Short Communication

ANALYSIS OF SURFACE AND VOLATILE COMPOUNDS OF FLOWER HEADS OF *FLOURENSIA THURIFERA* (MOL.) D.C.

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ABSTRACT

Surface compounds were obtained by a methylene chloride extraction of fresh flower heads of *Flourensia thurifera*. The methylene chloride extract was purified by column chromatography and analyzed by GC-MS, leading to the identification of several monoterpenes, sesquiterpenes and diterpenes. A hydrocarbon fraction of n-alkanes from C23 to C31 and C33 was also identified. Headspace analysis of the fresh flower heads was also carried out. Different proportions of the same mono- and some of the sesquiterpenes were identified in the volatile fraction. The presence of mono- and sesquiterpenes in the flower heads is in agreement with the fact that these families of compounds have been identified in other *Flourensia* species.

keywords: *Flourensia thurifera*.; Asteraceae; Flower heads; Methylene chloride extract; Headspace; GC-MS; Monoterpenes; Sesquiterpenes; Diterpenes; Hydrocarbons

INTRODUCTION

Flourensia thurifera (Mol.) D.C. (Asteraceae, Heliantheae) is a shrub with attractive yellow flowers from 8 to 10 cm in diameter. The plant is found in semi-arid land in Central Chile from Los Vilos (32°S latitude) to beyond the latitude of Concepción (37°S). It sometimes forms large and almost monospecific communities covering north facing slopes of hills¹, although it can grow among other species. The flowers of *F. thurifera* have a pleasant smell, and recently the plant has started to be domesticated for use in gardens with low water requirements².

In this communication, flower heads of *Flourensia thurifera* are studied by headspace analysis and methylene chloride extraction followed by GC-MS fractionating. The presence of mono- and sesquiterpenes in the flower heads is in agreement with the fact that these families of compounds have been identified in the leaf surface extract of *Flourensia cernua*³ and in the essential oil of *Flourensia oolepsi*⁴. Minor sesquiterpenoids and diterpenoids identified in the methylene chloride extract, were not detected in the headspace analysis, and the monoterpenoids were found in a greater proportion. These differences can be attributed to differences in the volatility of the compounds and plant control of the compartmentation.

EXPERIMENTAL

Plant Material

Flourensia thurifera (Mol.) D.C. (Asteraceae, Heliantheae) flower heads, were collected in November 2005, between Zapallar and Papudo (V Region, Chile 32° 30' S, 71° 30' W). Voucher specimens were deposited in the Herbarium of the National Natural History Museum, Santiago, Chile

Plant extraction

Fresh flower heads of *F. thurifera* (1.0 kg) were extracted by dipping the fresh plant material in 5.0 L of cold CH₂Cl₂ for 60 s^{5,6}. The extraction was repeated twice.

Column separation of the extracts

After removal of the solvent under vacuum, the residue (7.0 g, 0.7 %) was chromatographed on a silica gel column (70 g) using a pentane–methylene chloride–methanol step gradient (pentane, 75 mL; pentane-CH₂Cl₂, 99:1, 60 mL; pentane-CH₂Cl₂, 97:3, 90 mL; pentane-CH₂Cl₂, 1:1, 105 mL and CH₂Cl₂-MeOH 9:1, 270 mL. Fractions, each of about 15 mL, were monitored by TLC (silica gel HF₂₅₄; pentane-CH₂Cl₂, 98:2 and CH₂Cl₂-MeOH, 95:5) to afford 40 fractions regrouped after TLC into five new fractions; A: 1-5 (0.902 g), B: 6-9 (0.801g), C: 10-15 (0.998 g), D: 16-22 (1.319 g) and E: 23-40 (2.668 g). The regrouped fractions A, B and C were analysed in triplicate by GC-MS.

