Gas Chromatography-Mass Spectrometry Study of the Essential Oils of *Schinus longifolia* (Lindl.) Speg., *Schinus fasciculata* (Griseb.) I. M. Johnst., and *Schinus areira* L.

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The essential oil composition from the aerial parts of three Anacardiaceae growing in Bahía Blanca, Argentina was studied by gas chromatography and gas chromatography-mass spectrometry. The essential oils of *S. longifolia* and *S. fasciculata* have been studied for the first time. The major constituents were α-pinene (46.5%), β-pinene (15.1%) and α-phellandrene (10.1%) for *S. longifolia* and limonene (10.9%), β-phellandrene (6.16%) and α-phellandrene (5.6%) for *S. fasciculata.* The major components of the essential oil of *S. areira* were limonene (28.6%), α-phellandrene (10.1%), sabinene (9.2%) and camphene (9.2%) differing from the literature data. The essential oils from *S. areira* and *S. longifolia* exhibited a high biotoxicity in a brine shrimp assay with *Artemia persimilis.*

Key words: Schinus, Essential Oil, Biotoxicity

Introduction

The genus *Schinus* (family Anacardiaceae) comprises twenty-two species, six of them are endemic in Argentina. In the southwest of Buenos Aires province, particularly in the region of Bahía Blanca, three species of the genus *Schinus* can be found, *Schinus longifolia* (Lindl.) Speg., *Schinus fasciculata* (Griseb.) I. M. Johnst., and *Schinus areira* L. (Zuloaga and Morrone, 1999; Cabrera, 1965).

Schinus fasciculata is a thorny shrub known as "Molle Pispito". It is used in the folk medicine of the northwest of Argentina as an antitussive and for treatment of dysentery (Scarpa, 2004). This species is endemic in Argentina and can be found in Northern and Central provinces of our country and also in Paraguay and Bolivia. *Schinus longifolia* is a medium-size tree (5 m) that is frequently found in the area of Buenos Aires province, Argentina, and also in Brazil and Uruguay. Its leaves are used in the treatment of colds, as expectorant and laxative, the resin that exudates is used as emollient. No previous record on phytochemical studies of these two species has been found in the literature.

Schinus areira, previously named as *Schinus molle*, is an evergreen tree up to 10 m in height, locally known by its common names "Aguarybay" or "Gualeguay". Widely distributed in America it is well known by its several uses in the folk medicine, so far it is called "Balsamo sanalotodo" (cure-all balsam). Leaves, fruits, stems and bark, dried or fresh, are used to prepare infusions, ointments, cataplasms, beverages, collyrium, etc. which are used as purgative, diuretic, parasiticide, insecticide, vulnerary, topic disinfectant and for the treatment of rheumatism, stomach upset, menstrual disorders, bronchitis and conjunctivitis (Gupta, 1995). Besides, fruits are used as a substitute of "black pepper" and in the preparation of alcoholic drinks. The essential oil of *S. areira* has shown significant antibacterial and antifungal activity (Gundidza, 1993; Dikshit *et al.*, 1986). Limonene and α-pinene, components of the essential oil of *S. areira*, and the oil itself have been studied as allelopathic agents showing strong inhibitory activity of the root growth of *Zea mays* seedlings (Scrivanti *et al.*, 2003).

In the present work we are reporting for the first time the chemical composition of the essential oils of *S. longifolia* and *S. fasciculata.* Besides, the chemical composition of the essential oil of *S. areira* is analyzed and compared with previously reported data for *S. areira* from different locations

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in Argentina. The biotoxicity of these oils has been evaluated through the brine shrimp assay observing the percentage of mortality of *Artemia* nauplii at different oil concentrations.

Materials and Methods

General

Gas chromatography-mass spectrometry analyses were performed with a Hewlett-Packard 6890 chromatograph connected to a Hewlett-Packard 5972A mass spectrometer equipped with a capillary column (HP-5, 30 m x 0.25 mm, 0.25 mm film thickness). The carrier gas was helium with a flow of 1 ml/min. The GC oven temperature was held at 60 °C for 1 min, programmed at 5 °C/min to 250 °C, then held at this temperature for 15 min. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 350 amu. The temperature of the injection block was 250 °C.

GC analyses were performed on a Shimadzu G14B chromatograph with a flame ionization detector on a DB-1 column (50 m × 0.25 mm, 0.25 μm film thickness) and a Carbowax 20M column (50 m × 0.25 mm, 0.25 μm film thickness) with the same analytical conditions used for the GC-MS analyses.

Plant material

Aerial parts of *S. longifolia* and *S. areira* were collected in August 2003 at Bahía Blanca city, Buenos Aires province, Argentina. Aerial parts of *S. fasciculata* were collected in May 2004 at Puerto Cuatreros, near Bahía Blanca. The taxonomy of this material was determined by Lic. M. G. Murray. Voucher specimens are kept in the "Herbario del Departamento de Biología, Bioquímica y Farmacia – Universidad Nacional del Sur (BBB)" under the numbers MGM121 (*S. areira*), MGM401 and MGM402 (*S. fasciculata*) and MGM400 (*S. longifolia*).

