Composition of the Essential Oils from *Salvia dominica* L. and *Salvia hormium* L.Grown in Jordan

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ABSTRACT

In Jordan, *Salvia dominica* L. is widely distributed while *Salvia hormium* L. is scarcely found. The objective of this study was to determine the volatile oil composition of the fresh and dried aerial parts of these two species, collected during flowering time. The hydrodistilled volatile oils were analyzed by gas chromatography-mass spectrometry (GC/MS). The volatile oil of fresh and dry *S. dominica* was composed mainly of oxygenated monoterpenes (79.0% and 68.1%, respectively). The major components detected in the oils of fresh and dry *S. dominica* L. were linalool (31.4% and 18.3%, respectively) and alpha terpineol (25.4% and 15%, respectively). Mono- and sesquiterpenoids were detected in the volatile oils of fresh and dry *S. hormium* in nearly equal amounts. Alpha cadinene (7.8% and 11.4%, respectively) was the predominant constituent in oils obtained from fresh and dry plants. Eugenol (7.3%) was found only in the volatile oil of fresh *S. hormium*.

Keywords: Volatile oils, Oxygenated monoterpenes, Linalool, α-Terpineol, α-Cadinene, GC/MS.

INTRODUCTION

Salvia species (sage) belong to the family Lamiaceae (formerly Labiatae). *Salvia* is the largest and the most important genus of this family. Plants belonging to this genus show high diversity in their secondary metabolites as well as in their pharmacological effects.[1] The genus *Salvia* encompasses about 900 species, widespread throughout the world, and includes several ornamental, culinary and medicinal species.[2] Nineteen species of *Salvia* are reported to occur in the flora of Jordan.[3-4] Most *Salvia* species are inherently linked to local traditional medicine systems in their country of origin.

Salvia species, although bitter in taste, are used traditionally to treat various conditions such as colic, diarrhea, common cold, cough, flu, liver sickness, bacterial infections, febrile attacks, sores in the body, and abdominal trouble and used as a purgative. Also, they are used for alimentary and cosmetic purposes.[5-7] In Jordan, *S. dominica*, which isvery widely distributed, is used like *S. triloba* as an anti-colic and astringent plant remedy in the treatment of common cold, stomach pain and indigestion.[8]

*S. dominica*L. has a strong aromatic smell and reaches up to 80 cm in height. The erect stems are densely haired. The cream-colored corolla has a yellow lower lip. *S. hormium*L. with an erect stem reaches only up to 40 cm. The violet corolla, however, is about twice as long as the calyx. Both species are found mainly in the Mediterranean biogeographical zone of Jordan. *S. hormium* extends into the Irano-Turanian zone.[9]

Volatile oils from the two *Salvia* species, discussed in this paper, growing wild in Jordan, have not been previously studied. Some old surveys from countries of

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the region deal primarily with the isolation and structure determination of terpenoids from *S. hormium*in addition to major volatile compounds from *S. dominica*.[10-15]

Recently, the isolation of sesterterpene lactones from the crude extracts of *S. dominica*for qualitative and quantitative analysis has been reported.[16-17]In these recent studies, no emphasis was given to the volatile oil composition of *S. dominica.* To the best of our knowledge, no previous studies have been carried out on the chemical composition of the volatile oil of *S. hormium.* Hence, the present paper reports the analysis of the essential oils of these two *Salvia* species, *S. dominica*and *S. hormium* from the flora of Jordan, which were hydrodistilled from fresh and dried plantsand then analyzed using gas chromatography/mass spectroscopy (GC/MS).

EXPERIMENTAL

Plant Material

In two successive years (April 2009-April 2010),aerial parts of *S. dominica*(24 LABI-FMJ) and *S. hormium* (28 LABI-FMJ)were collected during the flowering period from the Jordan Valley. The collected plant material was taxonomically identified by Professor Dawoud Al-Eisawi (Department of Biological Science, University of Jordan). Voucher specimens were deposited in the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan.

