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Essential Oil Compositions of Two Populations of *Salvia samuelssonii* Growing in Different Biogeographical Regions of Jordan*

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**Dedicated to the memory of Professor Mohammed Meelad (Botanist), Umm Al-Qura University*

The composition of the essential oils of flowering aerial parts of *Salvia samuelssonii* Rech. fil. (Lamiaceae Section *Aethiopis*), collected in two different biogeographical regions, has been analyzed. Sample1, collected in a Mediterranean-like region, "As-Subayhi", contains mainly monoterpenes (54.2%), sesquiterpenes (27.6%) and phenylpropanoids (10.5%), while sample 2, collected in the Irano-Turanian region, "Al-Adasiyyah", contains mainly phenylpropanoids (30.6%), monoterpenes (24.9%) and sesquiterpenes (21.2%). In Sample1, the most representative constituents were sabinene (21.5%), *cis*-chrysanthenyl acetate (20.8%), germacrene D (9.3%) and myristicin (5.9%), while in sample 2, myristicin (24.1%), sclareoloxide (6.3%), and germacrene D (5.7%) were main constituents. The rate of oxygenated derivatives in the Irano-Turanian sample was higher than the Mediterranean sample. Myristicin is an unusual constituent of *Salvia* species.

Keywords: *Salvia samuelssonii*, Essential oil, Biodiversity, Chemotypes, GC.

The flora of Jordan is characterized by high biodiversity due to the climatic variation. It comprises four biogeographical regions (Mediterranean, Irano-Turanian, Saharo-Arabian and tropical) [1]. The Mediterranean region in Jordan is represented by mountain ranges and is characterized by fertile soils, the highest amount of rainfall, and the coldest temperatures. The Irano-Turanian region is represented by a narrow strip that surrounds the Mediterranean region, except in the north. It is characterized by medium rainfall and temperature, medium altitude and poor soil [1]. Twenty species of *Salvia* are recorded in the Jordanian flora [2], some of which are used as medicinal plants, edible herbs and as tea-flavoring herbs [3a-e].

S. samuelssonii Rech. fil. (Syn. *S. cognata* Sam.) is a perennial herb with a pleasant strong smell. This species is close to *S. palaestina* Benth., but differs in several morphological features, especially the size of the fruiting calyx, which is 25-30 mm wide in *S. samuelssonii*, while in *S. palaestina* it does not exceed 22 mm [4]. *S. samuelssonii* belongs to the section Aethiopis Benth. The essential oils of some Jordanian *Salvia* species belonging to this section, such as *S. syriaca* L., *S. spinosa* L., *S. palaestina* Benth. and *S. dominica* L., have been phytochemically investigated [3e,5a-c]. This paper reports the composition of the essential oil of two wild populations of *S. samuelssonii* growing in different biogeographical regions: sample 1, collected at As-Subayhi, a Mediterranean-like region, while sample 2 was collected at Al-Adasiyyah, an Irano-Turanean-like region. To the best of our knowledge, this is the first phytochemical study of this plant. Sample 1 contained mainly monoterpenes (54.2%), of which 27.3% were hydrocarbon derivatives and 26.9% oxygenated monoterpenes. Sesquiterpenes represented 24.6% of the oil of which the hydrocarbons formed 19.6% and the oxygenated sesquiterpenes 8.0%. The percentage of phenylpropanoids was 10.5%. Other non-terpenoid compounds, such as alcohols, aldehydes, ketones and esters were also present (Table 1).

The main component was the monoterpene hydrocarbon, sabinene (21.5%), followed by *cis*-chrysanthenyl acetate (20.8%), an oxygenated monoterpene. The non-oxygenated sesquiterpenes were represented by germacrene D (9.3%) and β-caryophyllene (4.9%), while the oxygenated ones are present in lower percentages, such as germacrene D-4-ol (3.3%). Among the phenylpropanoids, myristicin and anethole formed 5.9% and 3.2%, respectively.

