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Chemical Composition of the Essential Oils of *Salvia polystachia* and *Salvia microphylla* (Lamiaceae) Growing in Costa Rica

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Abstract. *Salvia*, with about 900 to 1000 species, is the largest genus in the Lamiaceae family, distributed both in the Old World and the American continent. This work aimed to study the chemical composition of the essential oils obtained by hydrodistillation from aerial parts of *Salvia polystachia* growing wild and *S. microphylla* cultivated in Costa Rica. The chemical composition of the oils was studied by capillary gas chromatography with a flame detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) using the retention indices on a 5 % diphenyl-95 % dimethylpolysiloxane capillary column in addition to mass spectral fragmentation patterns. One hundred twenty-three compounds were identified in the essential oil of *S. polystachia*, constituting about 91.9 % of the total oil. The major constituents were germacrene-D (25.8 %), (*E*)-caryophyllene (15.9 %), bicyclogermacrene (7.2 %), caryophyllene oxide (5.7 %), palustrol (3.2 %) and β-elemene (2.8 %). This is the first report on the chemical composition of the essential oil obtained from *S. polystachia*. Seventy-three compounds were identified in the essential oil of *S. microphylla*, constituting about 91.6 % of the total oil. The major constituents were (*E*)-caryophyllene (34.5 %), germacrene-D (8.4 %), *trans*-muurola-4(14),5-diene (6.5 %), caryophyllene oxide (4.7 %), germacrene B (4.2 %), β-pinene (3.2 %), γ-terpinene (3.2 %), β-gurjunene (3.2 %) and borneol (2.2 %). The presence in large amounts of the biologically active (*E*)-caryophyllene and its oxide, and other sesquiterpenes (e.g., germacrene D, *trans*-muurola-4(14),5-diene, bicyclogermacrene, and germacrene B) in the essential oils were characteristic. Both species can be classified within the sages whose essential oils have sesquiterpenoids as major constituents (sesquiterpene chemotype).
Keywords: *Salvia* spp.; essential oils; sesquiterpenoids; diterpenoids; (*E*)-caryophyllene, germacrene-D; GC-FID, GC-MS.

Resumen. El género *Salvia*, contiene cerca de 900 a 1000 especies, es el mayor de la familia Lamiaceae, distribuido tanto en el Viejo Mundo como en América. El objetivo de este trabajo fue estudiar la composición química de los aceites esenciales obtenidos mediante hidrodestilación de las partes aéreas de *Salvia polystachia* silvestre y de *S. microphylla* cultivada en Costa Rica. El aceite esencial se obtuvo mediante hidrodestilación con un equipo Clevenger modificado. La composición química de los aceites se analizó mediante las técnicas de cromatografía gaseoso-líquida con detector de ionización de llama (GC-FID) y de cromatografía gaseoso-líquida acoplada a un detector selectivo de masas (GC-MS). Para la identificación de los constituyentes se utilizaron índices de retención obtenidos en una columna capilar tipo DB-5 y se usaron los patrones de fragmentación de masas. En el aceite esencial de *S. polystachia* se identificaron en total 123 compuestos, correspondientes a 91.9 % de los constituyentes totales. Los compuestos mayoritarios fueron

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germacreno-D (25.8 %), (*E*)-cariofileno (15.9 %), biciclogermacreno (7.2 %), óxido de cariofileno (5.7 %), palustrol (3.2 %) y β-elemeno (2.8 %). Este es el primer informe acerca de la composición química de aceites esenciales de *S. polystachia*. En el aceite esencial de *S. microphylla*, se identificaron un total de 73 compuestos constituyendo el 91.6 % del aceite total. Los constituyentes mayoritarios fueron (*E*)-cariofileno (34.5 %), germacreno-D (8.4 %), *trans*-murola-4(14),5-dieno (6.5 %), óxido de cariofileno (4.7 %), germacreno B (4.2 %), β-pineno (3.2 %), γ-terpineno (3.2 %), β-gurjuneno (3.2 %) y borneol (2.2 %). La presencia en cantidad importante de (*E*)-cariofileno, biológicamente activo y su óxido, así como de otros sesquiterpenos (p. ej., germacreno D, *trans*-muurola-4(14),5-dieno, biciclogermacreno y germacreno B) en los aceites esenciales fue característica y, ambas especies, pueden clasificarse dentro del grupo de salvias cuyos aceites esenciales presentan sesquiterpenoides como constituyentes principales (quimiotipo sesquiterpénico).
Palabras clave: *Salvia* spp.; aceites esenciales; sesquiterpenoides; diterpenoides; (*E*)-cariofileno, germacreno-D; GC-FID; GC-MS.

Introduction

Lamiaceae is a family of flowering plants with about 236 genera and over 7173 species [1]. Plants of this family are mostly aromatic herbs or small shrubs with young stems, often four-angled. Many lamiaceous herbs are used as spices and for medicinal purposes, such as balm (*Melissa officinalis* L.), basil (*Ocimum basilicum* L.), lavender (*Lavandula angustifolia* Mill.), marjoram (*Origanum majorana* L.), mint (*Mentha* spp.), oregano (*Origanum vulgare* L.), perilla (*Perilla frutescens* (L.) Britton), rosemary (*Rosmarinus* officinalis L.), sage (*Salvia officinalis* L.), savory (*Satureja* spp.), and thyme (*Thymus vulgaris* L.). Some dried herbs or spices in Europe and Costa Rica are used in commercial blends named *Herbes de Provence* and *Italian Seasoning* [2].