Reference Substances

α- and β-pinenes, p-cymene, 1,8-cineol, limonene, linalool, borneol (Fluka, Buchs, Switzerland) eugenol, sabinene hydrate (Sigma-Aldrich, Buchs, Switzerland) were used as reference substances in GC/MS analysis.

Volatile Oil Preparation from Fresh Plants

From each collected batch of plants, 250 gm of fresh *S. dominica* and 150 gm of fresh *S. hormium* were coarsely powdered and hydrodistilled with 3L and 2L water, respectively, for 3 hours using a Clevenger- type apparatus. Distillation was carried out twice for each plant and the oils obtained for each were pooled, dried over anhydrous sodium sulphate (Na_2SO_4) and stored at 4ºC in amber glass vials before analysis.[18]

Volatile Oil Preparation for Dried Plants

Flowering aerial parts of each plant were air dried at room temperature in the shade for one week until a constant weight and subsequently assayed for essential oil composition. Samples of 130gm of dry *S. dominica* -equivalent to 250gm fresh plant- and 90gm of dry *S. hormium* -equivalent to 150gm fresh plant- were coarsely powdered and hydrodistilled separately using a Clevenger apparatus for 3 h. The extraction was repeated twice for dry plants and the oils obtained for each were pooled separately, dried over anhydrous Na_2SO_4 and stored at 4º C in amber glass vials until analysis.[18]

Gas Chromatography-Mass Spectroscopy Analysis (GC/MS)

About 1 µL aliquot of each oil sample, appropriately diluted in n-hexane, was subjected to GC-MS analysis. GC-MS analysis was performed using a Varian Chrompack CP-3800 GC/MS/MS-200 (Saturn, Netherlands) equipped with a split-less injector and DB-5 (5% diphenyl, 95% dimethyl polysiloxane) GC column (30m x 0.25mm ID, 0.25 µm film thickness). The column temperature was kept at 100˚C for 3 min and programmed to 250˚C at a rate of 10˚C/min and kept constant at 250˚C for 1 min. Flow rate of helium as a carrier gas was 1 mL min^{-1}. A hydrocarbon mixture of *n*-alkanes (C_8-C_{20}) was analyzed separately by GC/MS under the same chromatographic conditions using the same DB-5 column.

Qualitative and Quantitative Analysis

The volatile compounds were identified using built in libraries (Nist Co and Wiley Co, USA) and by comparing their calculated retention indices with literature values measured with columns with identical polarity or with authentic samples (α- and ß-pinenes, eugenol, limonene, linalool and sabinene hydrate).[19]A relative area percentage (as average percent contents) was obtained from GC-FID analyses assuming a unity response of all components.

RESULTS AND DISCUSSION

Hydrodistillation of the fresh and dried aerial parts of *S. dominica* yielded 1.9% and 1.3% volatile oil, respectively while the yield obtained from the fresh and dried aerial parts of *S. hormium* was 1.2% and 0.8%, respectively. The chemical constituents of these volatile oils, the percentage of each constituent and their retention indices (RI), together with the reported RI for each detected and identified constituent, are summarized in Table 1.[19]The RI of the sample components were calculated on the basis of homologous *n*-alkane hydrocarbons (C8-C20) analyzed under the same conditions.

The GC/MS analysis of the volatile oils from fresh and dry *S. dominica*indicated that the monoterpenes (87.8% and 72.4%, respectively) were the main class of the volatile oil. The oxygenated monoterpenes, mainly alcohols, were dominant. Linalool was the main alcoholic monoterpenoid in oils of fresh and dry plants (31.4% and 18.3%, respectively) followed by alpha terpineol (25.4% and 15%, respectively). In the oil from fresh plants, these two compounds comprised more than 50% of the volatile fraction. The strong aromatic smell of the fresh plants was an indication ofthe presence of a high percentage of linalool in this species. In the volatile oil obtained from dried *S. dominica*, in addition to linalool and alpha terpineol, dihydrocarveol and thymol were detected in concentrations of 10.7% and 7.5%, respectively. Thymol was absent in the fresh oil. Sesquiterpenes were the minor class of the volatile oil in *S. dominica*. Germacrene D (4.3%) was the main sesquiterpenoid in the distilled oil from fresh plants.

