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Volatile secondary metabolites from *Spilanthes americana* obtained by simultaneous steam distillation–solvent extraction and supercritical fluid extraction

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Abstract

Secondary volatile metabolites were isolated by simultaneous distillation–solvent extraction (SDE) and supercritical (CO₂) extraction (SFE) from flowers, leaves and stems of *Spilanthes americana* (Mutis) Hieron (Compositae). The extracts were analyzed by capillary gas chromatography using flame ionization, nitrogen–phosphorus (NPD) or mass spectrometric detection. Compounds were identified according to their Kováts indexes, mass spectra (EI, 70 eV), or by comparison with standard substances. The method of extraction (SDE, SFE) and the source of the plant material (flowers, stems, leaves) affected the composition of the extracts. SFE extracts from stems were rich (>40%) in sesquiterpenes (α - and β -bisabolones, caryophyllene and cadinenes), while those from leaves and flowers were abundant in nitrogenated (43 and 27%) and oxygenated (36 and 23%) compounds. N-(isobutyl)-2E,6Z,8E-decatrienamide, N-(2-methylbutyl)-2E,6Z,8E-decatrienamide, N-(isobutyl)-6Z,8E-decadienamide and N-(2-phenylethyl)-2E,6Z,8E-decatrienamide are examples of the nitrogenated compounds found. SDE extracts from stems, leaves, and flowers of *S. americana* contained sesquiterpene levels of 32, 28 and 20%, and a generally higher proportion of oxygenated compounds (28, 52 and 32%) and monoterpenes (27, 10 and 42%) than their SFE counterparts. Only trace-level contents of nitrogenated compounds were found using NPD in SDE extracts. Some heavy hydrocarbons (C_n>20) originated from flower pigments and waxes were isolated by SFE but not by SDE. SFE was both selective and highly efficient in the isolation of sesquiterpenes, heavy hydrocarbons and nitrogenated compounds (amides).

Keywords: Extraction methods; *Spilanthes americana*; Sesquiterpenes

1. Introduction

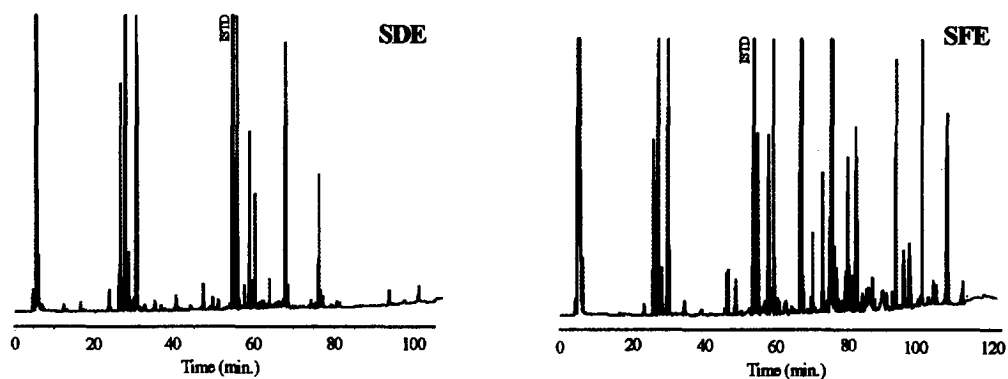
Spilanthes americana (Mutis) Hieron (family Compositae) commonly called in Colombia “guaca”, “guasca” or “quemadora”, is native to South America [1] and is used in Colombian cuisine [2]. Extracts from *S. americana* have been shown to

possess antiseptic and analgesic effects and insecticidal activity against adult mosquitoes, several leaf-eating insects and houseflies [3–6]. The plant produces a slight burning, paralytic effect on the tongue [6]. The extracts from *S. americana* flowers also have found application in the treatment of *Herpes simplex* and *Herpes zoster* [7].

