© 2012 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 11 (1): 86 - 90
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Identification of the components of a non polar extract of the aerial parts of *Dunalia spinosa* by GC-MS

[Identificación por CG-EM de los componentes de un extracto no polar de *Dunalia spinosa*]

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Abstract
This communication reports the chemical composition of the light petroleum ether extract from the leaves and stems of *Dunalia spinosa*. The aerial part of *D. spinosa* was extracted using light petroleum ether, which on evaporation yielded a residue. This residue was subsequently fractioned using CC chromatography on silica gel with petroleum ether, petroleum ether-CH_2Cl_2 gradient, CH_2Cl_2 and CH_2Cl_2-MeOH gradient. The fractions were analyzed using GC and GC-MS. A total of 34 compounds were identified accounting for 56.59% of the extracted mass. The unidentified compounds were isomers of branched chain alkanes and alkenes for which there were no documented compounds matching their calculated RI's. The following compounds were identified: n-Alkanes (65.88%) from C-14 to C-21 as the primary components. 1-Alkenes (1.15%): 1-tetradecene, 1-pentadecene, 1-hexadecene, 1-nonadecene. Aldehydes (1.29%): hexanal, nonanal and decanal. Monoterpenes (5.9%) sabinene, β-pinene, *p*-cymene, limonene, 1,8-cineol and 1.8-*trans*-sabinene. Sesquiterpenes (0.17%), caryophyllene oxide. Diterpenes (1.64%): Phytol. In addition, various compounds of note were identified, specifically pythone (4.98%), 7-oxabicyclo [4.1.0] heptane (5.85%) and methyl hexadecanoate (5.57%). This methodology is appropriate for the identification of complex compound mixtures in discarded fractions as in many phytochemical studies, these fractions provide a more complete profile of secondary metabolites in the studied species.

Keywords: *Dunalia spinosa;* Solanaceae; non polar extract; GC; GC-MS analisys

Resumen
En esta comunicación, se informa la composición química del extracto obtenido con éter de petróleo liviano de hojas y tallos de *Dunalia spinosa*. La parte aérea de *D. spinosa* se extrajo con éter de petróleo liviano y por evaporación del extracto se obtuvo un residuo que se fraccionó por cromatografía en columna de gel de sílice con un gradiente de éter de petróleo, éter de petróleo-CH_2Cl_2 y CH_2Cl_2-MeOH y las fracciones se analizaron por CG y CG-EM. Se identificó un total de 34 compuestos que dieron cuenta de un 56,59% de la masa del extracto, los compuestos no identificados correspondieron a isómeros de alcanos y alquenos de cadena ramificada de los cuales no se encontraron compuestos con IR coincidentes en las bases de datos. Los siguientes compuestos fueron identificados: n-Alcanos (65.88%) de C-14 a C-21 como componentes mayoritarios. 1-Alquenos (1.15%): 1-tetradeceno, 1-pentadeceno, 1-hexadeceno, 1-nonadeceno. Aldehidos (1.29%): hexanal, nonanal y decanal. Monoterpenos (5.9%): sabineno, β-pineno, *p*-cimeno, limoneno, 1.8-cineol y *trans*-sabineno. Sesquiterpenos (0.17%): oxido de cariofileno. Diterpenos (1.64%): fitol. Adicionalmente fueron identificados compuestos misceláneos, entre los que destacan, fitona (4.98%), 7-oxabiciclo [4.1.0] heptano (5.85%) y hexadecanoato de metilo (5.57%). Esta metodología es adecuada para que compuestos de fracciones que en muchos estudios fitoquímicos son desechadas, sean identificados, el estudio de estas fracciones permite completar el perfil de metabolitos secundarios de las especies estudiadas.

Palabras Claves: *Dunalia spinosa;* Solanaceae; extracto de compuestos no polares; CG-EM

Recibido | Received: December 31, 2011.
Aceptado en versión corregida | Accepted in revised form: January 16, 2012
Publicado en línea | Published online: January 30, 2012.
Declaración de intereses | Declaration of interests: Financial support for this work was provided by Universidad de Santiago de Chile (USACH), DICYT, 020941UM
Este artículo puede ser citado como / This article must be cited as: Javier Espinoza, Alejandro Urzúa, Javier Echeverría. 2012. Identification of the components of a non polar extract of the aerial parts of *Dunalia spinosa* by GC-MS. **Bol Latinoam Caribe Plant Med Aromat** 11(1): 86 - 90.