Collection of the headspace

For headspace analysis, flower heads (ca. 300 g fr.wt) were gathered and placed inside a glass jar equipped with an inlet and an outlet. At the inlet, a compressed air cylinder, containing synthetic air made from extra pure oxygen

and nitrogen with no detectable organic impurities, was attached through a regulator which controlled the air flow. At the outlet, a column was attached which contained Porapak Q (100 mg). Volatile entrainment (5 h with air flow of 0.5 L/min) commenced immediately after the flowers had been severed from the plants. After collection of the headspace, the compounds in the Porapak Q column were removed with CH₂Cl₂ (0.5 mL). The solution was stored at -18 °C, also under purified N₂, until GC-MS analysis. The experiment was performed in triplicate.

Analysis of the constituents

The methylene chloride fractions (A, B and C), that contained the terpenoids (TLC), and the headspace fractions, were analysed in triplicate by GC-MS, using a Fisons MD-800 equipped with a J & W DBS-MSD capillary column (15 m x 0.20 mm, 0.25 µm film thickness). The temperature of the injector was 295 °C and the temperature of the column was programmed to start at 60 °C, for 2 min, followed by a rise to 305 °C at 10 °C min⁻¹. Helium was the carrier gas at 7 lb. psi. Detection was done using QI and EI. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their retention indices (RI). Limonene, α-pinene, β-pinene, linalool, 1, 8-cineol, α-ocimene, α-cubebene, α-copaene, α-humulene, alloaromadendrene, β-caryophyllene, phytol, isophytol and n-alkanes were obtained from Aldrich-Sigma USA. The other compounds show their retention indexes and MS data in good agreement with both the literature⁷⁻¹⁰ and with data from the NIST library, with correlation indices larger than 97%.

RESULTS AND DISCUSSION

The results of the GC-MS analysis are given in Table 1. Analysis of the methylene chloride extract of fresh flower heads resulted in the identification of 36 constituents which collectively accounted for more than 82% of the analysed fractions (constituents detected 45: identified: 36).

Minor sesquiterpenoids and diterpenoids identified in the methylene chloride extract, were not detected in the headspace analysis, and the monoterpenoids were found in a greater proportion. These differences can be attributed to differences in the volatility of the compounds and plant control of the compartmentation¹¹.

The presence of mono- and sesquiterpenoids in the flower heads of *Flourensia thurifera* is in agreement with the chemistry of other *Flourensia* spp. The leaf surface extracts of *Flourensia cernua* shows the presence of mono- and sesquiterpenoids: camphene, β-myrcene, 3-carene, limonene, 1,8-cineole, borneol, *cis*-jasmone, β-caryophyllene, caryophyllene oxide and globulol. These were consistently present in different samples along with several unidentified compounds, some of which were tentatively identified as: β-cubebene, α-copaene, γ-cadinene, α-humulene and β-bourbonene³. From the essential oil of *Flourensia oolepsi*, a total of 36 terpenoids were identified and the main components were *t*-cadinol (10.5%), β-selinene (9.8%), linalool (8.2%) and β-eudesmol (8.0%)⁴.

Even though some of these molecules and structural types are repeated

among species, a clear terpene pattern implying chemosystematic correlations in these species of *Flourensia* could not be found.

Finally, flower heads of *Flourensia thurifera* are visited by a varied entomofauna and, although no systematic studies have been performed, the knowledge of the volatile components responsible of eventual odorous stimuli is important for future studies of interactions between *F. thurifera* and insects¹².

Table 1. Chemical composition of CH₂Cl₂ extract and headspace from flower heads of *F. thurifera*.

