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 ± 0.36%), \(\gamma\)-terpinene (14.0 ± 0.18%), \(p\)-cymene (5.2 ± 0.06%) and \(\beta\)-caryophyllene (3.0 ± 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; \(\gamma\)-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<sup>a</sup> | LRI<sup>b</sup> | (%)<sup>b</sup> | No. | Compounds* | LRI<sup>a</sup> | LRI<sup>b</sup> | (%)<sup>b</sup> |
|-----|------------|----------------|----------------|-------------|-----|------------|----------------|----------------|-------------|
| 1.  | Diacetone alcohol | 841 | 1364 | t | 38. | *cis*-p-Mentha-2,8-dien-1-ol | 1138 | - | t |
| 2.  | *trans*-3-Hexen-1-ol | 850 | - | t | 39. | *trans*-p-Mentha-2-en-1-ol | 1143 | 1592 | t |
| 3.  | *trans*-2-Hexenal<sup>c</sup> | 852 | 1217 | t | 40. | trans-Verbenol | 1146 | 1686 | t |
| 4.  | *cis*-3-Hexen-1-ol<sup>c</sup> | 854 | 1389 | 0.1 ± 0.01 | 41. | Camphor<sup>c</sup> | 1148 | 1519 | t |
| 5.  | 1-Hexanol<sup>c</sup> | 868 | 1359 | t | 42. | Camphene hydrate | 1152 | - | t |
| 6.  | 2-Methyl butyl acetate | 880 | 1122 | t | 43. | *iso*-Borneol | 1162 | 1667 | t |
| 7.  | Methyl hexanoate | - | 1188 | t | 44. | Pinocarvone | 1164 | - | t |
| 8.  | *α*-Thujene | 928 | 1024 | 0.5 ± 0.02 | 45. | Borneol<sup>c</sup> | 1169 | 1709 | 0.1 ± 0.00 |
| 9.  | *α*-Pinene<sup>c</sup> | 935 | 1018 | 0.2 ± 0.01 | 46. | *δ*-Terpineol | 1170 | 1679 | t |
| 10. | Camphene<sup>c</sup> | 950 | 1060 | t | 47. | 1-Nonanol | 1174 | - | t |
| 11. | *α*-Fenchene | - | 1053 | t | 48. | Terpinen-4-ol<sup>c</sup> | 1180 | 1608 | 0.7 ± 0.01 |
| 12. | Sabinene | 975 | 1118 | t | 49. | *p*-Cymene-8-ol | 1188 | - | t |
| 13. | *β*-Pinene<sup>c</sup> | 978 | 1105 | t | 50. | *α*-Terpineol<sup>c</sup> | 1194 | 1704 | t |
| 14. | 1-Octen-3-ol<sup>c</sup> | 980 | 1456 | 1.2 ± 0.05 | 51. | Dihydrocarveol | 1198 | - | t |
| 15. | 3-Octanone | 988 | 1255 | t | 52. | *trans*-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. | iso-Dihydrocarveol | - | 1788 | t |
| 18. | 3-Octanol<sup>c</sup> | 997 | 1399 | 0.1 ± 0.01 | 55. | *trans*-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*-Terpineinc<sup>c</sup> | 1018 | 1177 | 1.3 ± 0.02 | 58. | Piperitone | - | 1733 | t |
| 22. | *m*-Cymene | 1023 | - | t | 59. | *2E*-Decenal | 1265 | 1635 | t |
| 23. | *p*-Cymene<sup>c</sup> | 1027 | 1269 | 5.2 ± 0.06 | 60. | *n*-Decanol | 1276 | 1755 | t |
| 24. | Limonene<sup>c</sup> | 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<sup>c</sup> | **1293** | **2190** | **66.0 ± 0.36** |
| 26. | 1,8-Cineole<sup>c</sup> | 1036 | 1213 | 0.2 ± 0.04 | 63. | Carvacrol<sup>c</sup> | 1303 | 2219 | 0.4 ± 0.03 |
| 27. | *cis*-β-Ocimene | 1040 | 1237 | t | 64. | *n*-Undecanal | 1308 | - | t |
| 28. | *trans*-β-Ocimene | 1050 | 1252 | t | 65. | *cis*-Pinocarvyl acetate | 1310 | 1647 | t |
| 29. | *γ*-Terpineinc<sup>c</sup> | 1061 | 1245 | 14.0 ± 0.18 | 66. | 2-Methyl naphthalene | 1314 | - | t |
| 30. | *cis*-Sabinene hydrate | 1069 | 1471 | 0.1 ± 0.02 | 67. | Myrtenyl acetate | 1325 | - | t |
| 31. | *p*-Cymenene | - | 1437 | t | 68. | Piperitenone | 1346 | - | t |
| 32. | *α*-Terpinolene<sup>c</sup> | 1090 | 1282 | 0.1 ± 0.02 | 69. | Thymol acetate | 1358 | 1854 | 0.1 ± 0.04 |
| 33. | *trans*-Sabinene hydrate | - | 1555 | t | 70. | Eugenol<sup>c</sup> | 1362 | - | t |
| 34. | Linalool<sup>c</sup> | 1101 | 1552 | 0.2 ± 0.00 | 71. | *α*- Copaene | 1382 | 1494 | 0.1 ± 0.02 |
| 35. | Nonanal<sup>c</sup> | 1106 | 1395 | t | 72. | *β*-Patchoulenone | 1389 | 1486 | t |
| 36. | *β*-Thujone | 1119 | 1442 | 0.1 ± 0.01 | 73. | *β*-Bourbonene | 1392 | 1522 | t |
| 37. | *cis*-p-Mentha-2-en-1-ol | 1124 | - | 0.1 ± 0.00 | 74. | *cis*-Jasmone | 1401 | 1951 | t |
| No. | Compound                        | Retention Index | ± SD | n (%) | Retention Index | ± SD | n (%) |
|-----|---------------------------------|-----------------|------|-------|-----------------|------|-------|
| 75. | Dodecanal                       | 1408            | -    | 0.1 ± 0.01 | 100. α-Cadinol | 1663 | 2240 | 0.1 ± 0.04 |
| 76. | cis-α-Bergamotene               | 1420            | 1569 | t     | 101. Patchouli alcohol | 1671 | 2190 | t |
