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Essential oils from Bolivia. XVI. A first look at three endemic *Salvia*: *S. orbignaei*, *S. haenkei* and *S. retinervia*

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Abstract

The genus *Salvia*, within the Lamiaceae family, is estimated to include more than 900 species. Many are aromatic plants, and the composition of the essential oils of several species is yet to be reported. This study focuses on the compositional study and large quantitative variation amongst the essential oils (EO) from the leaves of three Bolivian endemic species of *Salvia*: *S. orbignaei*, *S. haenkei* and *S. retinervia*. Thus, one sample of *S. orbignaei* EO contains 71% of limonene and four others feature at least 40% of sesquiterpenes. While the main fraction of one sample of the EO of *S. haenkei* is characterized by sesquiterpenes, accounting for 56% of the total; a second sample shows a high fraction of oxygenated sesquiterpenes at 47%. As far as *S. retinervia* is concerned, β -caryophyllene is a major constituent, accounting for 22 and 31% of the oils. Finally, the identification of some kessyl or kessanyl esters is discussed.

Keywords *Salvia orbignaei*, *Salvia haenkei*, *Salvia retinervia*, essential oil, Kessyl esters

1. Introduction

The series of articles *Essential oils from Bolivia* covers an effort to gain deeper insight into the volatile constituents of a number of previously unstudied or scarcely examined species from the biodiverse Bolivian flora [1]. The focus was oriented towards the quality of various EOs from two perspectives: fundamental knowledge in a phytochemical and taxonomic perspective, and commercial potential.

In Bolivia, by the latest account [2], 28 species of sages endemic to the country and its immediate surroundings are described. Of the latter category, *S. orbignaei* is common from North to South around the valley of Cochabamba, in the vicinity the towns of Mizque and Totora and towards Sucre. Some records were also identified in northern Argentina. Colonies of two other sage species, *S. haenkei* and *S. retinervia*, have been observed in the same Bolivian regions, extending northwestward into southern Peru. None of these three species have been examined previously for their EOs, and the objective of the present study was to shed light on some endemic *Salvias* species of this Andean region and offer an insight into these plants' phytochemical composition.

2. Materials and Methods**2.1 Plant Materials**

The foliage of the following Lamiaceae was studied: *Salvia orbignaei* Benth. (syn. *S. cochabambensis* Rusby, *S. kuntzeana* Briq. and *S. chiarantha* Briq.), *S. haenkei* Briq. (syn. *S. avicularis* Briq.), and *S. retinervia* Briq. (syn. *S. chorianthos* Epling). These three *Salvias* among 25 others have been the subject of a very detailed report on their morphology, distribution and habitat [2]. Colonies of *S. orbignaei*, *S. haenkei* and *S. retinervia* has been observed in Cochabamba, Bolivia, along the highway that connects the provinces of Arani, Mizque and Aiquile. Foliage of *S. orbignaei* (samples D, E and F) were collected in the immediate vicinity of the town of Aiquile while all other samples, A, B and C, closer to the road from Arani to Mizque. *S. haenkei* (samples G and H) on the road from Arani to Mizque (Kewiñal hill) and *S. retinervia* (J and K) aprox. 10 km on the road from Mizque towards

Aiquile (Map 1). The characteristics of the harvesting sites are shown in Table 1. Voucher of specimens were authenticated from their morphological and anatomical features and have been deposited in the Herbario Nacional Forestal Martin Cardenas (BOLV) of the Universidad Mayor de San Simón,

Cochabamba, Bolivia. Voucher registration codes appear in Table 1. It should be noted that the Cochabamba region was subjected to a severe drought in 2023. This may have affected both quantitatively and qualitatively the essential oils.



Map 1: Map situating the localities where specimens of the endemic *Salvia* species were collected.

Table 1: Main characteristics of the *Salvia* accessions studied

Species	<i>S. orbignai</i>						<i>S. haenkei</i>		<i>S. retinervia</i>	
	A	B	C	D	E	F	G	H	I	J
Harvest month	03-2023	04-2022	03-2023	03-2020	12-2021	04-2022	03-2023	04-2022	04-2022	12-2021
Voucher #	MZ 7005		MZ-7084	MZ-7007		MZ-7009	MZ-7006		MZ-6951	
Geolocation	17°35'21" S, 65°45'13" W		17°34'26"S, 65°44'10"W	18°14'56"S, 65°09'51"W		18°13'03"S, 65°11'47"W	17°33'53"S, 65°43'11"W		18°00'19"S, 65°17'15"W	
Altitude ^a	2950		3130	2375		2310	3330		2155	
Kg foliage	1.4	3.5	1.4	1.5	1.8	2.8	3.5	2.8	4.2	1.8
mL EO	1.8	3.8	0.4	3.9	2.0	1.4	2.8	1.8	4.4	0.3
Yield (%)	0.13	0.11	0.03	0.26	0.11	0.05	0.08	0.06	0.11	0.02

^aMeters above sea level

2.2 Oil Isolation

The fresh aerial parts of the collected samples were divided into fractions of 0.5 kg, frozen and subjected to steam distillation for 3 hours, using a Clevenger-type apparatus. The oily layer collected on the top of the aqueous distillate was

separated and dried over anhydrous sodium sulfate. The EOs were kept in amber vials at 4 °C prior to analysis. Yield was calculated by dividing the mass of essential oil obtained by the mass of the raw material.

2.3 Oil Analysis

The GC-FID and GC-MS conditions used have previously been described elsewhere^[1]. Identification of the components was done by comparison of their retention indices with normal alkanes on both apolar and polar columns, (DB-5) and (DB-Wax) columns, RI_a and RI_p, from C7 to C40 using the linear formula of van den Dool and Kratz^[3] and by comparison of their mass spectra with literature data^[4-7] and with our own databases. Quantitative data were obtained electronically from GC-FID area percentages without correction (internal normalization).

3. Results & Discussion

3.1 EO of *S. orbignaei*

The results of the analyses of the essential oils of *S. orbignaei* are reported in Table 2. More than 150 compounds in total were identified in these EOs. The sesquiterpene family was the most prominent, although sample D contains only a little more than 15% sesquiterpenes vs 77% monoterpenes. In addition, the percentages of oxygenated sesquiterpenes varied from 2 to 35%. In all analyses, β -caryophyllene appeared to be an important constituent. Its percentage was between 8 and 43%. It was accompanied by bicyclogermacrene (10.7% in sample F) and γ -cadinene (about 12% in the A, B and E samples). Furthermore, τ -cadinol was present in samples A, B and E in percentages close to 18%, and caryophyllene oxide comprised 19% of sample C.

Numerous peaks most of them minor, featured unknown mass spectrum (MS) within the libraries used, some of which were more abundant and deserve to be reported for future reference. First, the presence of a MS identical to that of the α -cubebene was noticed. However, on the DB-5 nonpolar column it was located at 48 retention units higher than that of the actual α -cubebene. We therefore report it as " α -cubebene analog". Figures 1 - 4 display the MS of the other main unidentified peaks (U1-U4) observed in this series of EOs. The MS that appears in Fig. 1 was also observed in *S. haenkei*. The same signal with the same RI_a values previously was noted in commercial samples of *S. sclarea* analyzed in our laboratory^[8]. Figure 4 shows a MS also seen in an EO of *Myrica gale*, a circumboreal aromatic shrub found in bogs and near lakes^[9]. In addition, the MS of the unknown U4 (Figure 4) was observed in a EO of *Thymus arcticus*, also a Lamiaceae like *Salvia* sp.^[8].