Salvia (tribe Mentheae) is a cosmopolitan genus of about 900 to 1000 species distributed worldwide and predominantly occurring in the American continent (Central and South America, ca. 500 species), the Mediterranean basin, and Central Asia (ca. 250 species) and Eastern Asia (ca. 90 species) [3]. The characteristics of this genus are the flowers with only two stamens and the secreting glandular trichomes that accumulate complex essential oils composed mainly of terpenoids, which are stored in the subcuticular space within these anatomical structures [4-6]. The genus *Salvia* has been the subject of numerous chemical studies. It is a rich source of terpenoids, steroids, and polyphenolics [7-9].

Salvia is the only genus of Lamiaceae in Costa Rica (apart from the cultivated *Rosmarinus officinalis*, native to the Mediterranean region) whose flowers have a bilabial calyx and only two stamens. In Costa Rica, the genus *Prunella* is the only one that shares these morphological traits. *Salvia polystachia* Cav. is a perennial herb or subshrub, up to 2 m high, with a distributional range from Mexico to Honduras, Costa Rica, and Panama [10]. In Costa Rica, it is distributed in several habitats from 850 to ca. 2500 m of altitude, in tropical rainforests, tropical montane forests, oak forests, and disturbed areas where the plant is commonly known as "chan," "chirrite" and "jalacate". This species is recognized by its petiolate and ovate-lanceolate leaves up to 13 cm long and 7 cm wide with rounded or slightly cordate base, acuminate apex, and characteristic densely spiciform inflorescences with blue flowers [11]. *Salvia polystachia* belongs to subgenus *Calosphace,* containing ca. 500 species endemic to North, Central, and South America [12] and represents a well-defined natural group, strongly supported as monophyletic [3].

In Mexico, *S. polystachia* is popularly known as "chía" with *S. hispanica* L., *S. columbria* Benth., and another *Salvia* spp. Since pre-Hispanic times, the rural population has used the "chía" nutlets as food [13,14]. *Salvia polystachia* is used in traditional medicine as a purgative drug to treat dysentery and to stop diarrhea [11].

Phytochemical studies of *S. polystachia* from Mexico have been directed primarily to the extraction, isolation, and structural elucidation of *neo*-clerodane diterpenes (polystachynes) by spectroscopic methods [15-17]. Calzada and collaborators [18,19] studied the antiamoebic and antigiardial activities of crude acetone extract of *S. polystachia* and of four of its diterpenoid constituents, resulting in linearolactone

(=linearifoline) was the most active clerodane against both *Entamoeba histolytica* and *Giardia lamblia* trophozoites.

Several exotic species of sage are occasionally cultivated as ornamentals in Costa Rica, such as *Salvia leucantha* Cav. ("bandera"), and *S. microphylla* natives of Mexico. *Salvia microphylla* Kunth is an introduced subshrubby species with bright red flowers grown in the gardens of the University of Costa Rica campus. This species is native to Mexico, where it is commonly known as "mirto or toronjil" and it is also part of the subgenus *Calosphace*, used traditionally to relieve gastrointestinal troubles (stomachache, diarrhea, dysentery, cramps, and colic) and insomnia; and is used externally to treat rheumatism and skin problems [12]. Phytochemical studies of *S. microphylla* revealed the presence of sesquiterpenoids, diterpenoids, triterpenoids, and phenolic compounds [20-23].

In the literature, there are many studies on the chemical composition of essential oils of diverse species of *Salvia* growing in different countries [24-34]. The composition of the essential oils of *S. microphylla* cultivated in Italy (Giardini Botanici Hanbury, Capo Mortola, Ventimiglia) [35], Brazil (the Garden of Medicinal Plants of the Federal University, Lavras, Minas Gerais) [36], and Algeria (Blida, southwest of Algiers) [37] have been reported. Recently, two reports on the volatile chemical composition of *S. microphylla* cv. Hot Lips (with striking bicolor flowers with half-white and half-red petals that resemble crimson-red lips) cultivated in Italy (Sanremo) [38] and in the USA (Grover Beach, California) [39] were published.

To the best of our knowledge, there are no reports in the literature about the chemical composition of the essential oils from *S. polystachia*. The present paper is intended to contribute to the chemical knowledge of the essential oil from aerial parts of this species growing wild in Costa Rica. In this study, we also determine the chemical composition of the essential oils of *S. microphylla*, an introduced species cultivated in a garden in Costa Rica.

Experimental

Plant material

Aerial parts of a representative sample of a population of *Salvia polystachia* were collected in May 2015 in El Tirol, Province of Heredia (10°04'11''N, 84°05'07" W), at an elevation of 1850 m. A voucher specimen was deposited in the Herbarium of the University of Costa Rica (USJ 105600) (See **(A)**, Fig. 1).

