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# VOLATILE COMPOUNDS FROM *Haplophyllum myrtifolium*

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*The volatile fractions of the petroleum ether extract and of the alkaloid extract of* Haplophyllum myrtifolium *Boiss., endemic to Turkey, were trapped by using the Solid Phase Micro Extraction (SPME) technique and analyzed by GC/MS. Forty-seven compounds were characterized from the petroleum ether extract of the aerial parts with β-caryophyllene (14.6 %), decane (11.4 %), and β-phellandrene (7.0 %) as the major components. Chavibetol (21.9 %), eugenol (19.1 %), methyleugenol (10.8 %), trans-linalool oxide (7.1 %) and β-cyclocitral (6.0 %) were identified as the major components among the forty-two compounds characterized in the chloroform eluate of the alkaloid extract.*

**Key words**: *Haplophyllum myrtifolium*; *Rutaceae*; β-caryophyllene, chavibetol, GC/MS, Solid Phase Micro Extraction (SPME).

*Haplophyllum* (*Rutaceae*) species are a rich source of quinoline alkaloids and lignans [1–5]. The compounds described from the representatives of this genus possess a broad spectrum of pharmacological action [6–8]. *Haplophyllum* species are used in Sudanese and Mongolian folk medicine as an antipyretic and in the treatment of diarrhea and some forms of tumors [9–11]. All this indicates the promising properties of *Haplophyllum* species. *Haplophyllum myrtifolium* Boiss., a plant native to Turkey, was previously shown to contain lignans and quinoline alkaloids [12, 13]. In the present study on *H. myrtifolium*, the volatile fraction of the petroleum ether extract and another fraction obtained during column chromatographic separation of the total alkaloid extract were analyzed for the first time.

Forty-seven compounds were characterized from the petroleum ether extract, with β-caryophyllene (14.6%), decane (11.4%), and β-phellandrene (7.0%), as the main components. Chavibetol (21.9%), eugenol (19.1%), methyleugenol (10.8%), *trans*-linalool oxide (7.1%) and β-cyclocitral (6.0 %) were identified as the major components among the forty-two compounds characterized in the volatile fraction of the alkaloid extract. The compositions of the volatile compounds present in both extracts are shown in Table 1. Alkanes were used as reference points in the calculation of relative retention indices (RRI). The library search was carried out using the Wiley GC/MS Library and the TBAM Library of Essential Oil Constituents.

## EXPERIMENTAL

**Plant Material and Extraction**. The petroleum ether extract was obtained by percolation of the air-dried aerial parts of *H. myrtifolium* (720 g), collected during the flowering period from Honaz, Denizli, in June 2000. A voucher specimen, No 1250, is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University. The volatile fraction of the petroleum ether extract was trapped using Solid Phase Micro Extraction (SPME) [14] and analyzed by GC/MS. Moreover, a volatile fraction eluted by chloroform during the column chromatography of the alkaloid extract of the plant, collected in June 1994, was trapped using SPME and analyzed by GC/MS.

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TABLE 1. Compositions of the Volatile Fractions of the Petroleum Ether Extract and of the Alkaloid Extract of *Haplophyllum myrtifolium*, **%**