3.2 EO of *S. haenkei*

The results of the analyses *S. haenkei* are reported in Table 3. About 135 compounds were identified. Again, the two analyses were dissimilar. In sample G, we observed a mixture of monoterpenes (17%), oxygenated monoterpenes (16%) and sesquiterpenes (56%). On the other hand, sesquiterpenic compounds were the most important in sample H. Most of them were oxygenated: 47% in total. It must be noted that for this sample, many individually minor peaks were not reported due to low signal and mass spectral ambiguity due to numerous overlaps: *circa* 30% of total GC-FID signal was concerned by this observation. Nevertheless, a few constituents with unknown mass spectra (U1, U4-U6) were noteworthy owing to their greater abundance (Figures 1, 4-6), two of which were also present in the EO of *S. orbignaei* (Figures 1 and 4).

In the case of unknown U5 (Figure 5), the observed RI values are not in favor of an oxygenated sesquiterpene. The

difference between RI_p and RI_a values is insufficient to correspond to this molecular family. Thus, it is most probably to a sesquiterpene hydrocarbon, and its mass spectrum is probably contaminated by a trace amount of an oxygenated sesquiterpene. It is quite similar to that published for synthetic spirovetiva-1(10),7(11)-diene^[6]. Since the publication of this *Atlas*, the compound was reported in the essential oil of *Chrysopogon zizanoides* obtained in various countries^[10]. The RI reported values are 1523 and 1759, on apolar and polar columns, respectively. The first value agrees with that appearing in Table 3. The second one is a little higher than our value 1703. However, it is reasonable to question the likelihood of the presence of this molecule in this mixture. Its structure makes it closely related to β -vetivone: it was obtained by chemical degradation of this molecule^[11]. If it is therefore plausible to find it in an oil extracted from *C. zizanoides*, the EO of *S. haenkei* does not seem to be a favourable medium for the presence of such a molecule, and it could well be a confounding mass spectrum with very close retention behaviour. In addition, as with the unknown U4, the unknown U6 was also observed in the EO of *Thymus arcticus*^[8]. A MS observed in this essential oil appears in Figure 6.

3.3 EO of *S. retinervia*

The results for *S. retinervia* EO also appear in Table 3, since this oil features some qualitative similarities with that of *S. haenkei*. About 75 compounds were identified. In sample I and J, sesquiterpenes were the most important compounds: 52 and 69%, respectively. β -Caryophyllene was the most important compound: 22 and 31% in these samples, respectively. Bicyclogermacrene, germacrene D and α -humulene had percentages between 6.5 and 9.5%. The first sample (I) also contained monoterpenes (18.2%) including limonene (3.4%) and oxygenated sesquiterpenes (13%). Finally, accompanying a very weak yield in EO, one could note the presence of diterpenes (9%) in the last sample J. Two MS of unknowns U7 and U8 are shown in Figures 7 and 8. The pattern of the first spectrum shows some resemblance to *meta*-camphorene. However, based on literature the RI_a value of 1927 is too small compared to the reported value of 1953^[7]. On the other hand, the MS of U7 is also similar to that of β -springene as reported in literature^[12] but not so quite similar with the one available either in the NBS or NIST data basis^[7]. Unfortunately, the known RI_a for β -springene is not assured or unequivocal. At least one paper indicated a RI_a value = 1860^[13]. Others mentioned 1918 – 1923^[14-15]. In short, the identity of this compound remains at least uncertain. As for the last MS U8, it looks very similar to another obtained from EO of *S. sclarea*^[16].

Quantitatively, apart from sample D, whose essential oil yield reached 0.26%, the yields observed for the others rarely attained or exceeded 0.1%. As for the recorded percentages of each compound, apart from that of limonene again in the same sample (*circa* 72%), they were hardly individually remarkable. The chemical compounds themselves are relatively common in essential oils, including in the many analyses reported in the literature relating to the genus *Salvia*. Shyobunol is not a rare constituent in EOs as in *S. aurea* (0.1-0.9%) and *S. cinnabarina* (0.7%). These were cultivated in a Botanical garden in Ventimiglia, Italy and the last oil also contains 1-octen-3-yl acetate (2.7%)^[17]. Dihydroedulan I and II appear with minor percentages in *S. anatolica* as well as caryophylladienol I and II and caryophyllenol II^[18].

Table 2: Composition of the essential oils obtained from several samples of *Salvia orbignaei*

Identification	Ria ^a	Rip ^a	Sample					
			A	B	C	D	E	F
3-Methylcrotonaldehyde	781	1150			0.03			
3-Acetyl-3-methyl-cyclopentene	882	937		0.01	0.05			
Tricyclene	919	975	0.01	0.02			tr ^b	tr
α -Thujene	926	1003		0.01	0.01	0.05	tr	tr
α -Pinene	931	993	0.04	0.04	0.06	0.55	0.07	0.16
Camphene	944	1030	0.01	0.01	0.02	tr	tr	tr
Sabinene	971	1085	0.01	0.02	0.05	0.21	tr	0.05
β -Pinene	972	1067	0.03	0.03	0.04	0.20	0.05	0.15
Octen-3-one	978	1258		0.01		tr	tr	tr
1-Octen-3-ol	984	1420		0.02				
Octan-3-one	987	1220	0.06	0.02	0.04	tr	tr	tr
Myrcene	993	1135	0.06	0.10	0.02	1.47	0.16	0.28
Octan-3-ol	1000	1368		0.01			tr	
α -Phellandrene	1002	1128	0.02	0.02			tr	0.11
Δ 3-Carene	1008	1113	0.07	0.06	0.03	0.20	0.08	0.11
α -Terpinene	1015	1141	0.01	0.01		tr	tr	tr
<i>p</i> -Cymene	1022	1229	0.04	0.18	0.11	0.46	0.14	0.17
Limonene	1027	1159	0.63	2.28	0.61	71.90	2.97	0.87
β -Phellandrene	1027	1167			0.01			
1,8-Cineole	1027	1166	0.03	0.05	0.01	0.27	0.08	0.05
(<i>Z</i>)- β -Ocimene	1040	1207	0.27	0.15	0.01	0.12	0.29	0.29
(<i>E</i>)- β -Ocimene	1050	1222	1.23	0.49	0.02	0.74	1.13	1.77
γ -Terpinene	1058	1208	0.03	0.03		1.02	0.09	tr
Terpinolene	1086	1244	0.18	0.08		0.17	0.19	0.29
2-Nonanone	1093	1351	0.03	0.02	0.02	tr	tr	tr
Linalool	1102	1516	0.05	0.09	0.05	0.10	0.09	tr
2-Methylbutyl 2-methylbutyrate	1105	1254		0.01				
Nonanal	1105	1356	0.03	0.02		tr	tr	tr
Octen-3-yl acetate	1116	1351	0.02	0.02	0.06			
(<i>E</i>)-4,8-Dimethyl-1,3,7-nonatriene	1119	1282	0.02	0.03		tr	tr	
3-Methyl-3-butenyl isovalerate	1119	1336	0.01		0.03			
Octan-3-yl acetate	1128	1310	tr	0.01				
<i>p</i> -Cymen-8-ol	1185	1798		0.01	0.07			
2-Decanone	1194	1456	0.03	0.03	0.05	tr	tr	tr
(3 <i>E</i> ,5 <i>E</i>)-2,6-Dimethylocta-3,5,7-trien-2-ol	1212	1782		0.02				
(3 <i>Z</i>)-Hexenyl 2-methylbutyrate	1230	1442	0.01				tr	
(3 <i>Z</i>)-Hexenyl isovalerate	1235	1444	0.02	0.03	0.02			
Carvone	1239	1670			0.04			
Geraniol	1258	1806	0.04	0.04				
Bornylacetate	1283	1531	0.02	0.06	0.03			
Dihydroedulan I	1288	1442	0.04	0.05	0.04			
Theaspirane isomer I	1293	1452	0.03	0.06	0.07	tr	tr	tr
2-Undecanone	1294	1555	0.23	0.17	0.19	0.05	tr	0.16
Theaspirane isomer II	1307	1489	0.01	0.04	0.04	tr	tr	tr
Myrtenyl acetate	1323	1636	0.05	0.04			tr	
δ -Elemene isomer	1334	1433	0.06				0.08	0.02
δ -Elemene	1336	1437	0.47	0.13	0.04		0.62	0.16
Bicycloelemene	1336	1442				0.06		0.13
α -Cubebene	1348	1424	0.04	0.03	0.05	0.05		0.05
Cyclosativene II	1360	1442	0.04					
α -Copaene	1372	1452	0.71	0.54	0.56	0.49	0.52	0.56
β -Bourbonene	1380	1476	1.71	1.82	1.87	0.51	1.71	1.71
1,5-di- <i>epi</i> - β -Bourbonene	1382	1467	0.11	0.10	0.08	tr	0.12	0.10
β -Cubebene	1387	1498	0.18	0.13	0.15	tr	0.13	tr
β -Elemene	1390	1549	0.78	0.52	0.35	0.10	0.59	0.13