The essential oil of sample 2 contained a lower percentage of monoterpenes in comparison with sample 1, representing 24.9% of the oil, of which 15.7 were hydrocarbons (γ-terpinene 3.4% and α-phellandrene 2.9%), and 9.2% oxygenated compounds (4-terpineol 2.0%). Phenylpropanoids represented 30.6% of the oil. The major hydrocarbon sesquiterpenes were germacrene D (5.7%) and α-copaene (3.6%) (Table 1). Some variation was observed in the percentage of common compounds of the essential oils of the two populations. This variation could be considered as a result of biotic and abiotic factors, among them water stress. In our study the drastic variation in the composition, especially the presence of sabinene and *cis*-chrysanthenyl acetate in high percentage in sample 1 and their near absence in sample 2, could be attributed to genetic characteristics acquired over a long time as an adaptation process [6a,b].

A review of the composition of the essential oils of some other species of *Salvia* belonging to section Aethiopis [4,7a,b], such as *S. aethiopis* [8a-n], *S. vermifolia*, *S. atropatana, S. ceratophylla*, *S. chrysophylla*, *S. cyanescens*, *S. indica*, *S. limbata*, *S. spinosa*, and *S. syriaca* and *S. sclarea* [8a-n], showed that β-caryophyllene is the main sesquiterpene constituent of *S. aethiopis*, *S. atropatana*, *S. chrysophylla*, *S. limbata* and *S. spinosa*; germacrene D of *S. aethiopis*, *S. sclarea* and *S. syriaca*; and spathulenol of *S. cyanescens*, *S. syriaca* and *S. vermifolia*. The most frequent monoterpenes with the highest percentages were α-pinene, β-pinene and linalool.

Table 1: Essential oil composition of *S. samuelssonii* samples.