Aerial parts of *Salvia microphylla* were collected in August 2016 in the University of Costa Rica Campus, locality of Mercedes de Montes de Oca, Province of San José (9°56'16''N, 84°02'57" W) at an elevation of 1205 m. A voucher specimen was deposited in the Herbarium of the University of Costa Rica (USJ 111225). (See **(B)**, Fig. 1).

Fig. 1. (A) *Salvia polystachia* and, **(B)** *S. microphylla* blooming in Costa Rica. (Photografies by J. J. Araya and C. Chaverri).

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Essential oil extraction

Fresh aerial parts of *Salvia polystachia* (520 g) and *S. microphylla* (240 g) were submitted to hydrodistillation at atmospheric pressure using an all-glass Clevenger-type apparatus for 3 h. The distilled essential oils were collected and dried over sodium sulfate (Na_2SO_4, Merck KGaA, anhydrous GR for analysis), filtered, and stored at 0–10 °C in the dark for further analyses.

Gas chromatographic analysis (GC-FID)

The essential oils of *S. polystachia* and *S. microphylla* were analyzed by capillary gas chromatography with a flame ionization detector (GC-FID) using a Shimadzu GC-2014 gas chromatograph. The data were obtained on a 5 % diphenyl-95 %-dimethylpolysiloxane fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm; MDN-5S, Supelco). The GC integrations were performed with a LabSolutions, Shimadzu GCsolution™ Chromatography Data System software, version 2.3. Operating conditions used were carrier gas N_2, flow 1.0 mL/min; oven temperature program: 60 to 280 °C at 3 °C/min, 280 °C (2 min); sample injection port temperature 250 °C; detector temperature 280 °C; the split ratio was adjusted to 1:60.

Gas chromatography-Quadrupole mass spectrometry analysis (GC-MS)

GC-MS analyses were conducted with a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 apparatus and GCMSsolution™ software (version 1.21), with Wiley 139 and NIST computerized databases. The data were obtained with the same column described above. Operating conditions were carrier gas He, flow 1.0 mL/min; oven temperature program: 60–280 °C at 3 °C/min; sample injection port temperature 250 °C; transfer line temperature 260 °C; ionization voltage: 70 eV; ionization current 60 µA; scanning speed 0.5 s over m/z 38 to 400 Da range; the split ratio was adjusted to 1:70.

Compound identification

Identification of the constituents of the oils was performed using the retention indices, which were calculated employing a homologous series of *n*-alkanes on a 5 % diphenyl-95 % dimethylpolysiloxane type column [40], and by comparison of their mass spectra with those published in the literature [41] or those of our own homemade MS spectra library or comparing their mass spectra with those available in the NIST 107 and Wiley 139 computerized databases or in a web source [42]. To obtain the retention indices for each peak, 0.1 µL of the *n*-alkane mixture (Sigma, C_8–C_{32} standard mixture) was co-injected under the same experimental conditions reported above. Integration of the total chromatogram (GC-FID), expressed as area percent, without correction factors, has been used to obtain quantitative compositional data.

Results and discussion

The hydrodistillation of fresh aerial parts of *S. polystachia* from Costa Rica produced a colorless oil yielding 0.02 % (v/w). The essential oil contained 123 identified compounds, accounting for 91.9 % of the total composition (see the ***Sp*** column, Table 1). The essential oil was found to be a complex mixture consisting largely of sesquiterpene hydrocarbons (64.0 %) and oxygenated sesquiterpenes (20.3 %) with minor quantities of monoterpenoids, aliphatic compounds, and diterpenoids. The major constituents were germacrene D (25.8 %), (*E*)-caryophyllene (15.9 %), bicyclogermacrene (7.2 %), caryophyllene oxide (5.7 %), palustrol (3.2 %) and β-elemene (2.8 %) (see the Total Ion Chromatogram –TIC– in Fig. 2).

Table 1. Chemical composition of aerial parts essential oil of *Salvia polystachia* (***Sp***) and *S. microphylla* (***Sm***) from Costa Rica.

No.	[a]Compound	RI[b]	$RI_{Lit.}$[c]	Class	*Sp* (%)	*Sm* (%)	IM[d]
1	(*Z*)-Hex-3-en-1-ol	850	850	A		0.3	1,2
2	(*E*)-Hex-2-en-1-ol	854	854	A		tr	1,2