Cyperene	1393	1476	0.14	0.20	0.47		0.53	0.70
α -Cubebene analog	1393	1498	0.49	0.37	0.09			0.96
Isocaryophyllene	1402	1527		0.14	0.32	0.05	0.15	0.25
α -Gurjunene	1406	1485	0.04	0.04	0.03			
Sesquithujene	1406	1527	0.63	0.23	0.12			
α -Cedrene	1408	1514	0.10	0.11		tr	0.10	0.11
β -Cedrene	1408	1528					0.13	0.39
β -Ylangene	1416	1527	0.20	0.19				
β -Caryophyllene	1416	1549	11.80	13.60	27.50	8.30	13.90	43.50
Himachala-2,4-diene	1422	1555		0.07				
β -Copaene	1424	1530?	0.29	0.28	0.26	0.09	0.29	0.22
β -Gurjunene = Calarene	1427	1533	0.08	0.04	0.06			
γ -Elemene	1431	1595	0.60	0.40	0.27			
<i>trans</i> - α -Bergamotene	1434	1549	0.47	0.31	0.43			tr
α -Guaiene	1441	1590				0.05		0.24
6,9-Guaiadiene	1439	1563	0.40	0.13			0.10	
<i>trans</i> -Muuroala-3,5-diene	1442	1593	0.43	0.17				
α -Humulene	1448	1613	0.58	0.68	1.59	0.10	0.65	1.00
Rotundene	1452	1581	0.13	0.15	0.24	0.08		
9- <i>epi</i> - β -Caryophyllene	1457	1590					0.66	0.41
Geranylacetone	1454	1808				0.09		tr
<i>allo</i> -Aromadendrene	1455	1592	1.05	0.68	0.68			
<i>cis</i> -Muuroala-4(15),5-diene	1458	1619	1.24	0.96				
(<i>E</i>)- β -Farnesene	1458	1638	0.55	0.36	0.21			
γ -Gurjunene	1468	1603	0.18					
10- <i>epi</i> - β -Acoradiene	1470	1636	0.37	0.16				
γ -Muurolene	1473	1638	0.18			0.05	0.14	
GermacreneD	1477	1656	5.40	3.11	0.31	2.50	3.96	9.10
β -Selinene	1478	1661	0.17	0.10	0.16			
<i>ar</i> -Curcumene	1481	1727		0.11	0.12			
Phenylethyl isovalerate	1483	1931	0.23	0.25	0.78	tr	0.25	0.08
Viridiflorene	1492	1645	1.19	0.80	1.56		0.23	
Bicyclogermacrene	1492	1678	4.05	4.10	0.46	2.55	5.30	10.70
<i>epi</i> -Cubebol	1492	1840	0.06	0.08	0.49			
α -Muurolene	1497	1676			0.06			
Germacrene A analog	1499	1694		0.55				
Germacrene A	1499	1702	1.36	0.60	0.33	0.13	0.30	1.00
Cuparene	1499	1758	0.03	0.05				
Aromadendra-1(10),4(15)-diene	1501	1713						0.12
α -Alaskene	1508	1667			0.11			
β -Bisabolene	1512	1688	0.33		0.27		1.10	
β -Curcumene	1512	1694	0.58					0.06
γ -Cadinene	1512	1709	11.80	12.00	1.04	0.15	11.40	2.40
Cubebol	1511	1892		0.09	0.31			
<i>trans</i> -Calamenene	1518	1772	0.48	0.49	0.06	tr	0.36	tr
δ -Cadinene	1520	1707				tr	0.10	0.12
Kessane	1521	1709	1.38	0.90	0.36	0.45	0.96	0.17
Isocaryophyllene oxide?	1529	1852*				0.05		
(<i>E</i>)- γ -Bisabolene	1529	1709	0.17					
Selina-4(15),7(11)-diene	1529	1709	0.30	0.22	0.24			
α -Cadinene	1534	1738	0.33	0.29		tr	0.30	0.10
α -Calacorene	1539	1848		0.02				
(<i>E</i>)- α -Bisabolene	1542	1734	0.27	0.12				
Isocaryophyllene epoxide B	1545	1852			1.77	0.55		0.12
α -Elemol	1546	2029				tr	0.13	tr
Germacrene B	1551	1767	4.93	3.41	1.85	0.12	3.61	1.06
<i>cis</i> -Sesquisabinene hydrate	1553	1958	1.43	0.18				
Eudesma-5,7(11)-diene	1558	1766		0.09				

(E)-Nerolidol	1565	2001						0.07
Spathulenol	1572	2063	0.78	1.95	4.78	0.33	1.25	1.20
Caryophyllene oxide isomer	1575	1902	0.19	0.44	4.35			
Caryophyllene oxide	1575	1909	1.53	3.01	18.70	0.70	2.25	3.34
Globulol	1578	2015		0.26				
<i>trans</i> -Sesquisabinene hydrate	1588	2055	0.57				0.07	
Viridiflorol	1586	2024		0.77		tr	0.68	0.10
α -Cedrol	1593	2046	0.21	0.29		0.10	0.22	tr
Humulene epoxide II	1601	1963	0.10	0.15	0.65	tr	0.16	0.14
1,10-di- <i>epi</i> -cubenol	1610	1998	2.27	2.82	0.29	tr	2.48	0.27
Alismol	1623	2197	0.28	0.60	0.29		0.19	
β -Acorenol	1627	2102	0.41					
Caryophylla-4(12),8(13)-dien5 α -ol	1628	2228				0.06	0.18	0.19
Caryophylla-4(12),8(13)-dien5 β -ol	1632	2221				0.02	0.05	0.09
Isospathulenol	1633	2166			0.31			
τ -Cadinol	1641	2117	16.40	20.60	1.87	0.30	18.70	2.20
10- <i>epi</i> - β -Acorenol ^c	1643	2214?	0.14					
α -Cadinol	1650	2171	0.29	0.36			0.15	
Unknown U1	1661				0.43			
<i>ar</i> -Turmerone	1666	2187	0.20					
Valeranone	1670	2035		0.23			0.16	
β -Bisabolol	1667		0.27	0.12				
α -Turmerone	1670	2117	0.25					
Germacra-4(15),5,10(14)-trien-1-al	1678	2307				tr		0.06
α -Bisabolol	1681	2166	0.24	0.22			tr	
<i>epi</i> - α -Bisabolol	1683	2169	0.29	0.30			0.15	tr
Unknown U2	1698	1970	1.42	0.50				
β -Turmerone = Curlone	1702	2174	0.07					
Aromadendrane-4,10-diol	1713				0.30			
Oplopanone	1725	2451			0.09			
Unknown U3	1811	2226	1.76	0.99	0.77		0.98	
Unknown U4	1825	2518			0.24			
Phytone	1845	2095	0.11	0.12	0.20	tr		0.10
Kessyl propanoate	1850					0.02	0.02	tr
Kessyl isobutanoate ^d	1895	2370	0.23	0.43	0.10		0.35	tr
(5 <i>E</i> ,9 <i>E</i>)-Farnesylacetone	1913	2363		0.13		tr	0.14	0.12
Geranyl- α -terpinene	1949	2169	0.26	0.06		tr		0.10
Geranyl- <i>p</i> -cymene	1950	2240	0.06	0.11	0.07	tr	0.25	tr
Palmitic acid	1950	2865				tr		0.07
2-Phenylethyl nonanoate	1964	2420					0.10	
2-Phenylethyl 3-phenylpropanoate	2027	2840				tr	0.02	tr
Tricosane	2304	2300				0.01	0.02	0.02
Pentacosane	2505	2493					0.01	
Total (%)			91.7	91.3	84.3	95.0	82.3	88.2
Monoterpene hydrocarbons			2.5	3.5	1.0	77.1	5.2	4.3
Oxygenated monoterpenes			0.5	0.9	0.7	0.7	0.3	0.1
Sesquiterpene hydrocarbons			55.7	49.4	42.4	15.4	47.3	75.2
Oxygenated sesquiterpenes			28.8	34.0	35.3	2.1	27.7	8.0
Other oxygenated compounds			0.7	0.7	1.3	0.1	0.4	0.3
Diterpene hydrocarbons			0.7	0.9	0.5	tr	0.7	0.3