| 77. | β-Caryophyllene                 | 1428            | 1600 | 3.0 ± 0.03 | 102. β-Bisabolol | 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 | 1694 | -    | t |
| 81. | Germacrene-D                    | 1489            | 1712 | t     | 106. Geranyl tiglate | 1701 | 2098 | t |
| 82. | β-Selinene                      | 1494            | 1723 | 1.0 ± 0.00 | 107. (Z,E)-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. (5E,9Z)-Farnesyl acetone | 1891 | -    | t |
| 86. | trans-Calamenene                | -               | 1835 | t     | 111. 2-Heptadecanone | 1911 | -    | t |
| 87. | δ-Cadinene                      | 1530            | 1761 | 0.1 ± 0.07 | 112. Methyl hexadecanoate | 1924 | 2207 | t |
| 88. | cis-Sesquisabinene hydrate      | 1540            | 2078 | t     | 113. n-Hexadecyl acetate | 2007 | 2306 | t |
| 89. | Elemol                          | 1556            | 2087 | t     | 114. 13-epi-Manool oxide | 2010 | -    | t |
| 90. | Germacrene-B                    | 1561            | -    | t     | 115. Manool | 2067 | 2669 | t |
| 91. | Spathulenol                     | 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. n-Docosane | 2200 | 2200 | t |
| 95. | 1,10-Diepicubenol               | 1623            | 2066 | t     | 120. n-Tricosane | 2300 | 2300 | t |
| 96. | epoxyallo-Aromadendrene         | 1641            | -    | t     | 121. 3α-acetoxy manool | 2375 | -    | 0.1 ± 0.00 |
| 97. | α-Murolol                       | 1646            | -    | t     | 122. n-Tetracosane | 2400 | 2400 | t |
| 98. | δ-Cadinol                      | 1649            | 2181 | 0.3 ± 0.03 | 123. n-Pentacosane | 2500 | 2500 | t |
| 99. | α-Eudesmol                     | -               | 2231 | t     | 124. n-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

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*aCompounds are listed in their order of elution from HP-5 MS column; LRI=Determined linear retention index against mixture of n-alkanes (C8-C31) on HP-5 MS column; LRId=Determined linear retention index against mixture of n-alkanes (C8-C31) on DB-wax column; *aMean percentage calculated from flame ionization detector (FID) data and compounds higher than 1.0% are highlighted in boldface and their ± SD (n=2) are mentioned; *a*Identification were confirmed by co-injection/comparison with the LRI and mass spectra of standards; t=Trace (<0.05%).
| Species/Country | Major compounds (%) | References |
|-----------------|----------------------|------------|
| **P. cylindraceus** |                      |            |
| Yemen           | Thymol (68.5), α-terpinolene (5.3), β-selinene (4.7), β-caryophyllene (4.0), p-cymene (2.9), δ-cadinol (2.1). | Ali et al. 2012 |
| **P. tenuiflorus** |                      |            |
| Saudi Arabia    | Thymol (85.3).       | Smith et al. 1996 |
| **P. amboinicus** |                      |            |
| Egypt           | Carvacrol (15.9), thymol (12.7). | Khalid et al. 2014 |
| India           | Thymol (18.0), carvacrol (14.0), p-cymene (10.0). | Manjamalai et al. 2012 |
| Brazil          | Thymol (64.3), p-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), p-cymene (6.5), caryophyllene oxide (5.9), α-terpineol (3.3), β-selinene (2.0). | Senthilkumar & Venkatesalu 2010 |
| **P. marrubatus** |                      |            |
| Ethiopia        | Thymol (51.2), p-cymene (16.6), γ-terpinene (15.7), β-caryophyllene (2.9), α-terpinene (2.0). | Asres et al. 2012 |
| **P. ornatus**  |                      |            |
| Brazil          | β-Caryophyllene (9.6-62.4), eugenol (38.0), thymol (14.1). | de Albuquerque et al. 2007 |
| **P. melissoides** |                      |            |
| Brazil          | Carvacrol (41.3), p-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 along with 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 × 0.25 mm i.d., film thickness 0.25 μm, Agilent Technologies, USA). Supplementary
analyses were performed on a DB-Wax fused silica capillary column (polyethylene glycol, 30 m × 0.25 mm i.d., film
thickness 0.25 μm, 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 μl 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.

6
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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