SUPPLEMENTARY MATERIAL

A detailed study of the volatile components of *Plectranthus asirensis* of Saudi Arabian origin

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Abstract

Essential oil composition of *Plectranthus asirensis* grown in Saudi Arabia was chemically analysed for the first time by various gas chromatography techniques (GC–MS, GC–FID, Co-GC, LRI determination and database and literature searches) using two different stationary phase columns (polar and nonpolar). This analysis led to the characterisation of a total of 124 components representing 98.5% of the total oil composition. The results revealed that *P. asirensis* oil was mainly dominated by monoterpenoids (90.7%) in which most representative constituents were thymol ($66.0 \pm 0.36\%$), γ -terpinene ($14.0 \pm 0.18\%$), *p*-cymene ($5.2 \pm 0.06\%$) and β -caryophyllene ($3.0 \pm 0.03\%$). It is worth mentioning here that this is the first report on the phytochemical constituents of *P. asirensis*.

Keywords: Lamiaceae; *Plectranthus*, essential oils; thymol; γ -Terpinene; *P. asirensis*

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Supplementary Results

Table S1. Percentage composition of aerial parts essential oils of *Plectranthus asirensis* grown in the southern part of Saudi Arabia.

No.	Compounds [*]	LRI ^a	LRI ^p	(%) ^b	No.	Compounds [*]	LRI ^a	LRI ^p	(%) ^b
1.	Diacetone alcohol	841	1364	t	38.	<i>cis-p</i> -Mentha-2,8-dien-1-ol	1138	-	t
2.	<i>trans</i> -3-Hexen-1-ol	850	-	t	39.	<i>trans-p</i> -Mentha-2-en-1-ol	1143	1592	t
3.	<i>trans</i> -2-Hexenal ^c	852	1217	t	40.	<i>trans</i> -Verbenol	1146	1686	t
4.	<i>cis</i> -3-Hexen-1-ol ^c	854	1389	0.1 ± 0.01	41.	Camphor ^c	1148	1519	t
5.	1-Hexanol ^c	868	1359	t	42.	Camphene hydrate	1152	-	t
6.	2-Methyl butyl acetate	880	1122	t	43.	<i>iso</i> -Borneol	1162	1667	t
7.	Methyl hexanoate	-	1188	t	44.	Pinocarvone	1164	-	t
8.	α -Thujene	928	1024	0.5 ± 0.02	45.	Borneol ^c	1169	1709	0.1 ± 0.00
9.	α -Pinene ^c	935	1018	0.2 ± 0.01	46.	δ -Terpineol	1170	1679	t
10.	Camphene ^c	950	1060	t	47.	1-Nonanol	1174	-	t
11.	α -Fenchene	-	1053	t	48.	Terpinen-4-ol ^c	1180	1608	0.7 ± 0.01
12.	Sabinene	975	1118	t	49.	<i>p</i> -Cymene-8-ol	1188	-	t
13.	β -Pinene ^c	978	1105	t	50.	α -Terpineol ^c	1194	1704	t
14.	1-Octen-3-ol^c	980	1456	1.2 ± 0.05	51.	Dihydrocarveol	1198	-	t
15.	3-Octanone	988	1255	t	52.	<i>trans</i> -Piperitol	1210	1714	t
16.	β -Myrcene	993	1164	0.5 ± 0.11	53.	Shisofuran	1219	1999	t
17.	Dehydro-1,8-cineole	-	1191	t	54.	<i>iso</i> -Dihydrocarveol	-	1788	t
18.	3-Octanol ^c	997	1399	0.1 ± 0.01	55.	<i>trans</i> -Carveol	1224	1842	t
19.	α -Phellandrene	1006	-	0.2 ± 0.02	56.	Nerol	1229	1807	0.1 ± 0.03
20.	δ -3-Carene	1012	1146	0.1 ± 0.03	57.	Ascaridole	-	1870	t
21.	α-Terpinene^c	1018	1177	1.3 ± 0.02	58.	Piperitone	-	1733	t
22.	<i>m</i> -Cymene	1023	-	t	59.	2 <i>E</i> -Decenal	1265	1635	t
23.	<i>p</i>-Cymene^c	1027	1269	5.2 ± 0.06	60.	<i>n</i> -Decanol	1276	1755	t
24.	Limonene ^c	1031	1197	0.3 ± 0.12	61.	Bornyl acetate	1290	1585	0.1 ± 0.04
25.	β -Phellandrene	-	1203	0.1 ± 0.02	62.	Thymol^c	1293	2190	66.0 ± 0.36
26.	1,8-Cineole ^c	1036	1213	0.2 ± 0.04	63.	Carvacrol ^c	1303	2219	0.4 ± 0.03
27.	<i>cis</i> - β -Ocimene	1040	1237	t	64.	<i>n</i> -Undecanal	1308	-	t
28.	<i>trans</i> - β -Ocimene	1050	1252	t	65.	<i>cis</i> -Pinocarvyl acetate	1310	1647	t
29.	γ-Terpinene^c	1061	1245	14.0 ± 0.18	66.	2-Methyl naphthalene	1314	-	t
30.	<i>cis</i> -Sabinene hydrate	1069	1471	0.1 ± 0.02	67.	Myrtenyl acetate	1325	-	t
31.	<i>p</i> -Cymenene	-	1437	t	68.	Piperitenone	1346	-	t
32.	α -Terpinolene ^c	1090	1282	0.1 ± 0.02	69.	Thymol acetate	1358	1854	0.1 ± 0.04
33.	<i>trans</i> -Sabinene hydrate	-	1555	t	70.	Eugenol ^c	1362	-	t
34.	Linalool ^c	1101	1552	0.2 ± 0.00	71.	α -Copaene	1382	1494	0.1 ± 0.02
35.	Nonanal ^c	1106	1395	t	72.	β -Patchoulene	1389	1486	t
36.	β -Thujone	1119	1442	0.1 ± 0.01	73.	β -Bourbonene	1392	1522	t
37.	<i>cis-p</i> -Mentha-2-en-1-ol	1124	-	0.1 ± 0.00	74.	<i>cis</i> -Jasmone	1401	1951	t