INTRODUCTION

The genus *Dunalia* (Solanaceae) consists of seven species distributed along the Andes of South America and in Chile it is exclusively represented by *Dunalia spinosa* (Meyen) Dammer. Its common names are "Yara" and "Chumi-Chumi" (Marticorena and Quezada, 1985) and in folk medicine the leaves and fruits are used as an antiseptic (Castro *et al*., 1982). In an attempt to correlate the medicinal properties of this species with its chemical composition, the resinous exudate has been studied and has been found to contain lupeol, scopoletin, withaferin A, quercetin, β-sitosterol and dunaurone (Erazo *et al*., 2008).

The plant is located in Chile in Regions XV and I where it grows in extreme environmental conditions. These environments have significantly varied biotic and abiotic factors making this plant a good candidate for subsequent phytochemical studies focused on ecological chemistry (Riedemann *et al*., 2008; Shoonhoven *et al*., 2005).

This communication reports the chemical composition of the extract obtained using light petroleum ether from leaves and stems of *D. spinosa* due to potential interest in the non-polar compounds which normally accumulate in the plant epicuticle as primary protection against biotic and abiotic damage (Shoonhoven *et al*., 2005).

RESULTS AND DISCUSSION

From the aerial parts of *Dunalia spinosa* (95 g) a fraction was extracted using light petroleum ether and (1.37g, 1.46 %) was obtained.

Part of the extract was fractioned using CC and each fraction was subjected to extensive GC and GC-MS analysis, where a total of 34 compounds were identified accounting for 56.59% of the extracted mass.

The unidentified compounds corresponded to branched chain isomers of alkanes and alkenes for which there were no with compounds of the same type with matching RI's in the equipment data base. The composition of the identified compounds is listed in Table 1.

The following compounds were identified: n-alkanes (65.88%) from C-14 to C-21 carbon atoms.1-Alkenes (1.15%): 1-tetradecene, 1-pentadecene, 1-hexadecene, 1-nonadecene. Aldehydes (1.29%): hexanal, nonanal and decanal. Monoterpenes (5.9%): sabinene, β-pinene, *p*-cymene, limonene, 1.8-cineol and *trans*-sabinene. Sesquiterpenes (0.17%): caryophyllene oxide. Diterpenes (1.64%): phytol.

In addition, miscellaneous compounds of note were identified, specifically phytone (4.98%), 7-oxabicyclo [4.1.0] heptane (5.85%) and methyl hexadecanoate (5.57%).

The extract was characterized by the presence of straight chain alkanes with between 14 and 21 carbon atoms, with heneicosane ($C_{21}H_{44}$) accounting for over 40% of the fraction.

By comparison, the $C_{25}H_{52}$, $C_{27}H_{56}$, $C_{29}H_{60}$ and $C_{31}H_{64}$, principal epicuticular n-alkane hydrocarbons identified in *Haplopappus* and *Lithrea* (Urzúa *et al*., 2004; Urzúa *et al*., 2011), the n-alkanes identified in *Dunalia* were low molecular weight compounds.

In the diterpenoid fraction phytol was identified, this compound is universally present in plants as part of the chlorophyll molecule however the free form is less common.

