

Essential Oil composition from the aerial parts of Haplophyllum linifolium (L.) G. Don fil.

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Resumen

IÑIGO, A., PALÁ-PAÚL, J., PÉREZ-ALONSO, M.J. & VELASCO-NEGUERUELA, A. 2002. Composición química del aceite esencial de *Haplophyllum linifolium* (L.) G. Don fil. *Bot. Complutensis* 26: 79-83.

El aceite esencial extraído de las partes aéreas de *Haplophyllum linifolium* (L.) G. Don fil., una rutácea endémica de la Península Ibérica ha sido analizado cuantitativa y cualitativamente mediante CG capilar y CG/EM respectivamente. Se han identificado 61 compuestos que corresponden a un 95,5% del total del aceite esencial. Los componentes mayoritarios identificados en esta especie fueron: germacreno D (36,2%), biciclogermacreno (9,0%) y β -cariofileno (7,5%).

Palabras clave: *Haplophyllum linifolium* (L.) G. Don fil., Rutaceae, Aceite esencial, germacreno D, biciclogermacreno, beta-cariofileno.

Abstract

IÑIGO, A., PALÁ-PAÚL, J., PÉREZ-ALONSO, M.J. & VELASCO-NEGUERUELA, A. 2002. Essential Oil composition from the aerial parts of *Haplophyllum linifolium* (L.) G. Don fil. *Bot. Complutensis* 26: 79-83.

The volatiles isolated from the aerial parts of *Haplophyllum linifolium* (L.) G. Don fil., were studied by means of GC and GC/MS. Sixty-four components were characterized, amounting 95,5% of the oil. The major constituents found were germacrene D (36,2%), bicyclogermacrene (9.0%) and β -caryophyllene (7,5%).

Key Word: *Haplophyllum linifolium* (L.) G. Don fil., Rutaceae, essential oil composition, germacrene D, bicyclogermacrene, β -caryophyllene.

INTRODUCTION

The genus *Haplophyllum* A. Juss., belongs to the Rutaceae family with 8 species represented in Europe and *Haplophyllum linifolium* being endemic to Spain

(synonyms = *Haplophyllum hispanicum* (L.) Spach = *Ruta linifolia* L.) (Tutin et al., 1968).

The volatiles of *Haplophyllum* species have been the subject of three previous investigations. The oil of *Haplophyllum tuberculatum* (Forssk.) A. Juss., from Iran (Yari et al., 2000) was found to contain limonene (27,3%) and α -pinene (21,9 %) as major constituent. In other oil of this species (Brunke et al., 1991) the major components found was δ -3-carene (48,0%). Recently Kubeczka et al. (Kubeczka et al., 1995) in an oil of *Haplophyllum linifolium* gathered in Alicante (south east of Spain) found monoterpene hydrocarbons as main components of the fruit and aerial parts. The major constituents found were respectively α -pinene (8,8-7,6%), β -pinene (23,7-19,8%), sabinene (8,6-3,5%) and β -phellandrene (25,5-42,6%)

MATERIAL AND METHODS

Plant material

Plant material was collected at Vaciamadrid (near Madrid, Spain) on gypsum soil, June 2000. A voucher specimen, n.º 75500 MACB, has been deposited at the Herbarium of the Biology Faculty, Complutense University of Madrid, Madrid, Spain.

The aerial parts of the plant were subjected to steam distillation for 8hr using a Clevenger type apparatus according to the method recommended by the Spanish pharmacopeia, 1977. The oil was dried over anhydrous magnesium sulphate and stored at 4 °C in the dark. The yield was 0,14 ml/g based on dry weight of sample.

Gas Chromatography (GC)

Analytical GC was carried out on a Varian 3300 gas chromatograph fitted with a methyl silicone DB-1 capillary column (30 m, \times 0,25 mm i.d., 0,25 μ m film thickness). Carrier gas N₂, flow rate 1.5 mL/min. Split mode. Temperature was programmed from 95°-240 °C at 4 °C/min. Injector temperature 250 °C. Injection volume for the sample was 0,1 μ l. Detection was performed by FID.

Gas Chromatography - Mass Spectrometry (GC/MS)

Analysis was carried out in a Fisons 8000 gas chromatograph fitted with a methyl silicone SE-30 column (50 m \times 0,25 mm i.d., 0,25 μ m film thickness), coupled to a Fisons MD 800 detector. Column temperature was programmed from 70 °C to 240 °C at 4 °C/min. Injection was performed at 250 °C. Helium was used as carrier gas (0,5 ml/min.). Mass spectra were recorded in the scan mode at 70 eV (35-350 amu).