Isolation of the essential oils

The samples of *S. longifolia* (153 g), *S. fasciculata* (171 g) and *S. areira* (160 g) consisting of fresh picked aerial parts (leaves, fruits and stems) were subjected to hydrodistillation using a Clevenger type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate and stored at 4 °C under N_2. Essential oils yields were 0.23%, 0.26% and 2.22% for *S. longifolia*, *S. fasciculata* and *S. areira*, respectively, based on fresh weight of sample.

Identification of the compounds

The compounds were identified by gas chromatography by comparison of their retention indices (Kovats Indices) with retention times of known compounds and also by comparison of their mass spectra with those stored in the MS databases (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas. Results are summarized in Table I.

Brine shrimp bioassay

Each of the essential oils was tested at 1000, 100 and 10 μg of oil per ml. The concentrations were obtained by transferring the corresponding volume from the stock dichloromethane solutions to different wells, air drying overnight and redissolving in 3 ml of artificial sea water (35 g of sea salts "Marsal" per litre of deionized water). Brine shrimp eggs of the Argentine species *Artemia persimilis* obtained locally (Acuario Indico, Buenos Aires, Argentina) were hatched in artificial sea water at room temperature under continuous lighting. After an incubation time of 48 h, 100 μl of this solution containing 10–20 organisms were pipetted into each well and then incubated for 48 h under direct light at 24–26 °C. Then, each well was examined and the number of dead (*i.e.* nonmotile) nauplii was counted. Finally, 100 μl of methanol were added to each well and after 30 min the total number of shrimp in each well was counted. Triplicate assays were run for each concentration and control and the average value of the three assays was recorded. The controls were performed with the same amount of CH_2Cl_2 used for the dilutions of the samples, following the same procedure. Results are reported as percentage of mortality in Table II where M = (percentage of survival in the control – percentage of survival in the sample).

Results and Discussion

The hydrodistillation of the aerial parts of *S. longifolia* gave a colorless oil with a yield of 0.23% based on fresh weight. Thirteen compounds (98.19%), almost exclusively monoterpenes (93.35%), were identified and the major constituents were α-pinene (46.51%), β-pinene (15.13%), α-phellandrene (10.11%), limonene (7.70%) and β-myrcene (6.98%). All the identified compounds, their percentages and retention times are listed in Table I.

Table I. Constituents of essential oils from aerial parts of *Schinus longifolia, Schinus fasciculata* and *Schinus areira*.

Components[a]	RT [min]	Percentage *S. longifolia*	*S. fasciculata*	*S. areira*	Method of identification[b]
Tricyclene	6.25	1.50	–	2.20	GC-MS, RI
Hexanal	6.47	–	4.20	–	GC-MS, RI
Octane	6.51	–	1.10	–	GC-MS, RI
2-Hexenal (*E*)	6.85	–	1.21	–	GC-MS, RI
3-Hexen-1-ol (*Z*)	6.90	–	5.12	–	GC-MS, RI
1-Hexanol	7.53	–	8.03	–	GC-MS, RI, S
Nonane	7.89	–	7.60	–	GC-MS, RI
α-Pinene	8.52	46.51	3.84	6.51	GC-MS, RI, S
Camphene	9.12	5.26	–	9.16	GC-MS, RI
Sabinene	9.87	–	–	9.20	GC-MS, RI
β-Pinene	10.12	15.13	4.20	7.23	GC-MS, RI, S
β-Myrcene	10.71	6.98	–	5.98	GC-MS, RI
2-Carene	10.89	–	3.19	–	GC-MS, RI
α-Phellandrene	11.08	10.11	5.60	10.11	GC-MS, RI
Limonene	12.28	7.70	10.90	28.61	GC-MS, RI, S
β-Phellandrene	13.10	–	6.16	–	GC-MS, RI
3-Carene	13.90	0.16	1.78	0.16	GC-MS, RI
1-Octanol	14.13	–	0.46	–	GC-MS, RI, S
Nonanal	14.47	1.08	1.42	tr[c]	GC-MS, RI
2,6-Nonadienal (*E*,*Z*)	15.87	–	1.50	–	GC-MS, RI
Decanal	17.15	–	1.45	–	GC-MS, RI
1-Decanol	18.64	–	1.80	–	GC-MS, RI
2-Undecanone	20.10	–	1.17	–	GC-MS, RI
Exo-2-hydroxycineole acetate	21.46	–	–	0.20	GC-MS, RI
α-Cubebene	21.70	–	–	6.61	GC-MS, RI
α-Copaene	22.47	0.79	–	0.79	GC-MS, RI
β-Elemene	22.67	–	–	0.24	GC-MS, RI
1-Decene	22.72	–	12.34	–	GC-MS, RI
Caryophyllene	23.66	0.34	–	1.44	GC-MS, RI
Humulene	24.49	0.08	–	0.87	GC-MS, RI
Heptyl hexanoate	24.94	–	0.78	–	GC-MS, RI
Butyl hexanoate	26.85	–	2.48	–	GC-MS, RI
Germacrene B	27.78	2.55	–	4.05	GC-MS, RI
Globulol	28.24	–	–	0.81	GC-MS, RI
1-Dodecanol	29.98	–	9.21	–	GC-MS, RI
Spathulenol	32.86	–	–	0.42	GC-MS, RI
α-Santalol	34.16	–	–	0.17	GC-MS, RI
Kauren-18-ol acetate	34.82	–	–	0.15	GC-MS, RI

[a] Components are listed in order of elution on HP-5 (30 m) column.
[b] GC-MS: gas chromatography-mass spectrometry; RI: Kovats Index; S: standard.
[c] tr: traces < 0.05%.