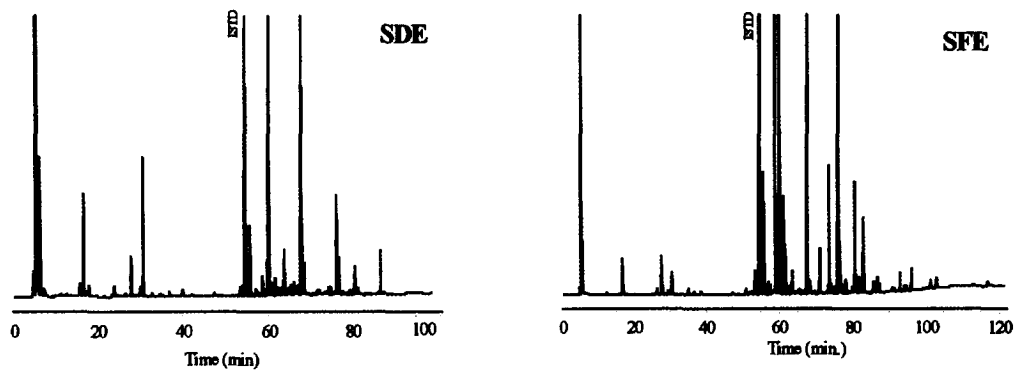
Research on the secondary metabolites from *S. americana* has focused on the isolation and identifi-

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FLOWERS



LEAVES



STEMS

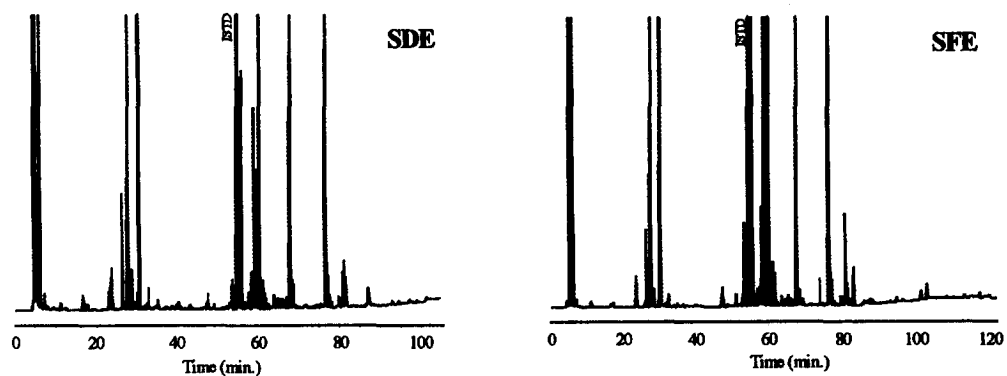


Fig. 1. Gas chromatograms of the extracts obtained by SDE and SFE from different parts of the Colombian *Spilanthes americana*. DB-1 (60 m×0.25 mm I.D.) column, FID, temperature programmed from 50°C (5 min hold) to 250°C at 2.5 °C min⁻¹.

cation of spilanthol [N-(isobutyl)-2E,6Z,8E-decatrienamido] [8], one of the biologically active metabolites with an insecticidal activity, isolated from the flowers of *S. americana* and other *Spilanthes* sp. However, little information exists about other *S. americana* secondary metabolites [4]. As this plant exhibits an ample range of biological activity and is widely used in folk medicine and the human diet, it is important to extend the study to other secondary metabolites. In the present work we isolated volatile secondary metabolites from different parts of *S. americana* (flowers, leaves, stems) using simultaneous distillation–solvent extraction (SDE) and supercritical fluid (CO₂) extraction (SFE). The extracts were analyzed by capillary gas chromatography with different detection systems [flame ionization detection (FID), nitrogen–phosphorus detection (NPD) or mass spectrometry (MS)]. Compounds were identified according to their Kováts indexes, mass spectra (EI, 70 eV) and by comparison with standard substances.

2. Experimental

2.1. Plant material

Spilanthes americana plants were gathered during the flowering period from an experimental plantation in Bucaramanga (Santander, Colombia) during January–May 1995. In all extractions only fresh and undamaged plant material was used. Volatile secondary metabolites from flowers, leaves or stems of *S. americana* were obtained by SDE or SFE immediately upon harvesting.

2.2. Essential oil extraction

2.2.1. Simultaneous steam distillation–solvent extraction

SDE was carried out in the micro-version apparatus, as described elsewhere [9]. Dichloromethane (chromatography grade reagent, Merck) and *n*-tetradecane (internal standard, reference substance for gas chromatography, Merck) were used as solvent and internal standard, respectively. For each extraction 25–30 g of plant material (flowers, leaves or stems) were placed in a 50-ml flask and subjected to

simultaneous SDE for 2 h, then, steam distillation was stopped and solvent extraction was continued for 15 min more.

2.2.2. Supercritical fluid extraction

A J&W Scientific High Pressure Soxhlet Extractor (J&W Scientific, Folsom, CA, USA) was used to obtain extracts from different parts (flowers, leaves or stems) of *S. americana* using supercritical CO₂ at 40–45°C and $7.24 \cdot 10^3$ – $7.58 \cdot 10^3$ kPa. The amount of sample was the same as in SDE. The extract obtained after 2 h of CO₂ recirculation followed by depressurization was dissolved in 2 ml CH₂Cl₂ containing 5 µl *n*-tetradecane as internal standard.