l.r.i [a]	l.r.i [b]	Constituents	As-Subayhi (Sample1)	Al-Adasiyyah (Sample 2)
854	1222	(*E*)-2-Hexenal	0.5	1.1
866	1352	1-Hexanol	tr	0.8
900		(*Z*)-4-Heptenal	tr	tr
910	1392	(*E,E*)-2,4-Hexadienal	tr	tr
928	1018	α-Thujene	0.4	0.4
931	1028	α-Pinene	0.3	1.9
953	1075	Camphene	tr	tr
957		Thuja-2,4(10)-diene	tr	-
961	1493	Benzaldehyde	tr	0.1
969	1455	*n*-Heptanol	tr	0.2
976	1116	**Sabinene**	**21.5**	1.3
980	1108	β-Pinene	0.4	1.1
985	1340	6-Methyl-5-hepten-2-one	tr	tr
988		3-Octanone	-	tr
991	1166	Myrcene	2.3	0.7
1005	1168	α-Phyllandrene	0.4	2.9
1015	1351	(*E,E*)-2,4-Heptadienal	-	2.9
1018	1180	α-Terpinene	0.3	0.7
1027	1243	*p*-Cymene	0.2	1.0
1031	1198	Limonene	0.6	1.2
1041	1209	1,8-Cineole	0.6	1.0
1044	1618	Phenyl acetaldehyde	tr	0.8
1051	1255	(*E*)-β-Ocimene	tr	0.1
1062	1246	γ-Terpinene	0.7	3.4
1070	1463	*cis*-Sabinene hydrate	0.4	0.2
1072	1563	*n*-Octanol	-	0.6
1089	1288	Terpinolene	0.2	0.3
1097	1468	*trans*-Sabinene hydrate	0.4	0.2
1099	1560	Linalool	0.3	0.7
1103	1382	Nonanal	0.6	1.0
1113		1,3,8-*p*-Mentha triene	-	tr
1123	1585	*cis*-*p*-menth-2-en-1-ol	0.6	0.2
1127	1507	α-Campholenal	tr	-
1140	1635	*trans*-Pinocarveol	0.2	-
1142	1633	*trans*-*p*-Menth-2-en-1-ol	0.1	-
1142	1654	*cis*-Verbenol	0.4	tr
1156	1597	(*E,E*)-2,4-Nonadienal	-	-
1158		β-Pinene oxide	tr	-
1160	1445	(*E*)-2-Nonenal	-	tr
1162	1450	*cis*-Chrysanthenol	0.6	-
1166	1665	δ-Terpineol	-	0.4
1173	1666	*n*-Nonanol	-	0.3
1179	1607	4-Terpineol	1.2	2.0
1185	1833	*p*-Cymen-8-ol	tr	0.2
1191	1684	α-Terpineol	1.9	2.9
1197	1669	Methyl chavicol	1.0	0.3
1205	1481	Decanal	0.3	0.3
1207	1716	Verbenone	tr	-
1214	1636	β-Cyclocitral	-	0.1
1235	1593	Methyl thymol	tr	-
1243	1448	Hexyl 3-methyl butanoate	0.4	0.4
1263	1532	***cis*-Chrysanthenyl acetate**	**20.8**	0.2
1270	1772	Decanol	-	tr
1283	1816	(*E*)-Anethole	3.2	2.0
1292	2187	Thymol	tr	tr
1299	1673	*trans*-Pinocarvyl acetate	tr	-
1300	2219	Carvacrol	tr	0.2
1306	1650	Undecanal	-	tr
1314	1703	Myrtenyl acetate	0.2	-
1316	1706	(*E,E*)-2,4-Decadienal	-	tr
1340	1690	Heptyl 2-methylbutyrate	0.2	-
1351	1461	α-Cubebene	0.2	0.7
1358	2187	Eugenol	tr	tr
1376	1477	α-Copaene	0.7	3.6
1384	1518	β-Bourbonene	0.3	0.6
1390	1545	β-Cubebene	0.2	0.7
1392	1593	β-Elemene	tr	-
1403	2020	Methyl eugenol	tr	0.2
1408	1724	Dodecanal	tr	0.3
1418	1598	β-Caryophyllene	4.9	1.6
1432	1590	β-Gurjunene	0.2	0.1
1441		2-Methyl butyl benzoate	-	0.4
1443	1636	Aromadendrene	-	tr
1454	1665	α-Humulene	2.0	0.2
1455	1842	(*E*)-Geranyl acetone	tr	0.1
1459	1661	(*E*)-β-Farnesene	tr	tr
1462		*cis*-Muurola-4-(14),5-diene	-	tr
1465		*trans*-Cadina-1(6),4-diene	-	tr
1477	1681	γ-Muurolene	0.2	0.2
1480	1695	**Germacrene D**	**9.3**	**5.7**
1485		(*E*)-β-Ionene	0.2	0.1
1490	1715	β-Selinene	-	tr
1494	1884	*epi*-Cubebol	-	0.4
1495	1737	Bicyclogermacrene	1.1	0.2
1500	1711	α-Muurolene	0.5	0.2
1505		α-Bulnesene	0.2	Tr
1509	1708	β-Bisabolene	-	tr
1513	1750	*trans*- γ-Cadinene	tr	tr
1520	2255	**Myristicin**	**5.9**	**24.1**
1532		*trans*-Cadina-4α-ol	-	0.2
1538	1680	α-Cadinene	tr	tr
1564	1952	β-Calacorene	-	tr
1570	1948	(*Z*)-Isoelemecin	-	0.2
1575	2062	Germacrene D-4α-ol	3.3	-
1581	1966	Caryophyllene oxide	1.2	2.8
1591		β-Copaen-4α-ol	-	0.2
1606	2101	β-Oplopenone	0.4	-
1621	2348	Dill apiole	0.4	3.8
1680		Khusinol	-	0.8
1736	2509	(*Z*)-Ligustilide	-	0.3
1758		Ambroxide	-	tr
1764	2655	Benzyl benzoate	-	tr
1876	2155	**Sclareoloxide**	1.5	**6.3**
1989	2444	Manoyl oxide	-	0.1
%			96.0%	90.6%

[a]l.r.i: linear retention index (apolar column); [b]l.r.i linear retention index (polar column), tr: trace quantity less than 0.05%

Table 2: Plants found growing close to *Salvia samuelssonii*.