^a RIa: Retention index on apolar (DB-5) column, RPp: Retention index on polar (DB-Wax) column ^b tr: Traces (<0.005%); ^c Tentative identification; ^d See text for details on identification

Table 3: Composition of the essential oils obtained from samples of *S. haenkei* and *S. retinervia*

Identification	Ria ^a	Rip ^a	Sample			
			<i>S. haenkei</i>		<i>S. retinervia</i>	
			G	H	I	J
3-Methylcrotonaldehyde	781	1150		0.04		
(2 <i>E</i>)-Hexenal	850	1171	0.02			
3-Acetyl-3-methyl-cyclopentene	882	937	0.03	0.16		
Tricyclene	919	975	0.03		2.57	0.10
α -Thujene	926	1003	0.07		tr ^b	tr
α -Pinene	932	995	4.23		0.77	0.24
α -Fenchene	944	1024	tr			
Camphene	944	1030	0.06		2.26	0.12
β -Pinene	973	1068	3.95		1.19	0.50
Sabinene	973	1085	0.53		0.49	0.08
3- <i>p</i> -Menthene	981	1050		0.04		
Octen-3-ol	984	1422	0.02		tr	
6-Methyl-5-hepten-2-one	988	1301		0.02		
Myrcene	993	1136	0.76		2.26	0.14
Octan-3-ol	1000	1368	0.04			
α -Phellandrene	1002	1128	0.09		2.24	tr
Pseudolimonene	1002	1131	0.02			
Δ^3 -Carene	1008	1113	0.13		tr	tr
α -Terpinene	1015	1141	0.02		tr	tr
<i>m</i> -Cymene	1022	1229	0.01			
<i>p</i> -Cymene	1022	1229	0.29		0.61	0.06
Limonene	1027	1160	3.86	0.13	3.40	1.54
1,8-Cineole	1027	1167	0.66		1.07	
(<i>Z</i>)- β -Ocimene	1040	1207	0.15		0.13	tr
(<i>E</i>)- β -Ocimene	1051	1222	2.57		2.19	0.48
γ -Terpinene	1058	1209	0.10		0.07	tr
<i>cis</i> -Sabinene hydrate	1066	1429	0.01			
Terpinolene	1086	1244	0.10		0.07	tr
<i>p</i> -Cymenene	1086	1387	0.04			
Nonan-3-ol	1100	1459	0.02			
Linalool	1102	1515	0.14	0.03	0.05	tr
Nonanal	1105	1356	0.02			
2-Mebutyl 2-methylbutyrate	1106	1258	0.03			
Octen-3-yl acetate	1116	1351	0.58	0.12		
(<i>E</i>)-4,8-Dimethyl-1,3,7-nonatriene	1119	1276	0.03		0.11	
α -Campholenal	1122	1437	0.02			
Octan-3-yl acetate	1128	1312	0.08	0.05		
Methyl octanoate	1128				0.06	
<i>cis</i> -Limonene oxide	1130	1394	0.01			
<i>trans</i> -Pinocarveol	1135	1609	0.07			
(<i>E</i>)-Myroxide	1139	1442	0.01			
<i>trans</i> -Verbenol	1144	1636	0.05			
Menthone	1151	1411	5.92			
Isomenthone	1159	1435	0.28			
<i>neo</i> -Menthol	1164	1558	0.06			
<i>cis</i> -Isopulegone	1171	1526	0.17			
Terpinen-4-ol	1174	1558	0.03			
Cryptone	1180	1600	0.02	0.10		
<i>p</i> -Cymen-8-ol	1186	1796	0.01			
Myrtenal	1189	1561	0.03	0.05		
α -Terpineol	1189		0.03			
Myrtenol	1195	1743	0.02			
(3 <i>E</i> ,5 <i>E</i>)-2,6-Dimethylocta-3,5,7-trien-2-ol	1213	1783	0.05			
β -Cyclocitral	1217	1580			0.07	0.06
Pulegone	1236	1584	8.18			
Methyl β -cyclogeranate	1236	1514				0.10
Carvone	1239	1670	0.11	0.06		
Linalyl acetate	1258	1522	0.05	0.06		
<i>neo</i> -Menthyl acetate	1274	1489	0.06			
Bornyl acetate	1283	1530	0.22	0.27		
Dihydroedulan I	1288	1444	0.03	0.04		
Theaspirane isomer I	1293	1452	0.40	0.07		tr
2-Undecanone	1298	1550			0.25	0.21
Theaspirane isomer II	1307	1491	0.02	0.03		