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No.	[a]Compound	RI[b]	$RI_{Lit.}$[c]	Class	*Sp* (%)	*Sm* (%)	IM[d]
3	Hexan-1-ol	863	863	A		tr	1,2
4	α-Thujene	925	924	M	tr	0.1	1,2
5	α-Pinene	932	932	M	0.1	0.5	1,2,3
6	Camphene	946	949	M		0.5	1,2
7	Benzaldehyde	956	952	A	tr		1,2
8	Sabinene	972	969	M	0.7	0.2	1,2
9	β-Pinene	977	974	M	0.2	3.2	1,2,3
10	Oct-1-en-3-ol	978	974	A	tr		1,2
11	Octan-3-one	988	979	A	tr	tr	1,2
12	Myrcene	989	988	M	tr	0.2	1,2
13	Octan-3-ol	996	988	A	tr	tr	1,2
14	Octanal	998	998	A	tr		1,2
15	α-Phellandrene	1001	1002	M	tr		1,2
16	(*Z*)-Hex-3-enyl acetate	1004	1004	A		tr	1,2
17	δ-3-Carene	1009	1008	M	0.1	tr	1,2
18	α-Terpinene	1016	1014	M	tr	0.1	1,2
19	*p*-Cymene	1023	1020	M	tr	0.8	1,2
20	Limonene	1027	1024	M	tr	0.1	1,2,3
21	β-Phellandrene	1030	1025	M	tr	0.1	1,2
22	1,8-Cineole	1032	1026	OM	tr	1.5	1,2,3
23	(*Z*)-β-Ocimene	1033	1032	M		tr	1,2
24	(*E*)-β-Ocimene	1044	1044	M		tr	1,2
25	γ-Terpinene	1055	1054	M	0.1	3.2	1,2
26	*cis*-Sabinene hydrate	1067	1065	OM	0.2	tr	1,2
27	*p*-Mentha-2,4(8)-diene	1081	1085	M	tr		1,2
28	Terpinolene	1082	1086	M	tr	0.1	1,2
29	Linalool	1093	1095	OM	0.3		1,2,3
30	*trans*-Sabinene hydrate	1098	1098	OM	0.2	tr	1,2
31	Nonanal	1100	1100	A	tr	tr	1,2
32	*trans*-Thujone	1111	1112	OM	tr		1,2
33	*trans*-Pinene hydrate	1115	1119	OM		tr	1,2
34	*trans*-Pinocarveol	1140	1135	OM		0.1	1,2
35	*cis*-*p*-Menth-2-en-1-ol	1122	1118	OM	tr		1,2

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No.	[a]Compound	RI[b]	$RI_{Lit.}$[c]	Class	*Sp* (%)	*Sm* (%)	IM[d]
36	Undec-1-yne	1127	1122	A	tr		1,2
37	*cis*-*p*-Mentha-2,8-dien-1-ol	1132	1133	OM	tr		1,2
38	*trans*-*p*-Menth-2-en-1-ol	1144	1136	OM	tr		1,2
39	Camphor	1147	1141	OM	tr	tr	1,2,3
40	(2*E*,6*Z*)-Nona-2,6-dienal	1151	1150	A	tr		
41	Camphene hydrate	1154	1145	OM	tr		1,2
42	(*Z*)-Isocitral	1158	1160	OM	0.1		1,2
43	Pinocarvone	1162	1160	OM	tr		1,2
44	Borneol	1166	1165	OM	tr	2.2	1,2,3
45	Terpinen-4-ol	1175	1174	OM	0.1	0.3	1,2,3
46	Thuj-3-en-10-al	1181	1181	OM	tr		1,2
47	Dill ether	1184	1184	OM	tr		1,2
48	α-Terpineol	1189	1186	OM	0.2	0.1	1,2
49	Myrtenol	1192	1194	OM	tr		1,2
50	Decanal	1201	1201	A	tr		1,2,3
51	*trans*-Piperitol	1208	1207	OM	tr		1,2
52	*trans*-Pulegol	1213	1213	OM	tr		1,2
53	β-Cyclocitral	1218	1217	OM	tr		1,2
54	Cumin aldehyde	1248	1238	OM	tr	tr	1,2
55	Pinocamphone	1247	1246	OM	tr		1,2
56	1-Octen-3-ol butanoate	1278	1280	A	0.1		1,2
57	Isobornyl acetate	1283	1283	OM	tr		1,2
58	Bornyl acetate	1291	1287	OM		tr	1,2
59	α-Terpinen-7-al	1287	1285	OM	tr		1,2
60	Dihydroedulan I	1294	1292	OM		0.2	1,2
61	(2*E*,4*E*)-Deca-2,4-dienal	1318	1315	A	tr		1,2
62	Bicycloelemene	1331	1330[e]	S	0.1		1,2
63	δ-Elemene	1334	1335	S	0.2		1,2
64	α-Cubebene	1345	1345	S		0.1	1,2
65	α-Ylangene	1367	1373	S	0.1	0.1	1,2
66	α-Copaene	1373	1374	S	1.6	1.3	1,2
67	β-Bourbonene	1381	1387	S	1.5	1.2	1,2
68	β-Elemene	1387	1389	S	2.8	0.3	1,2