The effect of plant-to-plant variability was averaged by performing independent extractions from flowers, leaves or stems of *S. americana*. The relative concentration values reported here correspond to the average of these extractions for each type of plant material.

2.3. Essential oil analysis

High-resolution gas chromatographic (HRGC) analysis of the samples was performed on a Hewlett-Packard (HP) (Palo Alto, CA, USA) 5890A Series II gas chromatograph equipped with split/splitless injector (250°C, split ratio 1:10), FID and NPD, operated at 250°C and 230°C, respectively. Chromatographic data were processed with an HP ChemStation 3365-II (Hewlett-Packard). The columns used were a DB-1 (J&W Scientific) cross-linked fused-silica capillary column (60 m×0.25 mm I.D.), coated with polydimethylsiloxane (0.25 µm phase thickness) and DB-WAX (J&W Scientific) fused-silica capillary column (60 m×0.25 mm I.D.), coated with Carbowax 20M (0.25 µm phase thickness). The oven temperature was programmed from 50°C (5 min hold) at 2.5 C° min⁻¹ to 250°C for the DB-1 column and from 50°C (5 min hold) at 2.5 C° min⁻¹ to 180°C for the DB-WAX column. Helium (AGA, 99.995%) was used as a carrier gas (inlet pressure 152 kPa) with linear velocity 35.7 cm s⁻¹ for both columns. Air and hydrogen flow-rates were 300 and 30 ml min⁻¹ for FID, and 100 and 5 ml min⁻¹ for NPD. For both detectors nitrogen was used as a make-up gas at flow-rates of 30 ml min⁻¹ (FID) and

Table 1
Chemical composition of *Spilanthes americana* extracts obtained by SDE and SFE