75.	Dodecanal	1408	-	0.1 ± 0.01	100.	α -Cadinol	1663	2240	0.1 ± 0.04
76.	<i>cis</i> - α -Bergamotene	1420	1569	t	101.	Patchouli alcohol	1671	2190	t
77.	β-Caryophyllene^c	1428	1600	3.0 ± 0.03	102.	β -Bisabolol ^c	1678	2146	0.1 ± 0.07
78.	β -Copaene	1434	-	t	103.	1-Tetradecanol	1679	2151	0.1 ± 0.07
79.	α -Humulene	1462	1673	0.3 ± 0.02	104.	Eudesma-4 (15),7-dien-1- β -ol	1683	2385	t
80.	γ -Murolene	1483	1692	t	105.	α -Bisabolol ^c	1694	-	t
81.	Germacrene-D	1489	1712	t	106.	Geranyl tiglate	1701	2098	t
82.	β-Selinene	1494	1723	1.0 ± 0.00	107.	(<i>Z,E</i>)-Farnesol	1730	2365	t
83.	α -Selinene	1502	1728	0.2 ± 0.03	108.	Aristolone	1788	2278	t
84.	α -Murolene	1506	-	t	109.	Eudesm-7(11)-en-4-ol, acetate	1849	-	t
85.	γ -Cadinene	1521	1762	t	110.	(<i>5E,9Z</i>)-Farnesyl acetone	1891	-	t
86.	<i>trans</i> -Calamenene	-	1835	t	111.	2-Heptadecanone	1911		t
87.	δ -Cadinene	1530	1761	0.1 ± 0.07	112.	Methyl hexadecanoate	1924	2207	t
88.	<i>cis</i> -Sesquisabinene hydrate	1540	2078	t	113.	<i>n</i> -Hexadecyl acetate	2007	2306	t
89.	Elemol	1556	2087	t	114.	13- <i>epi</i> -Manool oxide	2010	-	t
90.	Germacrene-B	1561	-	t	115.	Manool	2067	2669	t
91.	Spathulenol ^c	1586	2131	t	116.	1-Octadecanol	2084	2593	t
92.	Caryophyllene oxide	1592	1990	0.7 ± 0.04	117.	Oleic acid	2132	-	t
93.	Viridiflorol	1601	2093	t	118.	Octadecanoic acid	2177	-	0.1 ± 0.02
94.	Humulene epoxide II	1619	2047	0.1 ± 0.04	119.	<i>n</i> -Docosane	2200	2200	t
95.	1,10-Diepicubenol	1623	2066	t	120.	<i>n</i> -Tricosane	2300	2300	t
96.	<i>epoxyallo</i> -Aromadendrene	1641	-	t	121.	3 α -acetoxymanol	2375	-	0.1 ± 0.00
97.	α -Murolol	1646	-	t	122.	<i>n</i> -Tetracosane	2400	2400	t
98.	τ -Cadinol	1649	2181	0.3 ± 0.03	123.	<i>n</i> -Pentacosane	2500	2500	t
99.	α -Eudesmol	-	2231	t	124.	<i>n</i> -Hexacosane	2600	2600	t