Most constituents were tentatively identified by comparison of their GC retention index with those of authentic standard available in the author's laboratory or

with GC data previously published (5,6). Identification was confirmed when possible by comparison of their mass spectra with those stored in the MS database (NIST and WILEY libraries) and with mass spectra literature data (5,7,8). Component relative concentrations were obtained directly from GC peak areas.

RESULTS AND DISCUSSION

The identified compounds of the oil, their retention indices and percentage composition are given in Table 1 where the components are listed in order to their elution on the DB-1 column. The essential oil studied was dominated by sesquiterpene hydrocarbons; germacrene D (36,2%), bicyclogermacrene (9,0%) and β -caryophyllene (7,5%) being the major ones. Other minor components were β -pinene (5,9%), bornyl acetate (3,6%), β -phellandrene (3,4%) and α -pinene (3,0%).

Our results are not in accordance with those of Kubeczka *et al.* (1995) in that sesquiterpene hydrocarbons were the major components found in the oil from *Haplophyllum linifolium* gathered in Vaciamadrid whereas in the oil of *Haplophyllum linifolium* collected in Alicante the major constituents were found to be monoterpene hydrocarbons. It is worth noting that the oil of *Haplophyllum linifolium* has been reported to be used in folk medicine in the treatment of vitiligo, leucodermia and other skin diseases.

Tabla 1
Chemical composition of the oil of *Haplophyllum linifolium* (L.) G. Don fil.

Component	RI	Percentage
Tricyclene	932	0,2
α -thujene	934	0,1
α -pinene	942	2,9
camphene	957	1,2
sabinene	974	0,5
β -pinene	984	5,4
myrcene	984	0,6
α -phellandrene	1007	T
1,4-cineole	1017	T
α -terpinene	1020	0,1
p-cymene	1025	0,2
(Z)- β -ocimene	1027	0,2
β -phellandrene	1028	3,4
(E)- β -ocimene	1037	0,9
γ -terpinene	1054	0,2
terpinolene	1086	T
linalool	1088	0,6
1,3,8-menthatriene	1107	T
borneol	1161	T
terpinen-4-ol	1173	T

Tabla 1.
Chemical composition of the oil of *Haplophyllum linifolium* (L.) G. Don fil
(continuation)

<i>Component</i>	<i>RI</i>	<i>Percentage</i>
α -terpineol	1185	0,1
geraniol	1245	T
(E)-anethole	1271	T
Bornyl acetate	1274	3,6
p-cymen-7-ol	1286	0,1
δ -elemene	1336	0,1
α -cubebene	1349	0,1
α -copaene	1378	1,0
β -bourbonene	1386	1,9
β -cubebene	1391	0,1
α -gurjunene	1414	T
β -caryophyllene	1423	7,5
epi-bicyclosesquiphellandrene	1430	0,4
β -gurjunene	1444	0,2
α -humulene	1454	0,8
allo-aromadendrene	1463	0,6
γ -muurolene	1469	0,3
germacrene D	1485	36,2
epi-cubebol	1492	0,3
bicyclogermacrene	1498	9,0
(Z)- β -guaiene	1504	0,6
γ -cadinene	1510	0,5
δ -cadinene	1517	2,5
Cadina-1,4-diene	1528	0,1
α -cadinene	1534	0,1
germacrene B	1552	0,4
(E)-nerolidol	1556	0,3
Spathulenol	1571	1,8
caryophyllene oxide	1574	0,2
Globulol	1577	0,8
β -copaen-4- α -ol	1581	0,3
1,10-di-epi-cubenol	1600	1,2
epi- α -muurolol	1632	1,8
α -muurolol	1640	0,2
α -cadinol	1644	2,1
vulgarol isomer _	1668	1,1
vulgarol B	1747	0,6
14-hydroxy- α -muurolene	1788	0,3
tetradecanoic acid	1795	0,7
14-hydroxy- δ -cadinene	1802	0,1
Hexadecanoic acid	1946	1,3

RI = Programmed temperature retention index determined using the homologous series of n-alkanes t = traces (< 0,1%).

* Tentatively identified constituents

RI = 1668 MS, 70eV m/z (rel.int.): 220 [M]⁺ (43), 91 (100), 159 (100), 109 (99), 79 (76), 41 (57), 93 (67), 202 (18), 205 (7).

RI = 1747, 220 [M]⁺ (60), 105 (100), 91 (80), 159 (72), 93 (58), 81 (56), 41 (52), 205 (16), 202 (8)

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