2-Undecanol	1307	1692			0.08	
δ -Elemene isomer	1327	1430			0.14	0.13
Bicycloelemene	1334	1444	0.18			
δ -Elemene	1335	1437		0.09	1.50	1.30
α -Cubebene	1347	1425	0.04		0.16	0.15
α -Terpinyl acetate	1347	1646	0.18	0.37		
Cyclosativene II	1360	1435	0.01			
α -Copaene	1372	1452	0.68	0.58	3.26	4.02
β -Bourbonene	1380	1476	0.93	1.26	0.29	0.28
1,5-di- <i>epi</i> - β -Bourbonene	1382	1467	0.06	0.04	tr	tr
β -Cubebene	1387	1498	0.22	0.20	1.16	1.08
β -Elemene	1390	1549	1.13	1.06	0.57	0.69
Cyperene	1393	1476	0.43	0.38		
Isocaryophyllene	1401	1521	0.05	0.10		
α -Gurjunene	1404	1485	0.23			0.13
(<i>E</i>)- β -Damascone	1408	1758	0.05			
β -Ylangene	1416	1527	0.10			
β -Caryophyllene	1416	1549	9.45	2.59	22.30	31.20
β -Copaene	1424	1547	0.21	0.20	0.16	0.14
Aromadendrene	1431	1554	0.06	0.10	tr	0.06
<i>trans</i> - α -Bergamotene	1434	1549	0.15	0.41	0.05	
6,9-Guaiadiene	1439	1564	0.39		1.97	3.80
Sesquisabinene A	1441	1603		0.25		
α -Humulene	1448	1613	0.51	0.29	5.00	7.20
Rotundene	1452	1584	0.11	0.15		
<i>allo</i> -Aromadendrene	1455	1593	3.50	4.50	0.14	
9- <i>epi</i> - β -Caryophyllene	1457	1590			1.22	0.81
(<i>E</i>)- β -Farnesene	1459	1631	0.20			
γ -Gurjunene	1467	1603	0.10			
Selina-4,11-diene	1471	1625		0.19		
γ -Muurolene	1471	1639	0.19	0.25	0.22	0.10
α -Amorphene	1476	1638		0.08		
Germacrene D	1479	1659	23.70		4.50	7.10
<i>ar</i> -Curcumene	1480	1727		0.09		
β -Selinene	1482	1663	0.65	0.67		
Bicyclosiquiphellandrene	1485	1669			0.14	
α -Selinene	1490	1667	0.45	0.38		
<i>epi</i> -Cubebol	1490	1840		0.49	0.20	0.44
Viridiflorene	1493	1646	0.31	0.53		
Eremophilene	1493	1666	0.27			
Bicyclogermacrene	1493	1680	8.28		6.51	9.50
α -Muurolene	1497	1675		0.21	0.50	
Cuparene	1499	1758		0.13		
Germacrene A	1499	1703	1.36		0.61	0.76
γ -Cadinene	1510	1703	0.17	0.44	0.62	
Eremophila-1(10),7(11)-diene	1511	1680	0.25	0.30		
Cubebol	1511	1890	0.11	0.28	1.83	
<i>trans</i> -Calamenene	1518	1774		0.11		
δ -Cadinene	1520	1707	0.41		1.15	0.77
Unknown U5	1523	1703	0.38	0.18		
<i>trans</i> -Cadina-1,4-diene	1523	1703			0.07	
(<i>E</i>)- γ -Bisabolene	1529	1703	0.12		tr	0.11
Selina-4(15),7(11)-diene	1529	1719	0.46	0.34		
Isocaryophyllene oxide?	1529	1852*				0.16
α -Cadinene	1533	1735			0.07	0.05
Selina-3,7(11)-diene	1535	1719	0.09			
Isocaryophyllene epoxide B	1545	1852		2.69	0.25	0.19
α -Elemol	1546	2029	0.10		0.06	0.05
Germacrene B	1550	1764	0.33		0.23	
1- <i>nor</i> -Bourbonanone	1553				0.35	
Palustrol	1561	1866		0.16	tr	0.02
Germacrene D-4-ol	1575	1991	0.54		1.41	
Spathulenol	1572	2063	1.24	10.90	tr	0.50
Caryophyllene oxide isomer	1575	1901	0.15	5.00		
Caryophyllene oxide	1575	1908	0.67	19.80		1.04
Viridiflorol	1585	2023	0.10	0.58		0.58
Ledol	1593	1962	0.10	0.50	0.56	0.18
Humulene epoxide II	1601	1998	0.04	1.20	0.13	0.18
1,10-di- <i>epi</i> -Cubanol	1608	1996			0.05	0.03

Alismol	1624	2194			0.17	0.33
γ -Eudesmol	1625	2120				0.05
Caryophylla-4(12),8(13)-dien-5 α -ol	1628	2221			0.05	tr
Caryophylla-4(12),8(13)-dien-5 β -ol	1632	2228			0.04	
<i>allo</i> -Aromadendrene epoxide	1632	2015	0.45			
τ -Cadinol	1636	2112		0.72	0.53	0.23
α -Muurolol = Torreyol	1640	2141			0.10	
β -Eudesmol	1647	2150			tr	0.09
(3Z)-Caryophylla-3,8(13)-dien-5 α -ol	1649	2258		0.60		
α -Cadinol	1650	2166	0.12	0.62		1.16
<i>cis</i> -Calamene-10-ol	1654	2267		0.36		
Unknown U1	1660		0.49			
<i>trans</i> -Calamene-10-ol	1661	2306		0.24		
(3Z)-Caryophylla-3,8(13)-dien-5 β -ol	1667	2306		0.54		
Isocyperol	1669	2245		0.30		
Shyobunol	1683	2254	0.24		7.30	2.30
Oplopanone	1724	2451		0.33		
Unknown U4	1825	2518	0.07	0.96		
Phytone	1845	2095	0.12	0.78	0.10	0.13
Unknown U6	1893			0.68		
Unknown U7	1927				0.32	0.70
Unknown U8	1946	2206				0.10
Geranyl- α -terpinene	1949	2171	0.09		tr	0.12
Geranyl- <i>p</i> -cymene	1950	2239	0.03		4.70	9.00
Heneicosane	2104	2104	tr	tr	0.01	0.02
Tricosane	2305	2302	tr	tr	0.02	0.01
Pentacosane	2504	2499			0.01	0.02
Total (%)			95.6	68.4	90.5	90.7
Monoterpene hydrocarbons			17	0.2	18.2	3.3
Oxygenated monoterpenes			16	0.4	1.2	0.2
Sesquiterpene hydrocarbons			56.2	16.3	52.6	69.4
Oxygenated sesquiterpenes			4.2	47.7	13	6.5
Other oxygenated compounds			1.3	1	0.4	0.3
Diterpene hydrocarbons			0.2	0.8	4.9	9.3

^a RIa: Retention index on apolar (DB-5) column, RPp: Retention index on polar (DB-Wax) column ^b tr: Traces (<0.005%); ^c Tentative identification

3.4 Identification of kessyl esters

During this work, while exploring the numerous mass spectra of small peaks, not included in Tables 2 and 3, reaching percentages of less than 0.02%, a few of them caught our attention. They contained two main peaks at m/z value of 108 and 126 (Figure 9). These are typical of kessyl and kessanyl esters ^[5b]. The structural difference between these two esters is the position of the ester radical on the parent kessane molecule. Unfortunately, there are significant variations in both mass spectra and retention indices available in the literature for these constituents.

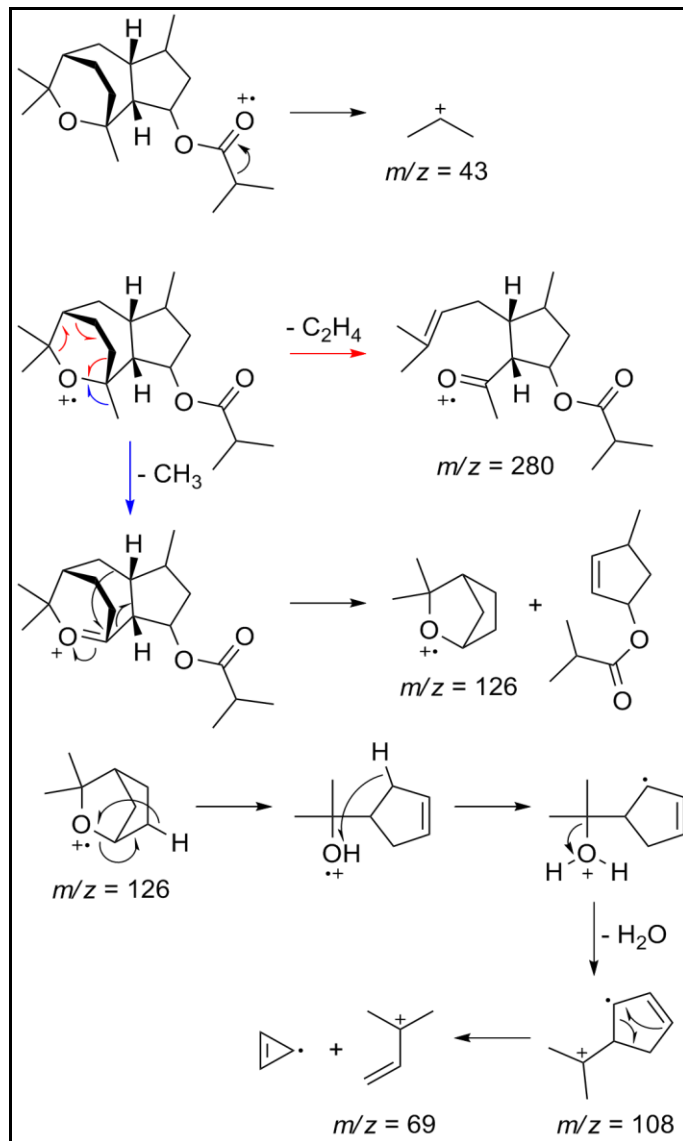
The RI value observed in this work for kessyl acetate on the apolar column corresponded to that reported by Lorenzo *et al* ^[19]. It should be added that in both present study and this reference, several common compounds have very consistent RI_a values (Table 4). In agreement with the rules governing retention index system ^[3], an increment of around 100 units should be observed from acetate to propanoate, therefore the RI(DB-5) value of 1845 would match the identification of kessyl propanoate and not kessanyl propanoate. On the same column kessyl butanoate should appear with a RI(apolar) around 1940 and that of the isobutanoate somewhere in between, in agreement with the 1896 value. The observed difference of 90 units between the values of RI(isobutanoate) and RI(isopentanoate) is also consistent with the same rules. Therefore, RI values suggest that *S. orbignaei* essential oil plausibly contains kessyl isobutanoate. Looking at the RI values obtained on the polar column, to our knowledge, there is at least one known value RI(kessane) = 1784 on a HP-Innowax column ^[18]. The present value on our DB-wax