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No.	[a]Compound	RI[b]	$RI_{Lit.}$[c]	Class	*Sp* (%)	*Sm* (%)	IM[d]
69	Cyperene	1397	1398	S	tr		1,2
70	(*Z*)-Caryophyllene	1400	1408	S		0.1	1,2
71	α-Gurjunene	1405	1409	S	1.6		1,2
72	**(*E*)-Caryophyllene**	1419	1417	S	**15.9**	**34.5**	1,2,3
73	β-Copaene	1428	1430	S	0.4		1,2
74	β-Gurjunene	1429	1431	S		3.2	1,2
75	*trans*-α-Bergamotene	1430	1432	S	tr		1,2
76	α-Guaine	1432	1437	S	0.3		1,2
77	γ-Elemene	1437	1439	S		0.6	1,2
78	Aromadendrene	1436	1439	S	tr	tr	1,2
79	6,9-Guaiadiene	1439	1442	S	tr		1,2
80	*cis*-Muurola-3,5-diene	1442	1448	S	0.1		1,2
81	α-Humulene	1452	1452	S	1.2	1.2	1,2,3
82	Dehydroaromadendrene	1457	1460	S	0.1		1,2
83	*cis*-Cadina-1(6),4-diene	1460	1461	S	tr		1,2
84	*cis*-Muurola-4(14),5-diene	1463	1465	S	tr		1,2
85	*9-epi*-(*E*)-Caryophyllene	1465	1464	S		0.4	1,2
86	4,5-di-*epi*-Aristolochene	1466	1471	S	0.5		1,2
87	γ-Gurjunene	1473	1475	S		tr	1,2
88	β-Chamigrene	1473	1476	S	0.5		1,2
89	**Germacrene D**	1481	1484	S	**25.8**	**8.4**	1,2
90	β-Selinene	1489	1489	S	tr		1,2
91	***trans*-Muurola-4(14),5-diene**	1491	1493	S		**6.5**	1,2
92	γ-Amorphene	1492	1495	S	0.5		1,2
93	**Bicyclogermacrene**	1495	1500	S	**7.2**		1,2
94	α-Muurolene	1500	1500	S	0.3	0.5	1,2
95	Germacrene A	1506	1508	S	0.7		1,2
96	γ-Cadinene	1511	1513	S	0.7		1,2
97	(*Z*)-β-Bisabolene	1513	1514	S		0.7	1,2
98	Cubebol	1514	1514	OS	0.3		1,2
99	δ-Cadinene	1516	1522	S	1.6	0.9	1,2,3
100	*trans*-Cadina-1,4-diene	1528	1533	S	0.2		1,2
101	*10-epi*-Cubebol	1532	1533	OS		0.2	1,2

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102	α-Cadinene	1534	1537	S	0.1		1,2
103	**Germacrene B**	1560	1559	S		**4.2**	1,2
104	(*E*)-Nerolidol	1561	1561	OS	0.3		1,2,3
105	**Palustrol**	1569	1567	OS	**3.2**		1,2
106	Dendrolasin	1570	1570	Misc	tr		1,2
107	Spathulenol	1578	1577	OS	1.6		1,2
108	**Caryophyllene oxide**	1583	1582	OS	**5.7**	**4.7**	1,2
109	Globulol	1590	1590	OS		tr	1,2
110	Viridiflorol	1592	1592	OS	0.5		1,2
111	Salvial-4(14)-en-1-one	1597	1594	OS	0.1	tr	1,2
112	Longiborneol (Juniperol)	1598	1599	OS	tr		1,2
113	Ledol	1605	1602	OS	1.7		1,2
114	β-Oplopenone	1605	1607	OS	tr		1,2
115	Humulene epoxide II	1610	1608	OS	0.1	0.7	1,2
116	1,10-di-*epi*-Cubenol	1615	1618	OS	0.9		1,2
117	Junenol	1619	1618	OS	0.2		1,2
118	1-*epi*-Cubenol	1627	1627	OS		0.1	1,2
119	Muurola-4,10(14)-dien-1-β-ol	1626	1630	OS	0.3		1,2
120	Caryophylla-4(12),8(13)-dien-5α-ol	1632	1639	OS	0.3	0.3	1,2
121	Caryophylla-4(12),8(13)-dien-5β-ol	1634	1639	OS		0.8	1,2
122	*epi*-α-Cadinol (τ-cadinol)	1638	1638	OS	0.3		1,2
123	*epi*-α-Muurolol (τ-Muurolol)	1643	1640	OS	0.5	tr	1,2
124	α-Eudesmol	1654	1652	OS		1.2	1,2
125	α-Cadinol	1658	1652	OS	1.3		1,2
126	Intermedeol	1666	1665	OS	0.6		1,2
127	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1670	1668	OS		0.4	1,2
128	Germacra-4(15),5,10(14)-trien-1α-ol	1685	1685	OS	1.2	0.6	1,2
129	Eudesma-4(15),7-dien-1β-ol	1689	1687	OS	0.8	0.3	1,2
130	*cis*-Thujopsenal	1707	1708	OS		0.1	1,2
131	Pentadecanal	1714	1715	A	0.1		1,2
132	Mint sulfide	1744	1740	SS	tr		1,2
133	Drimenol	1770	1766	OS	0.4		1,2
134	Hexadecan-2-one	1809	1809	A	tr		1,2