Components	CH ₂ Cl ₂ Extract		Headspace	
	RI	(%) ^a	RI	(%) ^a
Monoterpenes				
p-metha-1(7)-3-diene	923	0.6 (0.100)	923	2.6 (0.030)
α-pinene	939	0.4 (0.001)	934	3.9 (0.040)
β-pinene	977	0.6 (0.060)	977	2.7 (0.030)
limonene	1031	1.0 (0.100)	1031	3.7 (0.040)
3-thujene	1038	0.4 (0.010)	1035	3.3 (0.020)
α-ocimene	1044	10.1 (0.600)	1044	30.1 (2.200)
linalool	1098	2.1 (0.010)	1098	3.1 (0.30)
1,8 cineol	1228	0.3 (0.005)	1228	3.8 (0.20)
Sesquiterpenes				
α-cubebene	1350	19.1 (1.200)	1350	15.1 (1.400)
α-copaene	1375	0.6 (0.130)		
β-cubebene	1388	1.3 (0.120)	1388	1.1 (0.040)
β-caryophyllene	1413	7.4 (0.400)		5.7 (1.200)
1(10)-aristolene	1431	0.9 (0.010)		
α-humulene	1453	1.4 (0.020)	1453	0.9 (0.020)
aristolene	1458	1.3 (0.010)		
ishwarane	1460	1.3 (0.010)		
alloaromadendrene	1467	1.4 (0.010)		
germacrene D	1481	1.4 (0.010)		
β-selinene	1487	1.1 (0.040)		
γ-cadinene	1512	1.3 (0.100)		
3,7(11)-selinadiene	1531	1.3 (0.120)		
α-cadinene	1538	0.7 (0.010)		
germacrene B	1557	3.4 (0.080)	1557	3.6 (0.20)
spathulenol	1577	1.4 (0.100)		
Diterpenes				
phytol	1981	1.3 (0.100)		
isophytol	2210	1.7 (0.120)		
Hydrocarbons				
C ₂₃ H ₄₈	2300	0.9 (0.060)		
C ₂₄ H ₅₀	2400	0.4 (0.010)		
C ₂₅ H ₅₂	2500	1.7 (0.120)		
C ₂₆ H ₅₄	2600	0.3 (0.006)		
C ₂₇ H ₅₆	2700	3.2 (0.030)		
C ₂₈ H ₅₈	2800	0.4 (0.003)		
C ₂₉ H ₆₀	2900	12.1 (0.900)		
C ₃₀ H ₆₂	3000	3.2 (0.070)		
C ₃₁ H ₆₄	3100	2.1 (0.020)		
C ₃₃ H ₆₈	3300	0.3 (0.005)		

RI, retention indices (DB-5 column), for hydrocarbons (C_n × 100) according to Kovats; ^aarea of the identified compounds; mean of three analyses (SD in parentheses).

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REFERENCES

- Hoffmann J A. *Flora Silvestre de Chile, Zona Central*, 3° Ed. Ediciones Claudio Gay: Santiago Chile, 1995; pp. 238-239.
- Riedemann P, Aldunate G. *Flora Nativa de Valor Ornamental, Chile Centro*, Editorial Andrés Bello: Santiago Chile, 2001; pp. 344-345.
- Estell R E, Havstad K M, Fredrickson E L, Gardea-Torresdey L A. *Biochem. Syst. Ecol.* 22, 73 (1994).
- Priotti Z E, Zygadlo J A, Espinar L A, *J. Essent. Oil Res.* 9, 345 (1979).
- Urzúa A. *J. Chil. Chem. Soc.* 49, 137 (2004).
- Urzúa A, Santander R, Echeverría J, Rezende C M. *J. Chil. Chem. Soc.* 52, 1142-1144 (2007).
- Masry S, Abou-Donia A, Darwisch A F, Abou-Karam A M, Grenz M, Bohlmann F. *Phytochemistry* 12, 2953 (1984).
- Swing A A, Silverstein R M. *Monoterpenes Infrared, Mass, ¹H NMR, and ¹³C NMR Spectra, and Kovats Indices*, Aldrich Chemical Company, Inc.: Milwaukee USA, 1981.
- Connolly J D, Hill R A. *Dictionary of Terpenoids Volume 1, Mono- and Sesquiterpenoids*, Chapman and Hall: London U K, 1991.
- Vig O P. *J. Indian Chem. Soc.* 4, 159 (1972).
- Shoonhoven M L, A van Loon J A J, Dicke M. *Insect-Plant Biology (Chapter 4, Plant chemistry: endless variety)*, Oxford University Press: London U K, 2005; pp. 48-86.
- Shoonhoven M L, A van Loon J A J, Dicke M. *Insect-Plant Biology (Chapter 6, Host- Plant selection: how to find a host plant)*, Oxford University Press: London U K, 2005; 135-160.