column is RI = 1714 (Table 4). The great variability of the values reported on various polar columns makes a good interpretation difficult. However, the measured value is consistent with those reported in data base ^[5a]. The other values of the retention indices are consistent with the proposed identification. Furthermore, the study of the fragmentation pattern of the proposed kessyl isobutanoate allows to rationalize the formation of several of its characteristic ions, and in particular the minor ion m/z 280 that is formed due to the loss of a neutral molecule C₂H₄ by Retro Diels-Alder fragmentation. The structure of kessanyl isobutanoate would not allow the loss of C₂H₄, which is only possible from the kessyl backbone. The fragmentation mechanism of this compound is shown below (Scheme 1). Overall, this supports the tentative identification of kessyl butanoate.

Table 4: RI values reported in literature for kessane derivatives

Compound (molecular weight)	CP-SIL 5		DB-5/HP-5			DB-Wax	
	[19]	[21]	[22]	[23]	[20]	a	a
Reference							
Kessane (222)	1507	1507	1525	1507	1523	1520	1714
Kessyl alcohol (238)	1660		1691		1690		
Kessyl acetate (280)	1772	1772	1804	1772	1744	1749	2285
Kessanyl acetate (280)			1881	1827	1807		
Kessyl propanoate (294)						1845	2354
Kessyl isobutanoate (308)						1896	2370
Kessyl isopentanoate (322)	1986						
Kessyl hexanoate (336)	2070						

^a This work



Scheme 1: Proposed fragmentation pattern of kessyl isobutanoate molecular ion

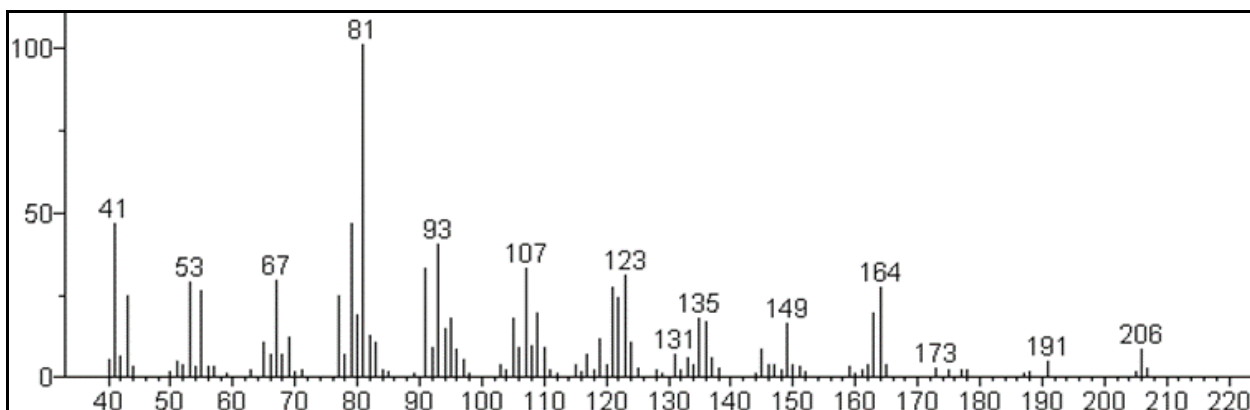


Fig 1: Mass spectrum of unknown compound U1 (RIa = 1661) observed in the EO of *S. orbignaei* and *S. haenkei*. EI-MS m/z : 81, 41(46), 79(46), 93(39), 91(33), 107(33), ... 164(26), ... 206(8).

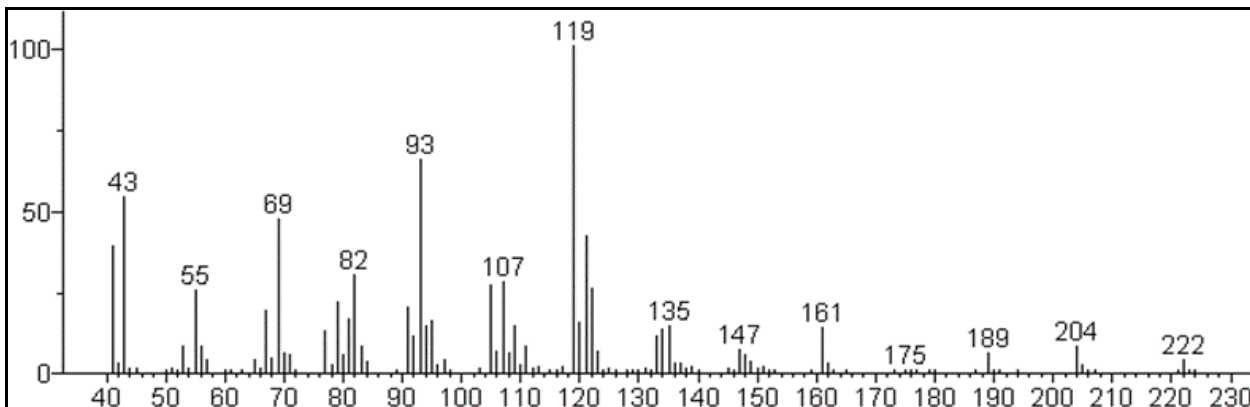


Fig 2: Mass spectrum of unknown compound U2 (RIa = 1698 and RIp = 1970) observed in the EO of *S. orbignaei*. EI-MS m/z: 119, 93(65), 43(54), 69(47), 121(42), 41(39), ... 222(4).

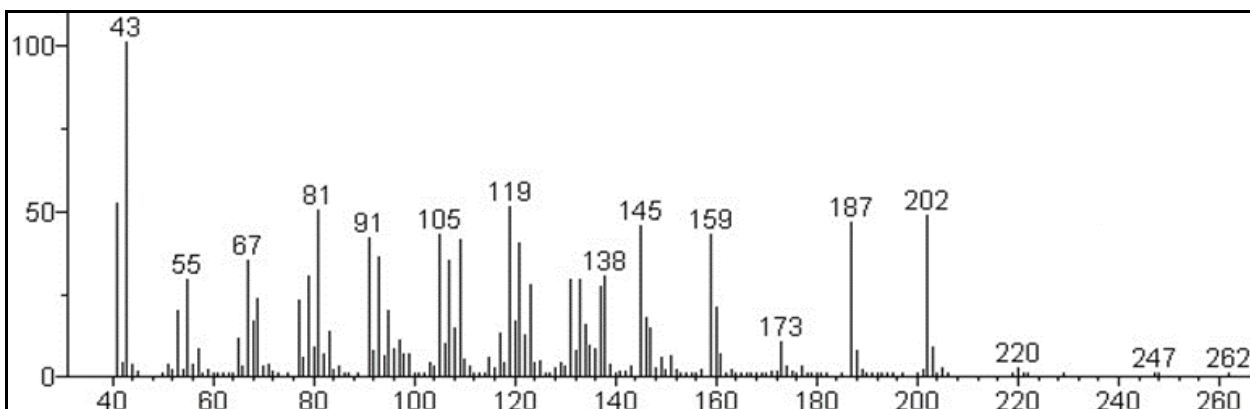


Fig 3: Mass spectrum of unknown compound U3 (RIa = 1811 and RIp = 2226) observed in the EO of *S. orbignaei*. EI-MS m/z: 43, 41(50), 81(49), 119(49), 202(46), 145(48), 187(43), 159(43), 91(40), ... 262.