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No.	**[a]Compound**	**RI[b]**	**RI$_{Lit.}$[c]**	**Class**	***Sp* (%)**	***Sm* (%)**	**IM[d]**
135	(5*E*,9*E*)-Farnesyl acetone	1909	1913	OS	tr		1,2
136	Phytol	1943	1942	OD	tr		1,2
137	Pimaradiene	1953	1948	D	tr		1,2
138	Hexadecanoic acid	1961	1959	A	1.8		1,2,3
139	Sandaracopimar-8(14),15-diene	1964	1968	D		0.6	1,2
140	Manoyl oxide	1992	1987	OD		0.3	1,2
141	Ethyl hexadecanoate	1997	1992	A		0.1	1,2
142	(*E*)-Biformene (labda-8(20),12,14-triene)	1998	1997	D	0.1		1,2
143	(*E*,*E*)-Geranyl linalool	2021	2026	OD	tr	tr	1,2
144	Abietatriene	2052	2055	D	0.2		1,2
145	(*Z*)-Phytol	2108	2114[f]	OD	0.6	tr	1,2
146	Nezukol	2129	2132	OD	tr		1,2
147	Oleic acid	2142	2141	A	0.8		1,2,3
148	Octadecanoic acid	2170	2172	A	1.2		1,2,3
149	Docosane	2200	2200	A		tr	1,2,3
150	8,13-Abietadien-18-ol	2319	2324	OD	0.2		1,2
151	*trans*-Ferruginol	2328	2331	OD	0.1		1,2
152	3-α-Acetoxy-manool	2351	2359	OD	0.1		1,2
153	Tetracosane	2400	2400	A	tr		1,2,3
154	Pentacosane	2500	2500	A	tr		1,2,3
	Chemical classes						
	Monoterpene hydrocarbons			M	1.2	9.1	
	Oxygenated monoterpenes			OM	1.1	7.6	
	Sesquiterpene hydrocarbons			S	64.0	64.2	
	Oxygenated sesquiterpenes			OS	20.3	9.4	
	Aliphatics			A	4.0	0.4	
	Diterpenes			D	0.3	0.6	
	Oxygenated diterpenes			OD	1.0	0.3	
	Others				tr		
	Identified components (%)				91.9	91.6	

[a]Compounds listed in order of elution from 5 % phenyl 95 % dimethylpolysiloxane type column. [b]RI = Retention index relative to C_8-C_{32} *n*-alkanes on the 5 % phenyl 95 % dimethylpolysiloxane type column. [c]Lit. RI = DB-5 [41,42]. [d]IM = Identification method: 1 = Experimental retention index; 2 = MS spectra; 3 = Standard. tr = Traces (<0.05 %). [e][43]; [f][44]. Major compounds are in boldface.

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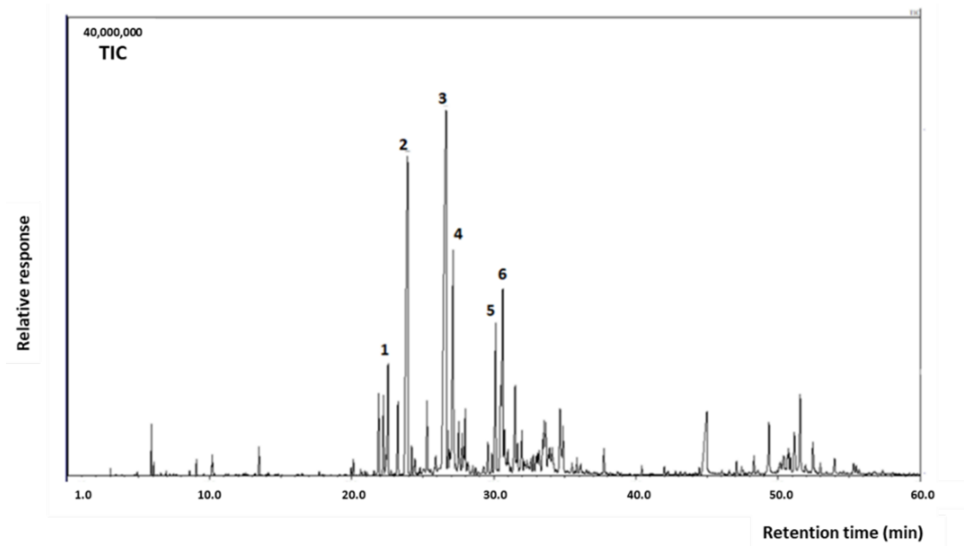

Fig. 2. GC-MS chromatogram (TIC) of *Salvia polystachia* oil: **1**. β-elemene; **2**. (*E*)-caryophyllene; **3**. germacrene D; **4**. bicyclogermacrene; **5**. palustrol; and **6**. caryophyllene oxide.

The hydrodistillation of fresh aerial parts of *S. microphylla* cultivated in Costa Rica produced a colorless oil yielding 0.13 % (v/w). The 73 identified oil compounds, accounting for 91.6 % of the whole volatiles, are summarized in Table 1 (see the ***Sm*** column). The essential oil composition consisted largely of sesquiterpene hydrocarbons (64.2 %), oxygenated sesquiterpenes (9.4 %), and monoterpene hydrocarbons (9.1 %), with minor quantities of monoterpenoids, aliphatic compounds, and diterpenoids. Seventy-three individual constituents were identified. The oil was dominated by (*E*)-caryophyllene (34.5 %), germacrene-D (8.4 %), *trans*-muurola-4(14),5-diene (6.5 %), with moderate amounts of caryophyllene oxide (4.7 %), germacrene B (4.2 %), β-pinene (3.2 %), γ-terpinene (3.2 %), β-gurjunene (3.2 %) and borneol (2.2 %), (see Fig. 3).