On the other hand**,** in the volatile oils obtained from fresh and dry *S. hormium*, the monoterpene and sesquiterpene fractions were present in quite similar amounts. The GC/MS analysis of the oil of fresh *S. hormium* resulted in the identification of twenty eight monoterpenoid and thirteen sesquiterpenoid compounds representing 34.1% and 32.2% of the total oil, respectively. Again, the oxygenated compounds were the dominating constituents among the mono- and sesquiterpenes. The former class was represented by the identification of 4-terpineol (5.1%), alpha terpineol (3.5%), gamma terpineol (2%), trans-carveol (2%) and cis-carvol (2%) as main constituents. As primary oxygenated sesquiterpenoids, tau-cadinol (5.8%), alpha-cadinol (5.4%) and spathulenol (5.1%) were detected. Nevertheless, the major compounds of this oil were alpha-cadinene (7.8%), a hydrocarbon sesquiterpene, followed by a phenylpropanoid compound eugenol (7.3%). The oil from the dried *S. hormium* contained considerably more mono- and sesquiterpenoid hydrocarbons than the oil obtained from fresh *S. hormium* (Table 1). Again, alpha-cadinene (11.4%) was found to be the major constituent of this oil while eugenol was detected only in a concentration of 1.1%. Beta-pinene (9.7%), trans-isolimonene (6%) and alpha-phellandrene (2.9%) were identified as the main monoterpenoid hydrocarbons, while 4-terpineol (3.6%) and thymol (2.7%) were detected as the main oxygenated monoterpenes. Additionally, major oxygenated sesquiterpenes were identified, namely,eudesmol-7-epi-alpha (6.5%), gamma-eudesmol (2.2%) and caryophyllene-14-hydroxy-9-epi-trans (2.2%).

The volatile oil composition of the popular and economically important *Salvia* species, such as *S. fruticosa*L. and *S. officinalis*L, has been largely investigated. For other species, however, as in the case of *S. dominica*and *S. hormium,*the available information is negligible.[20-23]This, in turn, renders the comparability between existing data and new studies not feasible.

Linalyl acetate and neryl acetate were identified previously as major components in *S. dominica*volatile oil.[10-12]In the current study, we were unable to identify even traces of linalyl acetate in the essential oil of the wild*S. dominica* in Jordan. Neryl acetate was detected in very low concentrations, namely 1.9% and 0.7%, in the oils of fresh and dry plants, respectively. Although linalool was previously identified in *S. dominica*species, no such report could be found in the literature on *S. dominica* native to Jordan.[10, 15] Nevertheless, variability in the yield and content of essential oils in the family Lamiaceae has been the topic of research in many publications. These variations have been attributed to genotype variety, environmental factors, stage of plant development, age of the plant, season of collection, method and conditions of drying, the part of the plant tissue analyzed and method of analysis.[20,23-26] To enrich the existing pool of data, further studies are planned to investigate volatile oil composition of both *Salvia* species collected from diverse geographic sites in Jordan and at different growth stages (pre-flowering, flowering and post-flowering).

Table 1:The components (in %) of the hydrodistilled volatile oils of fresh and dry*S .dominica* L. and *S. hormium*L. grown wild in Jordan