Peak No. on DB-1 column	Compound	Kováts indices ^a		GC peak area ^b (%)		SDE						
		DB-1	DB-Wax	SFE	SFE	Stems	Leaves	Flowers	Stems	Leaves	Flowers	
1	<i>n</i> -Hexane	600	600	-	-	-	-	-	0.04 ± 1 · 10 ⁻²	0.06 ± 2 · 10 ⁻²	-	-
2	2-Methyl-1-propanol	620	-	-	-	-	-	-	0.06 ± 2 · 10 ⁻³	-	-	-
3	2-Hexenal	835	-	-	0.60 ± 2 · 10 ⁻¹	-	-	-	0.11 ± 6 · 10 ⁻³	1.06 ± 9 · 10 ⁻¹	-	-
4	<i>n</i> -Propyl isobutyrate	844	-	0.04 ± 1 · 10 ⁻²	-	-	-	-	0.04 ± 6 · 10 ⁻³	0.06 ± 6 · 10 ⁻²	-	-
5	<i>cis</i> -3-Hexenol	849	-	-	-	-	-	-	-	0.13 ± 1 · 10 ⁻¹	-	-
6	α -Thujene	927	1022	0.23 ± 3 · 10 ⁻²	-	-	0.06 ± 3 · 10 ⁻⁴	-	0.26 ± 7 · 10 ⁻³	0.10 ± 8 · 10 ⁻²	-	0.23 ± 3 · 10 ⁻²
7	α -Pinene	963	1018	0.69 ± 1 · 10 ⁻¹	0.08 ± 8 · 10 ⁻³	-	0.75 ± 1 · 10 ⁻²	-	0.69 ± 2 · 10 ⁻²	-	-	2.28 ± 2 · 10 ⁻¹
8	Sabinene	970	1126	0.03 ± 6 · 10 ⁻³	0.08 ± 2 · 10 ⁻³	-	0.11 ± 1 · 10 ⁻²	-	0.06 ± 6 · 10 ⁻²	0.03 ± 1 · 10 ⁻²	-	0.34 ± 1 · 10 ⁻²
9	β -Pinene	981	1120	2.30 ± 3 · 10 ⁻¹	-	-	4.66 ± 4 · 10 ⁻¹	-	4.13 ± 2 · 10 ⁻¹	-	-	12.3 ± 1.00
10	β -Myrcene	988	1164	0.16 ± 2 · 10 ⁻²	0.33 ± 1 · 10 ⁻¹	-	0.21 ± 3 · 10 ⁻²	-	0.26 ± 3 · 10 ⁻³	0.36 ± 3 · 10 ⁻¹	-	0.58 ± 2 · 10 ⁻²
11	α -Phellandrene	992	1169	-	-	-	0.07 ± 7 · 10 ⁻³	-	0.05 ± 5 · 10 ⁻³	0.12 ± 6 · 10 ⁻³	-	0.09 ± 9 · 10 ⁻³
12	<i>cis</i> -Pinane	993	-	-	-	-	-	-	-	-	-	0.10 ± 1 · 10 ⁻²
13	Benzeneacetaldehyde	1004	-	-	0.14 ± 9 · 10 ⁻²	-	-	-	-	-	-	-
14	1,4-Cineole	1008	1199	0.09 ± 3 · 10 ⁻²	-	-	0.04 ± 9 · 10 ⁻³	-	0.09 ± 3 · 10 ⁻³	-	-	0.08 ± 1 · 10 ⁻²
15	1,8-Cineole	1017	1211	2.50 ± 3 · 10 ⁻¹	0.25 ± 9 · 10 ⁻³	-	0.08 ± 1 · 10 ⁻²	-	3.28 ± 4 · 10 ⁻²	0.19 ± 2 · 10 ⁻¹	-	6.70 ± 5 · 10 ⁻¹
16	β -Phellandrene	1018	1208	-	-	-	0.87 ± 2 · 10 ⁻³	-	-	-	-	1.78 ± 2 · 10 ⁻³
17	<i>p</i> -Cymene	1019	1272	3.00 ± 6 · 10 ⁻¹	0.90 ± 1.00	-	2.90 ± 4 · 10 ⁻¹	-	2.85 ± 2 · 10 ⁻³	1.46 ± 9 · 10 ⁻¹	-	0.05 ± 2 · 10 ⁻²
18	<i>trans</i> - β -Ocimene	1047	1246	0.09 ± 2 · 10 ⁻²	-	-	-	-	0.13 ± 5 · 10 ⁻³	0.06 ± 6 · 10 ⁻²	-	0.08 ± 9 · 10 ⁻³
19	Fenchone	1076	1391	-	0.07 ± 2 · 10 ⁻²	-	0.08 ± 7 · 10 ⁻⁴	-	0.04 ± 8 · 10 ⁻³	0.07 ± 5 · 10 ⁻²	-	0.07 ± 3 · 10 ⁻²
20	Myrcenol	1103	1584	-	-	-	-	-	-	0.06 ± 4 · 10 ⁻²	-	-
21	Neomenthol	1156	1551	-	-	-	-	-	-	0.08 ± 7 · 10 ⁻²	-	-
22	2-Undecanone	1270	1599	0.13 ± 3 · 10 ⁻²	-	-	0.22 ± 2 · 10 ⁻²	-	0.01 ± 1 · 10 ⁻³	0.12 ± 1 · 10 ⁻¹	-	0.22 ± 7 · 10 ⁻²
23	Isobornyl acetate	1275	1582	0.08 ± 1 · 10 ⁻²	-	-	0.04 ± 8 · 10 ⁻³	-	0.04 ± 4 · 10 ⁻³	-	-	0.17 ± 2 · 10 ⁻¹
24	Carvacrol	1307	2177	-	-	-	0.17 ± 3 · 10 ⁻²	-	0.03 ± 1 · 10 ⁻³	0.21 ± 2 · 10 ⁻³	-	-
25	Isoamyl <i>n</i> -heptanoate	1333	-	-	0.12 ± 5 · 10 ⁻³	-	0.04 ± 4 · 10 ⁻³	-	-	-	-	0.12 ± 3 · 10 ⁻²
26	α -Copaene	1373	1497	0.18 ± 2 · 10 ⁻²	0.25 ± 5 · 10 ⁻²	-	0.03 ± 4 · 10 ⁻³	-	0.26 ± 1 · 10 ⁻²	0.11 ± 9 · 10 ⁻²	-	-
27	Isobornyl propionate	1376	1681	0.64 ± 1 · 10 ⁻²	-	-	-	-	-	-	-	-
28	β -Cubebene	1386	1542	0.09 ± 2 · 10 ⁻²	0.05 ± 1 · 10 ⁻²	-	0.05 ± 7 · 10 ⁻³	-	0.04 ± 3 · 10 ⁻³	0.10 ± 9 · 10 ⁻²	-	0.09 ± 6 · 10 ⁻³
29	<i>trans</i> -Caryophyllene	1419	1607	2.71 ± 3 · 10 ⁻¹	1.42 ± 2 · 10 ⁻¹	-	1.70 ± 3 · 10 ⁻¹	-	1.80 ± 1 · 10 ⁻¹	0.78 ± 7 · 10 ⁻¹	-	3.6 ± 1.00