In the few studies carried out to date, quantitative and some qualitative differences in the chemical composition of essential oils have been observed. The oil of air-dried plants of *S. microphylla* cultivated in Italy [35], was rich in (*E*)-caryophyllene (10.8 %), bornyl acetate (9.1 %), 1,8-cineole (8.4 %), α-eudesmol (6.4 %), α-pinene (5.9 %), camphene (5.3 %), and β-eudesmol (5.3 %). The essential oil obtained from fresh leaves of plants cultivated in Brazil [36] presented as a major constituent (*E*)-caryophyllene (15.35 %), accompanied by α-eudesmol (14.06 %), β-eudesmol (8.74 %), γ-eudesmol (7.64 %), bicyclogermacrene (6.17 %) and isobornyl acetate (4.94 %), while the essential oil of *S. microphylla* from Algeria [37], contain (*E*)-caryophyllene (15.65-17.86 %), α-eudesmol (9.86-21.47 %), bornyl acetate (5.18-8.35 %), 1,8-cineole (1.98-7.50 %), γ-eudesmol (5.93-7.43 %), β-eudesmol (6.04-6.05 %), spathulenol (4.30-5.12 %), aromadendrene (4.90-5.39 %), and bicyclogermacrene (3.65-5.02 %). The chemical composition of the essential oil from the Costa Rican *S. microphylla* sample (University of Costa Rica campus) not previously studied is roughly similar to those formerly reported [35-37]. The Costa Rican essential oil is somewhat different in that neither bicyclogermacrene, spathulenol, γ-eudesmol, nor β-eudesmol was found. However, germacrene D and *trans*-muurola-4(14),5-diene were seen as important constituents not present in previously studied samples.

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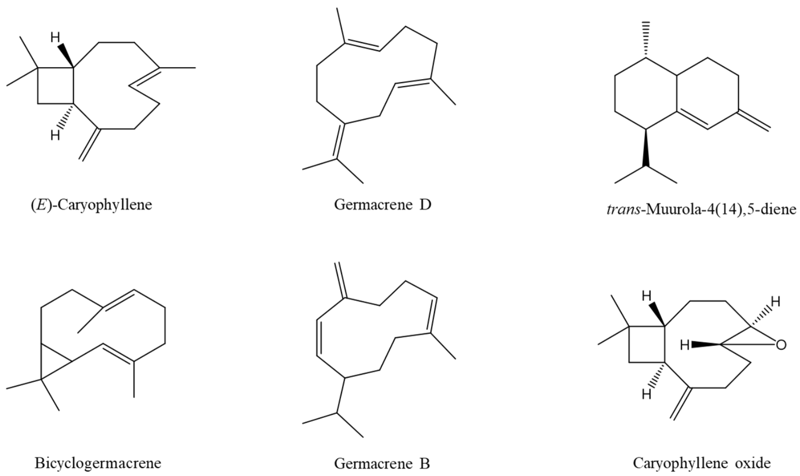

Fig. 3. Structure of the major sesquiterpenoid compounds identified in the essential oils of *Salvia polystachia* and *S. microphylla*, which occur in Costa Rica.

There are only two reports in the literature on the chemical composition of the essential oils of the garden cultivar Hot Lips. The essential oil extracted from leaves of *S. microphylla* cv. Hot Lips cultivated in California contains guaiol (24.6-26.3 %) as the major constituent, accompanied by α-eudesmol (15.6-19.9 %), (*E*)-caryophyllene (5.5-11.5 %), camphor (3.5-10.6 %) and 1,8-cineole (1.4-6.2 %) [39]. Guaiol is also present in the flower volatiles (4.0 %) of that cultivar growing in Italy (Research Centre for Vegetable and Ornamental Crops, Sanremo) [38], but in this case, the principal constituents were completely different (!): davana ether (16.3 %), hexahydrofarnesyl acetone (11.9 %), carvacrol (10.9 %), thymol (8.1 %), and (*E*,*E*)-farnesyl acetate (4.4 %). This difference could be due, on the one hand, to the fact that the flowers were specifically studied as a distinctly morphological part, and on the other hand, because an essential oil was not strictly extracted, since the volatile compounds were obtained by Headspace-Solid Phase Microextraction (HS-SPME) technique.

Although more than thirty-five compounds are shared by the Costa Rican oil of *S. microphylla* and the oil from cultivar Hot Lips [39], notable differences can be observed between them. In the studies carried out on *S. microphylla*, the major compound of the oil was (*E*)-caryophyllene, and the Costa Rican oil sample was the one that contained it in greater quantity (34.5 %). Some biological activities reported for this compound include analgesic, anti-inflammatory, antioxidant, neuroprotective, gastroprotective, anti-diabetic, antimicrobial, and antiproliferative effects. These properties have provided health benefits in several experimental models, such as analgesia, inflammation, anxiety disorder, depression, colitis, neurodegeneration, autoimmune diseases, metabolic ailments, osteoarthritis, and some types of cancer [45,46].