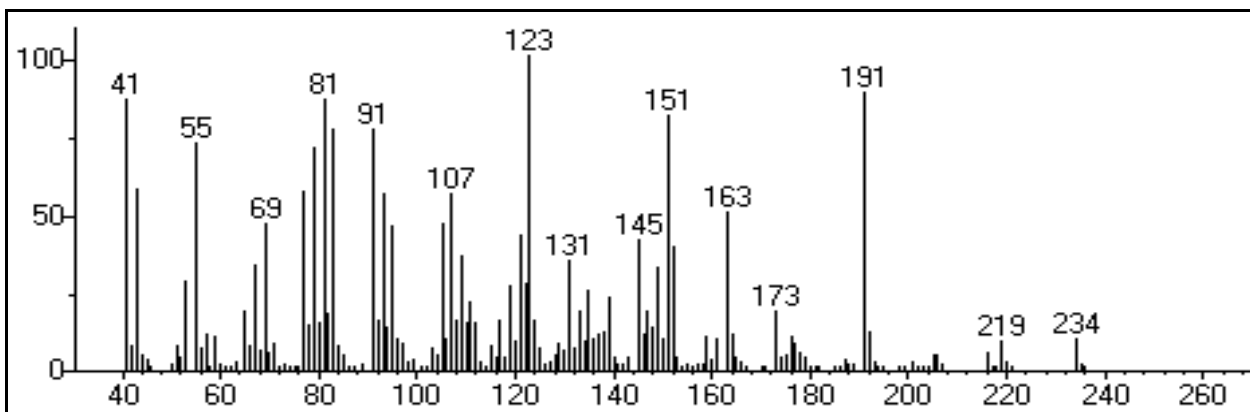


Fig 4: Mass spectrum of unknown compound U4 (RIa = 1825 and RIp = 2518) observed in the EO of *S. orbignaei* and *S. haenkei*. EI-MS m/z: 123, 191(88), 81(86), 41(86), 151(80), 91(76), ...

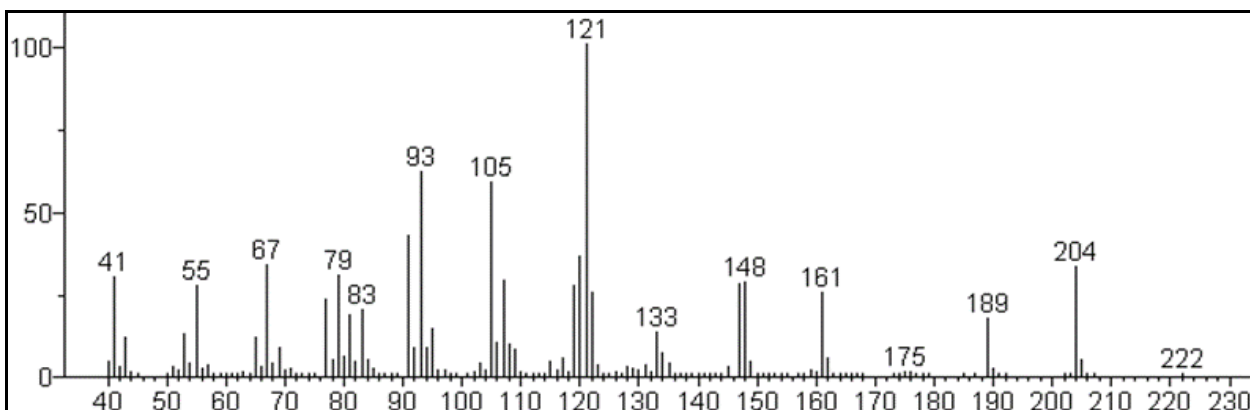


Fig 5: Mass spectrum of unknown compound U5 (RIa = 1523 and RIp = 1703) observed in the EO of *S. haenkei*. EI-MS m/z: 121, 93(61), 105(58), 91(42), 120(36), 67(33), 204(33).

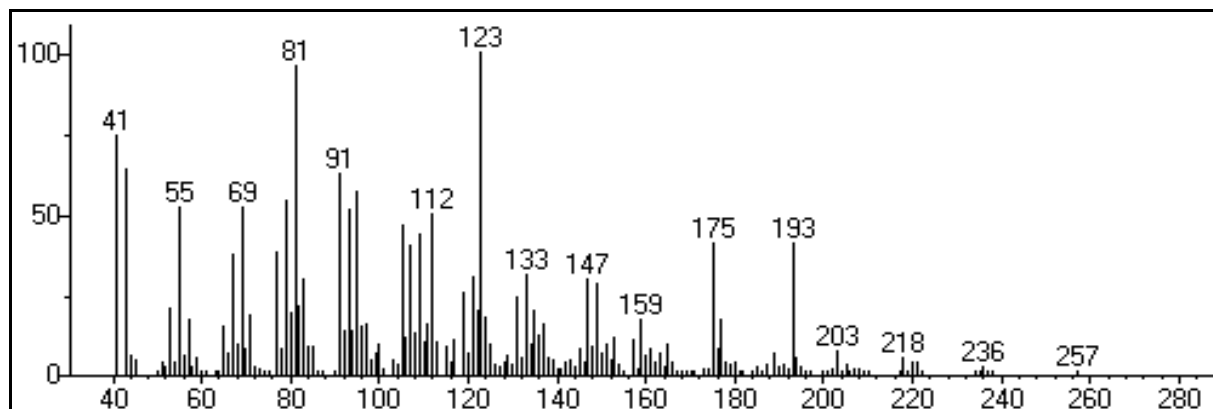


Fig 6: Mass spectrum of unknown compound U6 (RIa = 1893) observed in the EO of *S. haenkei*. EI-MS m/z: 123, 81(96), 41(74), 43(64), 91(62), 95(57), ...

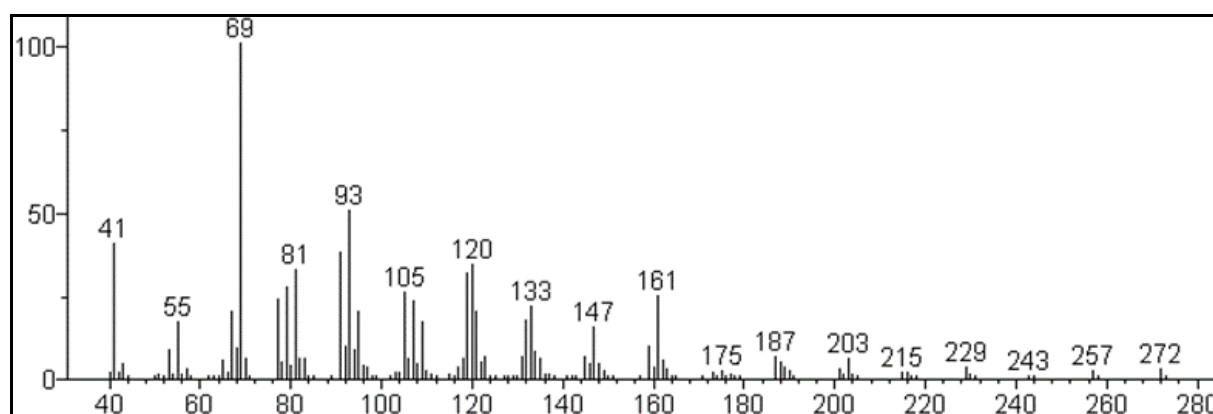


Fig 7: Mass spectrum of unknown compounds U7 (RIa = 1927) observed in the EO of *S. retinervia*. EI-MS m/z: 69, 93(50), 41(40), 91(38), 120(34), 81(32), 119(31), 79(27), 105(25), 161(25), ... 272(3).