Monoterpene hydrocarbons									22.5
Oxygenated monoterpenes									68.2
Sesquiterpene hydrocarbons									4.7
Oxygenated sesquiterpenes									1.3
Oxygenated aliphatic hydrocarbons									1.7
Diterpenes									0.1
Total identified									98.5
Oil yield (% , v/w-fresh weight basis)									1.3

*=Compounds are listed in their order of elution from HP-5 MS column; LRI^a=Determined linear retention index against mixture of *n*-alkanes (C8-C31) on HP-5 MS column; LRI^b=Determined linear retention index against mixture of *n*-alkanes (C8-C31) on DB-wax column; ^b=Mean percentage calculated from flame ionization detector (FID) data and compounds higher than 1.0% are highlighted in boldface and their $\pm$ SD (n=2) are mentioned; ^c=Identification were confirmed by co-injection/comparison with the LRI and mass spectra of standards; t=Trace (<0.05%).

Table S2. Presence of thymol in *Plectranthus* species reported from various parts of the world.

Species/Country	Major compounds (%)	References
<i>P. cylindraceus</i>		
Yemen	Thymol (68.5), α -terpinolene (5.3), β -selinene (4.7), β -caryophyllene (4.0), <i>p</i> -cymene (2.9), δ -cadinol (2.1).	Ali et al. 2012
<i>P. tenuiflorus</i>		
Saudi Arabia	Thymol (85.3).	Smith et al. 1996
<i>P. amboinicus</i>		
Egypt	Carvacrol (15.9), thymol (12.7).	Khalid et al. 2014
India	Thymol (18.0), carvacrol (14.0), <i>p</i> -cymene (10.0).	Manjamalai et al. 2012
Brazil	Thymol (64.3), <i>p</i> -cymene (10.3), γ -terpinene (9.9), β -caryophyllene (2.8).	Da Costa et al. 2010
India	Carvacrol (28.7), thymol (21.7), α -humulene (9.7), undecanal (8.3), γ -terpinene (7.8), <i>p</i> -cymene (6.5), caryophyllene oxide (5.9), α -terpineol (3.3), β -selinene (2.0).	Senthilkumar & Venkatesalu 2010
<i>P. marrubatus</i>		
Ethiopia	Thymol (51.2), <i>p</i> -cymene (16.6), γ -terpinene (15.7), β -caryophyllene (2.9), α -terpinene (2.0).	Asres et al. 2012
<i>P. ornatus</i>		
Brazil	β -Caryophyllene (9.6-62.4), eugenol (38.0), thymol (14.1).	de Albuquerque et al. 2007
<i>P. melissoides</i>		
Brazil	Carvacrol (41.3), <i>p</i> -cymene (17.4), γ -terpinene (10.1), thymol (7.9), carvacrol acetate (4.6), methyl thymol (3.0),	Mallavarapu et al. 2005

Figure S1. GC–FID chromatogram of aerial parts essential oils of *P. asirensis* on HP-5MS column. Numbering of identified peaks is given according to the serial number of compounds in Table S1.

Supplementary Materials

Experimental

1. Plant collection and identification

Aerial parts of *P. asirensis* were collected during the flowering stage in the month of March 2012 from Abha, southern part of Saudi Arabia. The identification of the plant species was confirmed by a botanical taxonomist from the Herbarium Division, College of Science, King Saud University, Riyadh, KSA. A voucher specimen of the plant material is maintained in our laboratory.

2. Isolation of the essential oil

Freshly collected aerial parts of *P. asirensis* was sliced into small pieces. The sliced fresh aerial parts of *P. asirensis* (250.0 g) were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus according to the European

Pharmacopoeia method (European Pharmacopoeia 1996) to give colorless oil. The essential oil obtained after the hydro-distillation was dried over anhydrous sodium sulfate and stored at 4°C until further use. The yield of the volatile oil derived from *P. asirensis* was 1.3% (v/w) on the fresh weight basis.