The analysis of the essential oil of *S. fasciculata* allowed us to identify twenty-three compounds (95.54%), among them limonene (10.90%), β-phellandrene (6.16%) and α-phellandrene (5.60%) were the major terpenes (Table I). It is to note that a high percentage of compounds like alcohols (24.62%), aldehydes (9.78%), esters (3.26%) and ketones (1.17%) was present in this hydrodistillated oil whereas a low content of monoterpenes (35.67%) and none sesquiterpenes were detected. In this case the essential oil was obtained with a yield of 0.26% based on fresh weight.

In the essential oil of *S. areira* (yield 2.22%) twenty-one compounds were identified (94.3%) (Table I). The major ones were limonene (28.61%), α-phellandrene (10.11%), sabinene (9.20%) and camphene (9.16%). These results differ from those reported for *S. areira* specimens collected in Córdoba province, middle region of Argentina, where the major component was α-pinene (85%) (Scrivanti *et al.*, 2003). For specimens collected in Mendoza province, West of Argentina, the composition was similar but with a higher percentage of limonene (45.95%) and a-phellandrene

(25.44%) (Wannaz *et al.*, 2003). Finally, our results also differ from those obtained in a study with several samples collected in Jujuy province, northwest of Argentina, where the main component of the essential oil of *S. areira* was β-phellandrene which was absent in our sample (Viturro *et al.*, 2003). Thus, for plants of *S. areira* growing in different regions of Argentina, a marked variability in the chemical composition of the essential oil can be observed. This variability may be attributed to different climatic and soil growing conditions.

Table II. Toxicity of the essential oils of *Schinus longifolia*, *Schinus fasciculata* and *Schinus areira* against *Artemia persimilis.*

	M (%)[a]		
Oil concentration [μg/ml]	1000	100	10
S. longifolia	94.4	63.0	12.9
S. fasciculata	0	0	0
S. areira	100.0	100.0	10.2

[a] Percentage of mortality after 48 h of exposure.

The shrimp bioassay is based on the ability to kill laboratory-cultured *Artemia* nauplii brine shrimp. This assay is considered a useful tool for preliminary assessment of toxicity (Solis *et al.*, 1993; McLaughlin *et al.*, 1991; Sorgeloos *et al.*, 1978). A good relationship has been found with the brine shrimp assay to detect antitumoral compounds in terrestrial plant extracts (Solis *et al.*, 1993; Meyer *et al.*, 1982; Mackeen *et al.*, 2000), and potentially cytotoxic metabolites in marine natural products (Carballo *et al.*, 2002). This encouraged us to test the essential oil of *S. longifolia*, *S. fasciculata* and *S. areira* with the brine shrimp assay. The results of the bioassay are summarized in Table II. After 48 h of exposure a mortality of 100% was observed for *S. areira* oil at concentrations of 1000 and 100 μg/ml. The oil of *S. longifolia* showed 94.4% mortality for an oil concentration of 1000 μg/ml and 63.0% mortality for 100 μg/ml. Both gave similar results (~ 10%) for the more diluted samples (10 μg/ml). On the other hand, the oil of *S. fasciculata* showed no activity (100% of survival) in the brine shrimp assay. These results could be explained considering the differences in the chemical composition of the essential oils of these *Schinus* species which are shown in Table III. While the essential oil of *S. fasciculata* gave a low percentage of monoterpenes (35.67%) and no sesquiterpenes could be detected, *S. longifolia* and *S. areira* gave higher contents of monoterpenes, 93.35% and 79.16%, respectively, and sesquiterpenes, 3.76% and 15.40%, respectively. The results of this bioassay suggest that the essential oils of *S. areira* and *S. longifolia* are good candidates for further evaluation of cytotoxicity and antitumoral activity.

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Table III. Percentage composition of grouped components of essential oils of *Schinus longifolia*, *Schinus fasciculata* and *Schinus areira*.

Grouped components	*S. longifolia* (%)	*S. fasciculata* (%)	*S. areira* (%)
Monoterpene hydrocarbons	93.35	35.67	79.16
Sesquiterpene hydrocarbons	3.76	–	15.40
Esters	–	3.26	0.35
Aldehydes	1.08	9.78	tr[a]
Ketones	–	1.17	–
Alcohols	–	24.62	–
Aliphatic and olefinic hydrocarbons	–	21.04	–
Total identified	98.19	95.54	94.93

[a] tr: traces < 0.05%.

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