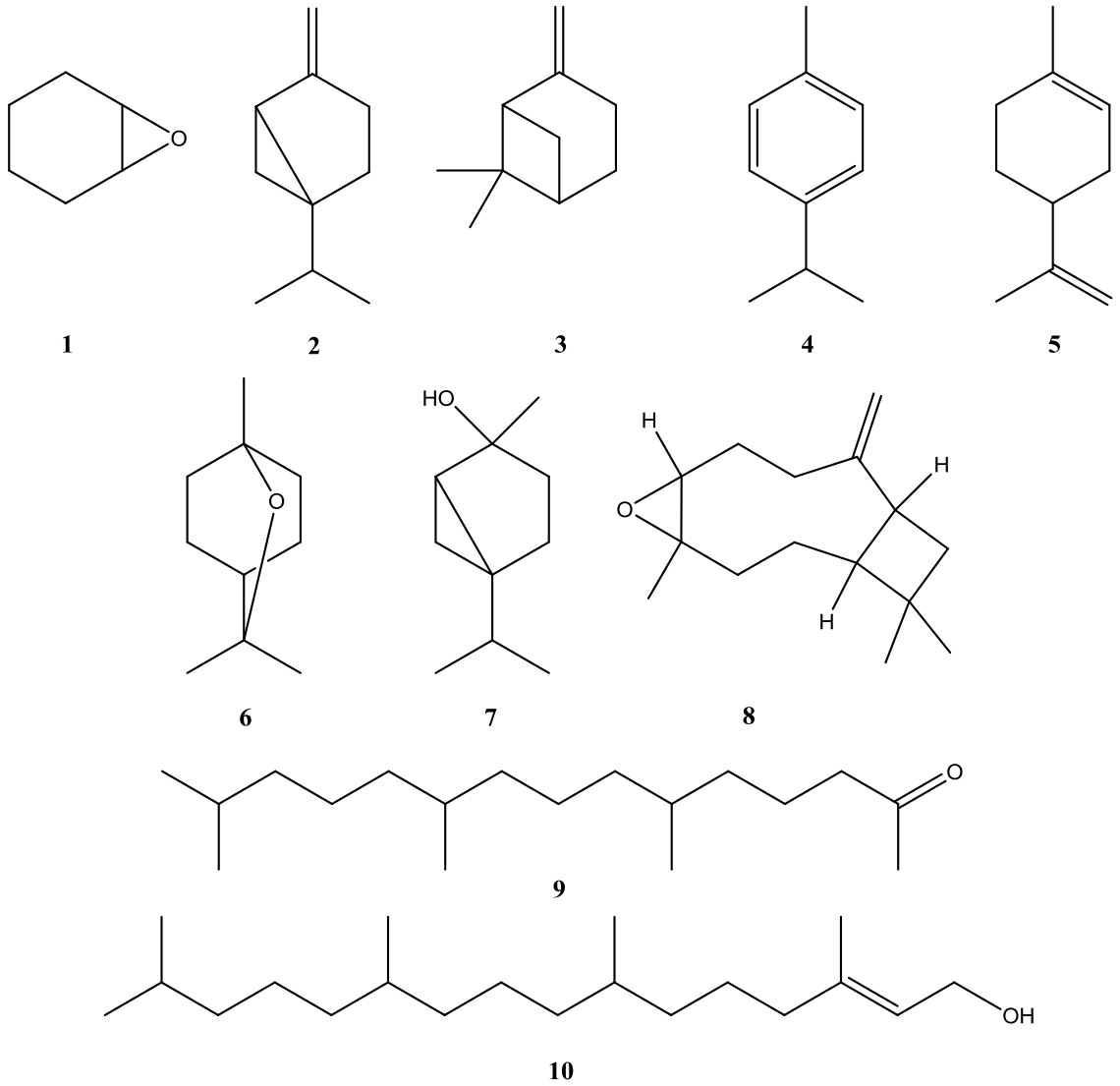
In addition, six hydrocarbon monoterpenes (Table 1) were identified. These compounds have been described as playing different roles; as semiochemicals in insect-plant relationships, as kairomones, allomones and as synomones (Shoonhoven *et al*., 2005; The pherobase, 2012)

This methodology is appropriate to identify complex mixtures of compounds from discarded fractions. In many phytochemical studies, considering these fractions provides a more complete profile of the secondary metabolites of the studied species (Urzúa *et al*., 2004; Urzúa *et al*., 2011).

TABLE 1
Composition of the non polar extract of aerial parts of *Dunalia spinosa*