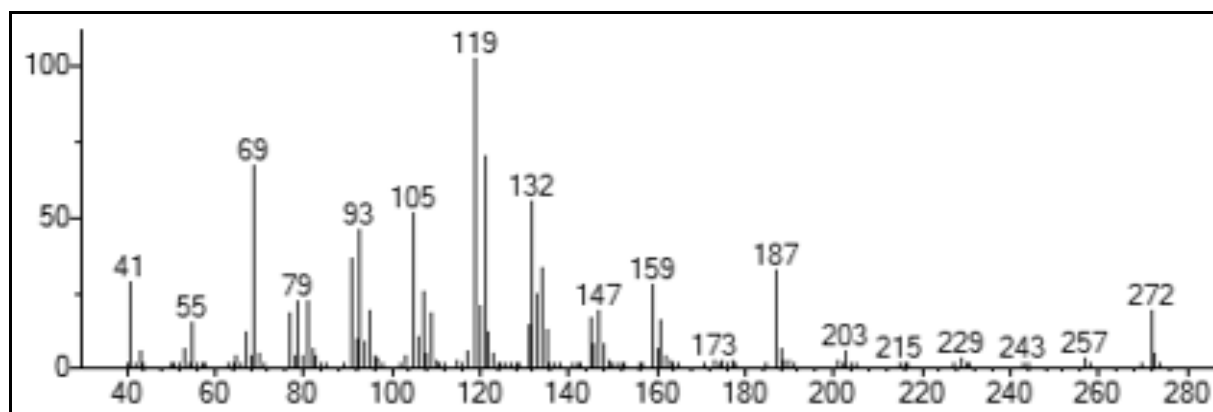


Fig 8: Mass spectrum of unknown compounds U8 (RIa = 1946 and RIp = 2206) observed in the EO of *S. retinervia*. EI-MS m/z: 119, 121(69), 69(66); 132(53), 93(44), 91(35), 134(32), 187(31), 41(28), ... 272 (15).

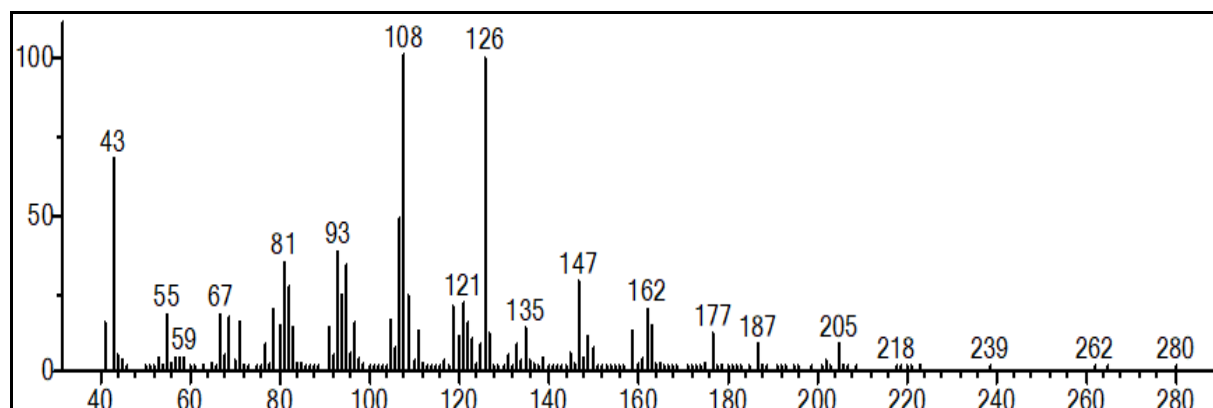


Fig 9: Tentatively identified kessyl isobutanoate mass spectrum in the essential oil of *S. orbignaei*. EI-MS m/z: 108, 126(98), 43(66), 107(48), 93(38), 81(34), 95(33), 109(24), ... 280(1).

3.5 Comparison with other American *Salvia*

Reported analyses of *Salvia* EOs appear to be less numerous in the Americas compared to those from Eurasia. Table 5 gives an overview of several works carried out in the Americas for spontaneous and grown species of sages. The

observed compositions are relatively different from each other, suggesting that the genus is home to a large diversity of biosynthetic pathways with regards to volatile constituents. Several of the compounds detected were also found in the EOs reported in the present study.

Table 5: Main compounds observed sage spontaneous or grown in Americas

<i>Salvia</i> species	Location	Main compound(s) (%)	Reference
<i>S. hispanica</i>	Southern Texas ^s	β -Caryophyllene (36)	[24]
	Southern California ^s	Globulol (22)	
	Northwestern Argentina ^s	Globulol (13)	
<i>S. sclarea</i>	Uruguay ^c	Linalyl acetate (38-48), germacrene D (8-18)	
<i>S. guaranitica</i>	Uruguay ^c	Germacrene D (25), β -elemene (20)	[27]
<i>S. leucantha</i>	Venezuela ^s	Bornyl acetate (24), β -gurjunene (15), β -caryophyllene (14), dill apiol (11)	[28]
<i>S. leucantha</i>	Dallas Arboretum, Texas ^c	Limonene (35), α -pinene (17)	[29]
<i>S. coccinea</i>		Viridiflorol (19)	
<i>S. farinacea</i>		1-Octen-3-ol (30), (Z)-3-hexenal (23)	
<i>S. greggii</i>		1,8-Cineole (22), borneol (17)	
<i>S. longispicata</i>		(Z)-3-Hexenal (24), 1-octen-3-ol (50)	
<i>S. madrensis</i>		(Z)-3-Hexenal (53),	
<i>S. roemeriana</i>		α -Pinene (20), limonene (49)	
<i>S. splendens</i>		(Z)-3-Hexenal (36), 2,5-dimethoxy- <i>p</i> -cymene (19)	
<i>S. apiana</i>	Poplarville, Mississippi ^s	1,8-Cineole (72)	[30]
<i>S. elegans</i>		Borneol (17), β -eudesmol (10)	
<i>S. leucantha</i>		Bornyl acetate (11), caryophyllene oxide (14)	
<i>S. palaefolia</i>	Colombia ^s	β -Caryophyllene (32), caryophyllene oxide (32)	[31]

Note: Several studies on various aspects of the EO of *S. officinalis* in Brazil and elsewhere are not included in this table. ^s: spontaneous or endemic; ^c: cultivated.

4. Conclusions

To say the least, the analyses were relatively dissimilar from one sample to another. Apart from the differences between each of the three species, several uncontrolled factors can certainly explain this disparity in compositions. The particularly intense drought experienced in the Cochabamba region during the summer of 2023 was probably not without effect. The location and the maturation of the plants and most likely the presence of various chemotypes must be considered as possible explanatory factors.

More detailed research involving the study of the various parameters just mentioned will be necessary to have a better understanding of these species with regards to their production of aromatic constituents.

5. Conflicts of interest

The authors declare no conflict of interest.

6. Authors contributions

Conceptualization, Guy Collin; Formal analysis, Alexis St-Gelais, Eliana M. Maldonado, Samuel Siles-Alvarado, and Guy Collin; Investigation, Alexis St-Gelais, and Eliana M. Maldonado; Methodology, Alexis St-Gelais; Resources, Alexis St-Gelais and Gloria Saavedra; Supervision, Eliana M. Maldonado and Guy Collin; Visualization, Alexis St-Gelais and Samuel Siles-Alvarado; Writing – original draft, Alexis St-Gelais and Guy Collin; Writing – review & editing, Eliana M. Maldonado and Samuel Siles-Alvarado.

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