| RRI | Compound | A | B | RRI | Compound | A | B |
|---|---|---|---|---|---|---|---|
| 1000 | Decane | - | 11.4 | 1562 | Octanol | - | 0.1 |
| 1032 | $\alpha$-Pinene | - | 1.9 | 1573 | (E,E)-3,5-Octadien-2-one | 0.3 | - |
| 1076 | Camphene | - | 1.0 | 1588 | Bornylformate | - | 0.7 |
| 1118 | $\beta$-Pinene | - | 1.0 | 1597 | Bornylacetate | - | 2.5 |
| 1132 | Sabinene | - | 0.4 | 1602 | 6-Methyl-3,5-heptadien-2-one | 0.4 | - |
| 1159 | $\delta$-3-Carene | - | 0.7 | 1611 | Terpinen-4-ol | 0.3 | - |
| 1174 | Myrcene | - | 2.0 | 1612 | $\beta$-Caryophyllene | - | 14.6 |
| 1176 | $\alpha$-Phellandrene | - | 2.0 | 1638 | $\beta$-Cyclocitral | 6.0 | - |
| 1200 | Dodecane | - | 0.5 | 1658 | (Z)-Ocimenol | 0.8 | - |
| 1203 | Limonene | - | 1.8 | 1684 | Ethylbenzoate | 1.6 | - |
| 1218 | $\beta$-Phellandrene | - | 7.0 | 1687 | $\alpha$-Humulene | - | 1.0 |
| 1246 | (Z)-$\beta$-Ocimene | - | 0.9 | 1690 | 4-Methyl-4-vinylbutyrolactone | 0.3 | - |
| 1248 | Ethylhexanoate | - | 0.8 | 1706 | $\alpha$-Terpineol | 0.9 | 0.6 |
| 1255 | $\gamma$-Terpinene | - | 0.3 | 1726 | Germacrene-D | - | 0.4 |
| 1266 | (E)-$\beta$-Ocimene | - | 1.4 | 1769 | Unknown 3 | 1.2 | - |
| 1280 | *p*-Cymene | - | 1.0 | 1797 | *p*-Methylacetophenone | 0.3 | - |
| 1285 | Isoamylisovalerate | - | 0.7 | 1800 | Ethylphenylacetate | 1.5 | - |
| 1290 | Terpinolene | - | 0.2 | 1834 | Ethylsalicylate | 0.4 | - |
| 1300 | Tridecane | 0.1 | 0.3 | 1838 | $\beta$-Damascenone | 1.4 | - |
| 1327 | (Z)-3-Hexenylacetate | - | 0.4 | 1854 | Germacrene-B | - | 0.5 |
| 1330 | Unknown 1 | 2.4 | - | 1864 | *p*-Cymen-8-ol | 0.1 | - |
| 1345 | Ethylheptanoate | - | 0.3 | 1868 | (E)-Geranylacetone | 0.1 | - |
| 1348 | 6-Methyl-5-hepten-2-one | - | 0.8 | 1870 | Hexanoic acid | - | 0.2 |
| 1360 | Hexanol | - | 0.3 | 1878 | Guaiacol | 0.3 | - |
| 1365 | Unknown 2 | 1.7 | - | 1889 | Unknown 4 | 1.0 | - |
| 1391 | (Z)-3-Hexenol | - | 1.0 | 1896 | Benzyl alcohol | - | 0.2 |
| 1400 | Tetradecane | 0.2 | - | 1900 | 1,10-Oxidocalamene | 0.1 | - |
| 1400 | Nonanal | - | 0.6 | 1925 | 2,3,4-Trimethylbenzaldehyde | 0.1 | - |
| 1418 | (Z)-2-Hexen-1-ol | - | 0.8 | 1937 | Phenethyl alcohol | - | 0.4 |
| 1444 | Ethyloctanoate | - | 0.5 | 1984 | Benzothiazole | Tr. | - |
| 1450 | *trans*-Linalool oxide (Furanoid) | 7.1 | - | 1988 | 2-Phenylethyl-2-phenylbutyrate | 0.1 | - |
| 1474 | *trans*-Sabinene hydrate | - | 0.2 | 2008 | Caryophyllene oxide | - | 0.3 |
| 1478 | *cis*-Linalool oxide (Furanoid) | 2.2 | - | 2012 | (Z)-Ethylcinnamate | Tr. | - |
| 1480 | Nerol oxide | 3.0 | - | 2030 | Methyleugenol | 10.8 | 0.8 |
| 1482 | (Z)-3-Hexenyl-2-methylbutyrate | - | 0.2 | 2053 | Anisaldehyde | 0.1 | - |
| 1484 | Nerylethyl ether | 0.1 | - | 2104 | Dimethylanthranilate | 0.1 | - |
| 1492 | Cyclosativene | - | 0.5 | 2144 | Unknown 5 | 1.5 | - |
| 1497 | $\alpha$-Copaene | - | 1.5 | 2157 | (E)-Ethylcinnamate | 0.4 | - |
| 1503 | 2-Methoxy-3-sec-butylpyrazine | - | 0.1 | 2179 | 3,4-Dimethyl-5-pentylidene-2(5H)-furanone | 0.5 | - |
| 1519 | Geranylethyl ether | 0.7 | - | 2186 | Eugenol (2-Methoxy-4-(2-propenyl)phenol) | 19.1 | - |
| 1529 | Dill ether | 0.1 | - | 2217 | Chavibetol (2-Methoxy-5-(2-propenyl)phenol) | 21.9 | - |
| 1535 | $\beta$-Bourbonene | - | 0.3 | 2269 | Heliotropine (Piperonal) | 0.4 | - |
| 1553 | Linalool | 1.4 | 3.9 | | **Total** | **83.2** | **69.0** |

A: The alkaloid extract. B: The petroleum ether extract. RRI: Relative Retention Indices. Tr: Trace ( 0.1%).

Unknown 1 (RRI: 1330): EIMS, 70 eV, *m/z* ($I_{rel}$.,%) 183 (0.5), 171 (0.3), 152 (0.5), 137 (0.8), 125 (0.8), 111 (2.6), 100 (1.9), 87 (100), 81 (4.6), 67 (6.1), 59 (91.2). Unknown 2 (RRI: 1365): EIMS, 70 eV, *m/z* ($I_{rel}$.,%) 183 (1.1), 153 (0.4), 137 (1.1), 125 (0.6), 111 (2.4), 100 (1.9), 93 (5.8), 87 (100), 67 (6.9), 59 (90.2). Unknown 3 (RRI: 1769): EIMS, 70 eV, *m/z* ($I_{rel}$.,%) 172 (25.7), 158 (12.5), 157 (100), 142 (47.8), 128 (10.2), 127 (3.8), 115 (12.7), 77 (8.5), 69 (6.2). Unknown 4 (RRI: 1889): EIMS, 70 eV, *m/z* ($I_{rel}$.,%) 152 (38.7), 137 (10.9), 123 (1.0), 109 (5.9), 95 (17.9), 79 (6.9), 67 (12), 57 (100). Unknown 5 (RRI: 2144): EIMS, 70 eV, *m/z* ($I_{rel}$.,%) 188 (2.3), 174 (12.6), 173 (100), 158 (6.7), 144 (8.1), 129 (21.1), 115 (11.8), 105 (3.9), 91 (4.9), 77 (5.5), 65 (2.9).

The **conditions of the GC/MS analyses** are shown below.
System: Hewlett-Packard G1800A GCD
Column: Innowax FSC column (60 × 0.25mm ⌀, with 0.25 mm film thickness)
Carrier Gas: Helium (1 ml/min)
**GC Temperatures**
Injection: 250°C
Column: 60°C for 10 min, 4°C /min to 220°C; 220°C for 10 min, 1°C /min to 240°C (Total 80 min)
Mass range: *m/z* 35–425
Electron energy: 70 eV

**Headspace-SPME**. SPME fibre (Supelco) precoated with a 100 mm layer of polydimethysiloxane (PDMS-red) was used. The coated fibre is accommodated in a hollow, stainless steel needle which allows easy injection. The vial containing the plant extract was sealed with parafilm. The fibre was pushed through the plastic film for exposure to the headspace of the extract for 15 min at 50°C. The fibre was then inserted into the injection port of the GC/MS for the desorption of the adsorbed volatile compounds for analysis.

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