RT	RI	Compounds	%	%*
11,44	802,5	Hexanal	0,38	0,68
13,63	864,6	7-Oxabicyclo[4.1.0]heptanes (**1**)	3,31	5,85
14,09	876,4	*p*-Xylene	0,17	0,31
14,72	891,9	2-Cyclohexen-1-ol	0,96	1,69
16,43	941,1	2-Cyclohexen-1-one	2,09	3,70
17,24	963,9	(E)-2-Heptenal	0,06	0,11
17,89	981,4	Sabinene (**2**)	0,10	0,18
18,05	985,6	β-Pinene (**3**)	0,48	0,84
18,28	991,5	6-methyl-5-Hepten-2-one	0,39	0,68
19,63	1032,6	*p*-cymene (**4**)	0,90	1,59
19,78	1037,2	Limonene (**5**)	1,40	2,47
19,94	1042,2	Eucalyptol (**6**)	0,40	0,70
20,78	1067,4	*trans*-Sabinene (**7**)	0,07	0,12
22,15	1108,1	Nonanal	0,27	0,47
25,25	1211,3	Decanal	0,08	0,14
30,33	1393,6	1-Tetradecene	0,10	0,17
30,53	1400,8	Tetradecane	0,23	0,40
32,03	1461,5	6,10-dimethyl-5,9-Undecadien-2-one	0,33	0,57
32,86	1493,9	1-Pentadecene	0,04	0,06
33,05	1500,7	Pentadecane	1,49	2,64
33,97	1521,6	Benzoic acid, 4-ethoxy-, ethyl ester	0,15	0,26
35,26	1594,3	1-Hexadecene	0,15	0,26
35,43	1600,7	Hexadecane	0,33	0,58
35,74	1615,4	Caryophyllene oxide (**8**)	0,09	0,17
37,68	1701,0	Heptadecane	0,64	1,12
39,82	1800,7	Octadecane	0,74	1,31
40,85	1851,4	Phytona (**9**)	2,82	4,98
41,76	1894,4	1-Nonadecene	0,38	0,66
41,90	1900,9	Nonadecane	7,53	13,31
42,55	1928,7	Hexadecanoic acid, methyl ester	3,15	5,57
44,19	1997,1	Hexadecanoic acid, ethyl ester	0,13	0,23
44,29	-	Eicosane	1,83	3,24
47,25	-	Heneicosane	24,49	43,28
47,93	-	Phytol (**10**)	0,93	1,64
NI	-	Not identified branched hydrocarbons	43,41	-
		Total	100,00	100,00

FIGURE 1



Most relevant compound in the non polar extract of the aerial parts of *D. spinosa*

EXPERIMENTAL

General

The non polar compounds analysis was performed using gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). Qualitative analysis was performed using a Thermo Scientific Trace GC Ultra linked to a ISQ quadrupole mass spectrometric detector with an integrated data system (Xcalibur 2.0, Thermo Fisher Scientific Inc. USA); quantitative analysis was carried out using a Shimadzu GC-9A gas chromatograph fitted with a FID-9 detector (Shimadzu Corporation, Kyoto, Japan). The same capillary column (Rtx-5MS, film thickness 0.25 μm, 60m x 0.25 mm, Restek Corporation, Bellefonte, PA. USA) was used in both instruments.

Plant material

Aerial parts of *Dunalia spinosa* (Meyen) Dammer were collected near Arica (XV Region, Chile) at an altitude of 2900 m over the average sea level during the flowering season, November 2005. Voucher specimens were deposited in the Herbarium of the Laboratory of Chemical Ecology, Universidad de Chile, Santiago, Chile. The aerial parts were dried in an oven with circulating air at 40º C for 24 h and milled.

Extraction of the non polar compounds and analysis

Aerial parts of *Dunalia spinosa* (95 g) were extracted by dipping the dry milled plan material in 500 mL of light petroleum ether (35 - 60º C) for 24 h. The extraction was repeated twice to assure the total extraction of the components. Elimination of the solvent gave the fraction of non polar compounds (1.37 g, 1.46 %). Part of the extract, (400 mg) was fractionated by CC (silica gel) using pentane, pentane – CH_2Cl_2 and CH_2Cl_2 – MeOH step gradients. Fractions eluted with pentane (25.5 mg), pentane-CH_2Cl_2 (9:1), (3 mg), pentane- CH_2Cl_2 (7:3), (94.2 mg), pentane-CH_2Cl_2 (6:4), (37.2 mg), CH_2Cl_2 (9.9) and CH_2Cl_2-MeOH (97:3), 179.1 were submitted to extensive GC-MS analysis. The non polar compounds analysis was performed by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS) using the instrumentation described above. The operating conditions were as follows: on-column injection; injector temperature, 250° C; detector temperature, 280° C; carrier gas, He at 1.25 ml/min; oven temperature program: 40º C for 5 min, increase to 260º C at 5º C/min, and then 260º C for 5 min. The mass detector ionization employed an electron impact of 70 eV. Recording conditions employed a scan time of 1.5 s and a mass range of 40 to 400 amu. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST08 library database, and by comparison of their retention index with those reported in the literature (Adams, 2007), for the same type of column or those of commercial standards, when available.

ACKNOWLEDGEMENTS

Financial support for this work was provided by Universidad de Santiago de Chile (USACH), DICYT, 020941UM

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