Mediterranean region	Irano-Turanean region
Sarcopoterium spinosum (L.) Spach.	*Salsola jordanicola Eig.*
Silybum marianum (L.) Gaertn	*Verbascum jordanicum* Murb.
Sideritis pullulans Vent.	*Acacia albida Delile*
Quercus ithaburensis Decne.	*Blepharis ciliaris* (L.) L.B. Burtt
Quercus coccifera L.	*Retama raetam* (Forssk.) Webb.
Capparis spinosa L.	*Anchusa strigosa* Banks. & Sol.
Teucrium polium L.	*Gundelia tournefortii L.*
Ballota undulata (Fresen.) Bentham	*Teucrium polium* L.
Salvia dominica L.	*Ballota undulata* (Fresen.) Bentham
Urginea maritima (L.) Baker	*Salvia dominica* L.
Varthemia iphionoides Boiss. & Blanche	*Urginea maritima* (L.) Baker
	Varthemia iphionoides Boiss. & Blanche

Table 3: Overlapping of Mediterranean and Tropical flora.

Mediterranean flora	Irano-Turanian flora	Tropical region flora
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These percentages in *S. samuelssonii* are in agreement for β-caryophyllene and germacrene D, while spathulenol is absent. Furthermore α-pinene, β-pinene and linalool are present in negligible quantities. Sabinene, which was abundant in the analyzed oils, was detected as a main constituent only in *S. limbata*. None of the above mentioned species contained either *cis*-chrysanthenyl acetate or myristicin as main constituents. Indeed myristicin has been found only in one *Salvia* species, *Salvia anatolica* [9], which does not belong to the section Aethiopis.

These results suggest the presence of two distinct chemotypes: a sabinene-chrysanthenyl acetate chemotype and a myristicin-sclareoloxide chemotype. The *in situ* flora growing around the collected samples, summarized in Table 2, show some species growing in both the Mediterranean and Irano-Turanian regions. In addition, some species of the tropical region flora were also found in the Irano-Turanian region. This means that the Irano-Turanian region constitutes an overlapping region of the Mediterranean flora on one side and the tropical flora on the other (Table 3). This gradient of climatic variation could cause a gradient of chemical variation of chemical components of essential oils and other secondary metabolites. From a phytochemical point of view this variation of secondary metabolites could be useful to detect and isolate higher numbers of bioactive compounds from the same species growing in different environmental conditions.

Experimental

Plant material: Flowering aerial parts of *S. samuelssonii* (sample 1) were collected in As-Subayhi (Al Balqa Province) while sample 2 was collected in Al-Adasiyyah 20 Km NE of the Dead Sea, in April

2009, the plant was identified by Ammar Bader. A voucher number Jo-It 2009/3EO specimen was deposited in the Herbarium of the Laboratory of Pharmacognosy at Umm Al-Qura University, Saudi Arabia. After air drying at room temperature, the plant material was ground, and 50 g of each sample was submitted to hydrodistillation in a Clevenger-like apparatus for 2 h. The essential oils were stored in sealed vials under refrigeration prior to analysis.

GC and GC-MS analyses: The GC analyses were performed with a HP-5890 Series II instrument equipped with DB-WAX and DB-5 capillary columns (30 m x 0.25 mm, 0.25 µm film thickness); analytical conditions: temperature program of 60ºC for 10 min, followed by an increase of 3ºC/min to 220ºC; injector and detector temperatures 250ºC; carrier gas helium (2 mL/min); detector dual FID; splitless injection. For both the columns, identification of the chemicals was performed by means of their Linear Retention Indices (LRI) relative to the series of *n*-hydrocarbons, and by computer matching against commercial and homemade MS library built up from pure substances (Sigma-Aldrich, Extrasynthese, Fluka and Supelco) and components of known essential oils and MS literature data [10a-d]. GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures at 220 and 240ºC, respectively; oven temperature was programmed from 60ºC to 240ºC at 3ºC/min; carrier gas helium at 1 mL/min; splitless injection.

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