30	Terpenyl propionate	1428	1747	$0.08 \pm 4 \cdot 10^{-2}$	$0.12 \pm 5 \cdot 10^{-2}$	$0.08 \pm 1 \cdot 10^{-2}$	$0.06 \pm 1 \cdot 10^{-2}$	-	$0.14 \pm 1 \cdot 10^{-1}$
31	α -Cedrene	1435	1601	$0.11 \pm 2 \cdot 10^{-2}$	$0.13 \pm 2 \cdot 10^{-2}$	$0.03 \pm 4 \cdot 10^{-4}$	$0.06 \pm 5 \cdot 10^{-3}$	$0.07 \pm 4 \cdot 10^{-2}$	$0.12 \pm 3 \cdot 10^{-2}$
32	α -Amorphene	1452	1687	$0.15 \pm 2 \cdot 10^{-2}$	$0.06 \pm 2 \cdot 10^{-3}$	$0.10 \pm 1 \cdot 10^{-2}$	$0.13 \pm 1 \cdot 10^{-3}$	-	$0.30 \pm 3 \cdot 10^{-1}$
33	α -Guaiene	1453	1668	$0.06 \pm 7 \cdot 10^{-3}$	$0.11 \pm 3 \cdot 10^{-2}$	$0.02 \pm 1 \cdot 10^{-3}$	$0.04 \pm 2 \cdot 10^{-3}$	$0.05 \pm 4 \cdot 10^{-2}$	$0.25 \pm 7 \cdot 10^{-2}$
34	β -Selinene	1464	1733	-	-	$0.09 \pm 2 \cdot 10^{-2}$	-	-	$1.84 \pm 2 \cdot 10^{-1}$
35	Humulene	1469	1699	$0.66 \pm 1 \cdot 10^{-1}$	4.71 ± 2.00	-	$0.28 \pm 3 \cdot 10^{-2}$	$0.27 \pm 1 \cdot 10^{-1}$	-
36	<i>cis</i> - β -Farnesene	1474	1622	$0.05 \pm 6 \cdot 10^{-3}$	-	-	$0.06 \pm 6 \cdot 10^{-3}$	-	-
37	(Z)-6-Pentadecen-1-ol	1480	1769	3.41 ± 1.00	$0.06 \pm 2 \cdot 10^{-3}$	$1.60 \pm 3 \cdot 10^{-1}$	$0.90 \pm 1 \cdot 10^{-2}$	$0.09 \pm 7 \cdot 10^{-2}$	$2.03 \pm 4 \cdot 10^{-3}$
38	Germacrene D	1486	1721	$2.53 \pm 3 \cdot 10^{-1}$	-	$0.11 \pm 2 \cdot 10^{-2}$	$0.14 \pm 2 \cdot 10^{-2}$	-	$0.29 \pm 8 \cdot 10^{-2}$
39	1-Pentadecene	1491	-	$0.39 \pm 1 \cdot 10^{-1}$	-	$0.22 \pm 3 \cdot 10^{-2}$	$0.18 \pm 1 \cdot 10^{-3}$	-	$0.50 \pm 4 \cdot 10^{-3}$
40	Germacrene B	1492	-	-	$0.99 \pm 3 \cdot 10^{-1}$	-	-	$0.37 \pm 3 \cdot 10^{-1}$	$0.44 \pm 9 \cdot 10^{-2}$
41	α -Bisabolene	1494	2007	$0.12 \pm 2 \cdot 10^{-2}$	$0.55 \pm 2 \cdot 10^{-1}$	$0.13 \pm 2 \cdot 10^{-2}$	$0.07 \pm 9 \cdot 10^{-3}$	$0.15 \pm 2 \cdot 10^{-1}$	-
42	β -Bisabolene	1500	1735	13.6 ± 1.00	7.4 ± 2.00	$2.30 \pm 3 \cdot 10^{-1}$	$7.07 \pm 6 \cdot 10^{-2}$	4.14 ± 3.00	$1.28 \pm 9 \cdot 10^{-3}$
43	α -Farnesene	1507	-	$0.06 \pm 1 \cdot 10^{-2}$	$0.13 \pm 5 \cdot 10^{-3}$	$0.04 \pm 3 \cdot 10^{-3}$	$0.07 \pm 2 \cdot 10^{-2}$	-	-
44	Citronellyl butyrate	1511	1765	$0.13 \pm 1 \cdot 10^{-2}$	-	-	-	-	-