Other main constituents of the oil were germacrene-D, *trans*-muurola-4(14),5-diene, caryophyllene oxide, and germacrene B. The volatiles from the cultivar Hot Lips growing in the USA and Italy contained guaiol, which appears to be a characteristic biomarker compound. The previously studied essential oils from S. microphylla do not contain guaiol [35-37], as does our oil sample of this species growing in Costa Rica.

Additionally, the essential oil of *S. polystachia* contained some diterpenoids (1.3 %) as minor constituents: phytol, abietatriene, (*E*)-biformene, 8,13-abietadien-18-ol, *trans*-ferruginol, 3α-acetoxy-manool, pimaradiene, and nezukol, whereas the essential oil of *S. microphylla* presented sandaracopimar-8(14),15-diene and manoyl oxide. Diterpenoids isolated from *Salvia* spp. are the largest class of terpenoids found in this genus [9] and, in the subgenus *Calosphace* they are both ubiquitous and characteristic [12]. Diterpenoid classes identified belong to four chemical skeletons: phytane, abietane, pimarane, and labdane (see the structures of cyclic diterpenoids identified, in Fig. 4).

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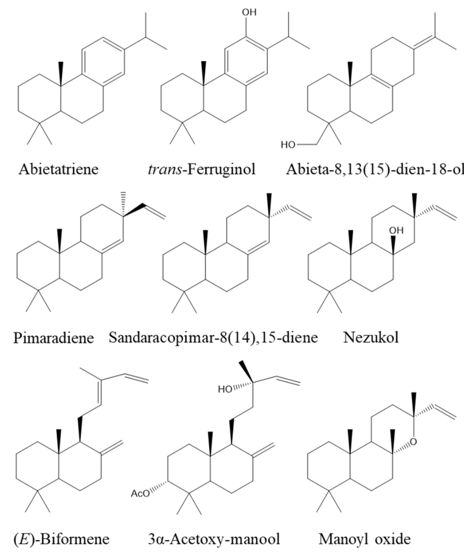

Fig. 4. Structures of cyclic diterpenoids identified in the essential oils of *Salvia polystachia* and *S. microphylla* from Costa Rica.

The essential oils of these two *Salvia* species from Costa Rica seem to be part of the group of *Salvia* oils containing mainly sesquiterpenoids with (*E*)-caryophyllene, germacrene D, bicyclogermacrene, and caryophyllene oxide as some of the principal constituents. Some examples are *S. aethiopis* L. [24,47-50], *S. amplexicaulis* Lam. [51], *S. cardiophylla* Benth. [52], *S. chionantha* Boiss. [53], *S. chloroleuca* Rech. f. & Aellen [53,54], *S. compressa* Vent. [55], *S. grossheimii* Sosn. [56], *S. guaranitica* A.St.-Hil. ex Benth. [57], *S. leucantha* Cav. [58], *S. longipedicellata* Hedge [51,59], *S. nemorosa* L. [49], *S. nubicola* Wall. ex Sweet [60], *S. palaestina* Benth. [61], *S. sclareopsis* Bornm. ex Hedge [62], *S. verbascifolia* M. Bieb. [62], *S. verticillata* L. [50,63], and *S. xanthocheila* Boiss. ex Benth. [54,64]. Jassbi et al. [65] and more recently, Asgarpanah [66] classified the species of the genus *Salvia* growing in Iran into four categories according to their essential oil composition (GC-MS and GC-FID analyses) depending upon their major components: a) monoterpene chemotype, b) mono and sesquiterpene chemotype, c) sesquiterpene chemotype with caryophyllane type-C-skeleton as the predominant compounds, and germacrane, aromadendrane, cadinane, and other sesquiterpene classes as the other major constituents and, d) GLV (green leaf volatiles) chemotype. Most species examined in Iran are represented by the sesquiterpene chemotype which is prevalent there. The publications included in these studies indicated that the species of the *Salvia* genus showed that there is a large chemical polymorphism. These chemical differences could be due to biotic (genetic and biological differences) and abiotic conditions (which include all environmental factors) and those resulting from human activities, including extraction and analysis methods.

Conclusions

The chemical composition of the essential oil obtained from the aerial parts of a wild natural population of *Salvia polystachia* growing in Costa Rica has been determined for the first time. Sesquiterpenoids dominated the chemical composition of the oil (84.3 %), with germacrene D, (*E*)-caryophyllene, bicyclogermacrene, and caryophyllene oxide as principal constituents. The chemical composition of essential oils obtained from the aerial parts of cultivated *S. microphylla* in Costa Rica was studied for the first time. The essential oil also consists mainly of sesquiterpenoids (70.4 %). The main sesquiterpene encountered was (*E*)-caryophyllene. This was accompanied by lesser amounts of germacrene

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D, trans-muurola-4(14),5-diene, caryophyllene oxide, and germacrene B. Both species can be classified within the sages whose essential oils have sesquiterpenoids as major constituents (sesquiterpene chemotype).

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