RI reported	RI experimental	Compound	*S.dominica* fresh (%)	*S.dominica* dry (%)	*S.hormium* fresh (%)	*S.hormium* dry (%)
900	900	n-nonane	-	-	-	2.1
930	925	α- thujene	0.2	-	0.4	-
939	947	α- pinene	-	-	2.4	-
954	954	Camphene	-	-	1.0	-
967	963	n-heptanol	-	-	15.4	-
979	970	Octen-3-ol	-	-	3.2	2.0
975	976	Sabinene	0.9	1.0	-	1.2
979	979	β- pinene	0.3	-	-	9.7
991	983	3-octanol	-	-	2.5	1.2
991	985	2,3-dehydro,1,8-cineol	0.4	-	-	-
985	987	Isolimonene trans	-	-	1.1	6.0
1003	1002	α- phellandrene	-	-	1.8	2.9
1005	1005	Hexenyl acetate	-	-	-	0.9
1017	1015	α- terpinene	2.5	-	0.9	0.7
1026	1020	*o*-cymene	0.4	2.0	0.8	2.0
1029	1023	Limonene	-	-	0.4	0.8
1031	1028	1,8-cineol	2.5	0.8	1.1	1.5
1031	1032	Δ- 3-carene	1.7	-	-	-
1037	1034	β- ocimene cis	1.0	-	-	-
1042	1042	Benzene acetaldehyde	0.4	2.3	3.0	1.9
1060	1057	γ- terpinene	0.2	0.6	0.2	-
1070	1071	Sabinene hydrate <cis>	-	-	0.8	0.6
1073	1067	Linalool oxide <trans>	1.3	0.9	-	-
1089	1081	Terpinolene	1.5	0.7	-	-
1091	1091	Linalool <dehydro>	31.4	18.3	-	-
1114	1111	Thujone, trans	-	-	-	0.5
1121	1121	Sabina ketone <dehydro>	0.4	-	-	-
1122	1124	Menth-2-en-1-ol, <cis-para>	-	-	0.7	0.6
1139	1140	Pinene hydrate <cis>	-	-	-	0.5
1145	1144	Menth-3-en-8-ol, <para>	-	-	0.8	-

RI reported	RI experimental	Compound	*S.dominica* fresh (%)	*S.dominica* dry (%)	*S.hormium* fresh (%)	*S.hormium* dry (%)
1160	1160	ß-terpineol, <cis-dehydro>	-	-	0.5	-
1166	1165	δ - terpineol	-	-	1.0	-
1169	1175	Borneol	0.3	-	1.1	0.7
1177	1179	4-terpineol	0.8	1.0	5.1	3.6
1187	1190	Neoisomenthol	-	-	-	1.5
1189	1194	α- terpineol	25.4	15.0	3.5	0.5
1199	1204	γ- terpineol	-	-	2.0	-
1204	1207	*p*-cymen-9-ol	-	-	0.6	-
1207	1208	Piperitol <trans>	-	-	0.7	-
1217	1218	Carveol <trans>	1.5	1.3	2.0	0.5
1229	1229	Dihydrocarveol, <neo-iso>	1.6	10.7	-	-
1229	1233	Carveol <cis>	1.6	5.7	1.6	0.7
1253	1248	Geraniol	3.1	3.8	-	-
1263	1260	Carvone oxide <cis>	-	-	0.7	-
1276	1265	Carvone oxide <trans>	-	-	0.5	-
1289	1281	Bornyl acetate	1.3	-	0.6	0.5
1290	1290	Thymol	-	7.5	-	2.7
1291	1291	p-cymene-7-ol	-	-	0.8	-
1295	1295	p-menth-1-en,9-ol	-	-	1.3	-
1338	1330	Δ- elemene	0.3	-	-	-
1349	1342	α- terpinyl acetate	4.7	2.5	-	-
1359	1353	Eugenol	1.2	-	7.3	1.1
1359	1359	Dihydrocarveol acetate	1.0	-	-	-
1362	1365	Neryl acetate	1.9	0.6	-	-
1391	1387	β- elemene	0.5	-	-	-
1419	1419	Caryophyllene <trans>	-	-	0.3	-
1434	1425	ß- gurjunene	0.7	0.7	-	-
1441	1441	Aromadendrene	-	-	0.3	-
1485	1485	Germacrene D	4.3	0.8	-	-
1500	1500	Bicyclogermacrene	2.9	0.6	-	-
1514	1516	γ- cadinene	0.3	-	-	0.7
1523	1521	Δ- cadinene	-	-	0.7	1.3
1522	1524	Selinen-<7-epi-alpha>	-	-	0.8	3.8
1523	1529	Sesquiphellandrene <beta>	-	-	-	2.9
1535	1535	Cadina-1(2),4-diene (trans)	-	-	-	0.5
1539	1540	α- Cadinene	-	-	7.8	11.4

