3	-	-
12	terpinen-4-ol	1174	tr	-	-
13	*a*-terpineol	1186	4.6	-	-
14	neral	1235	-	-	3.4
15	carvone	1237	-	-	4.2
16	geranial	1264	-	-	6.3
17	carvacrol	1298	tr	tr	tr
18	*a*-cubebene	1345	-	tr	-
19	hexadecanoic acid/ palmitic acid	1959	-	-	25.5
20	octadecanoic acid/stearic acid	2170	-	-	19.5

Constituents listed in order of elution from an HP-5 MS column.
RI, retention indices on the HP-5 MS column relative to C_9-C_{23} *n*-alkanes.
Identification based on the following scales: I; retention index, MS, mass spectrum obtained from the libraries of the gas chromatography-mass spectrometry system and from the literature.
tr, trace (<0.1%)

Chemical composition of the infusions volatiles

The infusion of *S. officinalis* was richer in 1,8-cineole and camphor (53.6%, 25.8% respectively) components. In *S. fruticosa* the oxygenated monoterpenes *cis*- and *trans*-thujone (61.2%, 24.3%) were identified as the main infusion volatiles, followed by 1,8-cineole (14.5%).

Palmitic (25.5%) and stearic acid (19.5%) on the other hand, were the main metabolites of the volatile fraction in the infusion of *M. officinalis*, whereas the main metabolites of the essential oil, geranial (6.3%) and neral (3.4%), appeared in lesser amounts. Carvone, an oxygenated monoterpene, presented in *M. officinalis* essential oil (5.6%), was identified in a considerable amount (4.2%) also in the infusion volatiles.

Conclusions

Oxygenated monoterpenes were the dominant group of compounds of commercially available *Salvia* essential oils; however the chemical analysis showed significant deviation from the literature data. Specifically the chemical profile of the sample of Albanian origin, declared as *S. officinalis*, was similar to the chemical profile of *S. fruticosa*, according to published data. In a similar way, the essential oil analysis of sage of Greek origin, botanically identified as *S. fruticosa*, resembled to the chemical profile of *S. officinalis*.

Noteworthy is the fact that caryophyllene oxide, one of the most abundant metabolite of *Melissa officinalis* essential oil, was not detected in the analysis of *M. officinalis* infusion volatiles, probably due to hydrolysis during extraction.

The fact that our experimental data, specifically the chemical analyses of the *Salvia* samples are generally not in accordance to the literature data, indicates an inaccurate botanical identification of the distributed raw material in the market of plant drugs. Hence, we believe that an accurate botanical identification and classification of the distributed plant material would be of great significance and extremely critical in order to ensure safe handling of all plant drugs and to avoid unintentional mishandling of potentially unsafe pharmaceutical plant material.

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Hemijski sastav etarskih ulja *Salvia officinalis*, *S. fruticosa*, *Melissa officinalis* i njihovih infuzuma

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Sažetak: U ovom radu ispitivan je sastav isparljivih komponenti komercijalno dostupnih vrsta *Salvia officinalis*, *Salvia fruticosa* i *Melissa officinalis*. Osušeni listovi biljaka razdvojeni su u dve grupe. Prva grupa je podvrgnuta hidrodestilaciji (za dobijanje etarskog ulja), a druga je korišćena za pripremu infuzuma. Hemijski sastav etarskog ulja prve grupe uzoraka i infuzum druge grupe ispitivan je primenom GC-FID i GC-MS tehnike. U etarskim uljima sva tri uzorka nađene su velike količine oksidovanih monoterpena (51,5%, 45,9%, 56,1%). U etarskom ulju *Salvia officinalis* su bili dominantni 1,8-cineol (27,5%) i kamfor (11,5%), u etarskom ulju *Salvia fruticosa* α- and β-tujon (16,5%, 16,4%) i 1,8-cineol (8,8%), dok je u etarskom ulju *Melissa officinalis* nađen najveći sadržaj seskviterpena kariofilen-oksida (14,9%) i oksidovanih monoterpena, geraniala (12,2%), nerala (11,2%) i citronelala (6,7%). U isparljivim frakcijama infuzuma najzastupljenije su bile iste komponente koje su dominirale i u etarskim uljima ispitivanih vrsta biljaka: *S. officinalis* (1,8 -cineol 53.6% i kamfor 25,8%) i *S. fruticosa* (α- tujon 61,2%, β-tujon 24,3%, 1,8-cineol 14,5%), dok su u infuzumu *M. officinalis* dominantne komponente bile palmitinska (25.5%) i stearinska kiselina (19,5%).

Ključne reči: etarska ulja, hemijski sastav, infuzumi, *Melissa officinalis*, *Salvia fructicosa*, *Salvia officinalis*

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