3. Chemicals

Analytical-grade acetone (Sigma-Aldrich, Germany) was used for the dilution of oil sample. Pure and enriched fractions of volatile compounds such as thymol, β -caryophyllene, α -terpinene, α -pinene, β -pinene, camphene, 1,8-cineole, α -terpinolene, linalool, nonanal, camphor, borneol, limonene, terpinene-4-ol, α -terpineol, eugenol, and α -bisabolol alongwith others were available in our laboratory and used for comparison/co-injection analysis.

4. Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Essential Oils

The essential oil was analyzed using a GC–MS and GC–FID equipped with two columns, one of which was polar (DB-Wax), and the other was nonpolar (HP-5MS). GC–MS was performed on an Agilent single-quadrupole mass spectrometer with an inert mass selective detector (MSD-5975C detector, Agilent Technologies, USA) coupled directly to an Agilent 7890A gas chromatograph which was equipped with a split–splitless injector, a quickswap assembly, an Agilent model 7693 autosampler and a HP-5MS fused silica capillary column (5% phenyl 95% dimethylpolysiloxane, 30 m $\times$ 0.25 mm i.d., film thickness 0.25 μ m, Agilent Technologies, USA). Supplementary analyses were performed on a DB-Wax fused silica capillary column (polyethylene glycol, 30 m $\times$ 0.25 mm i.d., film thickness 0.25 μ m, Agilent Technologies, USA). The HP-5MS column was operated using an injector temperature of 250°C and the following oven temperature profile: an isothermal hold at 50°C for 4 min, followed by a ramp of 4°C/min to 220°C, an isothermal hold for 2 min, a second ramp to 280°C at 20°C/min and finally an isothermal hold for 15 min. Conversely, the DB-Wax column was operated using an injector temperature of 250°C and the following oven temperature profile: an isothermal hold at 40°C for 4 min, followed by a ramp of 4°C/min to 220°C and an isothermal hold for 10 min.

Approximately 0.2 μ l of oil sample diluted in acetone (5% solution in acetone) was injected using the split injection mode; the split flow ratio was 10:1. The helium carrier gas was flowed at 1 ml/min. The GC–TIC profiles and mass spectra were obtained using the ChemStation data analysis software, version E-02.00.493 (Agilent). All mass spectra were acquired in the EI mode (scan range of m/z 45–600 and ionization energy of 70 eV). The temperatures of the electronic-impact ion source and the MS quadrupole were 230°C and 150°C, respectively. The MSD transfer line was maintained at 280°C for both polar and nonpolar analysis. The GC analysis was performed on an Agilent GC-7890A dual-channel gas chromatograph (Agilent Technologies, USA) equipped with FID using both polar (DB-Wax) and nonpolar (HP-5MS) columns under the same conditions as described above. The detector temperature was maintained at 300°C for both polar and nonpolar analyses. The relative composition of the oil components was calculated on the basis of the GC–FID peak areas measured using the HP-5 MS column without using correction factor. Results are reported in Table S1 according to their elution order on the HP-5MS column.

5. Retention indices

A mixture of a continuous series of straight-chain hydrocarbons, C8-C31 (C8-C20, 04070, Sigma-Aldrich, USA and C20-C31, S23747, AccuStandard, USA) was injected into both polar (DB-Wax) and nonpolar (HP-5MS) columns under the same conditions previously described for the oil samples to obtain the linear retention indices (LRIs) (also referred to as linear temperature programmed retention indices [LTPRI]) of the oil constituents provided in Table S1. The LRIs were computed using van den Dool and Kratz's equation (Dool & Kratz 1963).

6. Identification of volatile components

GC–FID chromatogram of aerial parts essential oils of *P. asirensis* with identified peaks of major components on HP-5MS column is shown in Figure S1. The identification of components was done by matching their mass spectra with the library entries (WILEY 9th edition, NIST-08 MS library version 2.0 f as well as the Adams and Flavor libraries) of a mass spectra database as well as by comparing their mass spectra and linear retention indices (LRI) with published data obtained using both polar and nonpolar columns (Davis 1990; Adams 2007; Babushok et al. 2011; NIST 2015; El-Sayed 2015; Acree 2015) and the co-injection of authentic standards available in our laboratory.

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