RI reported	RI experimental	Compound	*S.dominica* fresh (%)	*S.dominica* dry (%)	*S.hormium* fresh (%)	*S.hormium* dry (%)
1550	1549	Elemol	-	-	-	1.6
1559	1557	Cadinene ether trans	-	-	0.3	-
1578	1584	Spathulenol	0.2	1.9	5.1	1.2
1583	1590	Caryophyllene oxide	-	-	3.5	0.8
1632	1633	γ- Eudesmol	-	0.8	1.1	2.2
1640	1643	*Tau*-cadinol	-	0.9	5.8	1.2
1651	1651	β- eudesmol	0.4	-	-	-
1654	1657	α- cadinol	-	2.0	5.4	1.3
1664	1662	Eudesmol (7-epi-alpha)	-	-	-	6.5
1670	1666	Caryophyllene 14 hydroxy-9-epi-E	-	-	0.5	2.2
1680	1680	Khusinol	-	-	0.2	1.3
		Total identified	**99.1**	**82.4**	**97.6**	**85.8**
		Monoterpenoids	87.8	72.4	34.5	37.7
		Monoterpenoid hydrocarbons	8.8	4.4	9.1	23.4
		Oxygenated monoterpenoids	79.0	68.1	25.4	14.4
		Sesquiterpenoids	9.5	7.8	32.4	38.9
		Sesquiterpene hydrocarbons	8.7	2.2	10.6	20.9
		Oxygenated sesquiterpenes	0.8	5.6	21.8	18.0
		Phenylpropanoids	1.4	-	7.5	1.1
		Miscellaneous	0.4	2.2	23.2	8.1
		Unidentified	0.9	17.6	2.4	14.2

* Retention indices (RI) calculated on (DP-5MS) column,

** Percentage is given as the average of two independent measurements. Compounds are listed based on their elution order on the corresponding column and calculated relative peak area.

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استخلاص الزيوت العطرية من *Salvia dominica* و *Salvia hormium* التي تنمو في الأردن وتحليلها.

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ملخـص

تنتشر *Salvia dominica* في الأردن على نحو واسع بعكس *Salvia hormium* التي يندر توافرها. والهدف من هذا البحث هو استخلاص الزيوت العطرية لنبتتين من البيئة الأردنية، ودراسة الفرق في تركيبهما إذا كانت طازجة أو مجففة، وقد تم دراسة تركيبهما باستخدام الفصل الكروماتوغرافي للغاز- والمطياف الكتلي (GC-MS).

يتكون الزيت الطّيار المستخاص من العينات الجافة والطرية *Salvia dominica* على نحو رئيس من oxygenated monoterpenes (78,9% و 68,1 % على التوالي). المكونات الرئيسة المستخلصة من زيوت العينات الجافة والطرية *Salvia dominica* هي linalool (31,4 % و 18,3 % على التوالي) alpha terpineol (25,4 % و 15% على التوالي).

وجد كذلك -Mono و sesquiterpenoids في الزيوت الطيارة للعينات الجافة والطرية *S. hormium* بكميات متساوية تقريبا. وكان Alpha cadinene (7,8% و 11,4% على التوالي) المكون السائد في الزيوت المستخلصة من النباتات الجافه والطرية. وجد Eugenol فقط في الزيت الطّيار المستخاص من *S. hormium* الطرية وبنسبة 7,3%.

الكلمـات الدالـة: الزيوت العطرية، الكروماتوغرافي، النباتات الجافة، الزيوت الطيارة، المطياف الكتلي.

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