45	β -Sesquiphellandrene	1514	1773	$0.12 \pm 1 \cdot 10^{-2}$	$0.08 \pm 1 \cdot 10^{-2}$	$0.03 \pm 4 \cdot 10^{-3}$	$0.08 \pm 8 \cdot 10^{-3}$	$0.18 \pm 1 \cdot 10^{-1}$	$0.09 \pm 1 \cdot 10^{-2}$
46	δ -Cadinene	1522	1780	$0.22 \pm 1 \cdot 10^{-1}$	1.1 ± 1.00	$0.15 \pm 2 \cdot 10^{-2}$	$0.15 \pm 7 \cdot 10^{-3}$	-	$0.08 \pm 2 \cdot 10^{-2}$
47	Geranyl butyrate	1531	1871	$0.20 \pm 5 \cdot 10^{-2}$	$0.50 \pm 1 \cdot 10^{-1}$	$0.10 \pm 2 \cdot 10^{-2}$	$0.12 \pm 3 \cdot 10^{-3}$	$0.24 \pm 2 \cdot 10^{-1}$	$0.09 \pm 2 \cdot 10^{-2}$
48	Nerolidol	1554	2043	-	$0.08 \pm 3 \cdot 10^{-2}$	$0.08 \pm 2 \cdot 10^{-3}$	-	$0.07 \pm 6 \cdot 10^{-2}$	$0.08 \pm 3 \cdot 10^{-2}$
49	Caryophyllene alcohol	1566	-	-	-	0.05 ± 0.00	-	$0.11 \pm 1 \cdot 10^{-1}$	$0.06 \pm 6 \cdot 10^{-2}$
50	Caryophyllene oxide	1567	2008	-	$0.24 \pm 9 \cdot 10^{-2}$	$0.09 \pm 3 \cdot 10^{-4}$	-	$0.60 \pm 5 \cdot 10^{-1}$	$0.28 \pm 1 \cdot 10^{-2}$
51	α -Cedrene epoxide	1586	1978	$0.15 \pm 1 \cdot 10^{-1}$	-	$0.02 \pm 3 \cdot 10^{-4}$	$0.11 \pm 4 \cdot 10^{-4}$	$0.07 \pm 4 \cdot 10^{-2}$	-
52	Geranyl isopentanoate	1600	1919	$0.10 \pm 1 \cdot 10^{-2}$	$0.10 \pm 1 \cdot 10^{-2}$	$0.04 \pm 7 \cdot 10^{-4}$	$0.08 \pm 4 \cdot 10^{-3}$	$0.06 \pm 4 \cdot 10^{-4}$	$0.08 \pm 9 \cdot 10^{-3}$
53	Citronellyl pentanoate	1606	1883	$0.07 \pm 3 \cdot 10^{-3}$	$0.08 \pm 2 \cdot 10^{-2}$	$0.09 \pm 2 \cdot 10^{-2}$	$0.11 \pm 9 \cdot 10^{-3}$	$0.06 \pm 4 \cdot 10^{-4}$	-
54	Cedrol	1609	2065	$0.09 \pm 8 \cdot 10^{-2}$	-	$0.03 \pm 9 \cdot 10^{-3}$	$0.11 \pm 2 \cdot 10^{-2}$	$0.20 \pm 2 \cdot 10^{-1}$	-
55	Terpenyl pentanoate	1616	1921	$0.14 \pm 7 \cdot 10^{-2}$	-	-	$0.13 \pm 8 \cdot 10^{-2}$	-	-
56	Not identified	1626	-	-	-	-	-	$0.11 \pm 1 \cdot 10^{-1}$	-
57	Cadalene	1641	-	-	-	-	-	$0.13 \pm 10 \cdot 10^{-2}$	-
58	Geranyl pentanoate	1642	1964	$0.07 \pm 9 \cdot 10^{-3}$	$0.05 \pm 9 \cdot 10^{-3}$	-	$0.10 \pm 9 \cdot 10^{-3}$	$0.10 \pm 5 \cdot 10^{-2}$	-
59	5-Phenyl-2,4-pentadienyl acetate	1654	-	$3.16 \pm 6 \cdot 10^{-1}$	13.9 ± 2.00	$8.75 \pm 9 \cdot 10^{-1}$	$2.64 \pm 2 \cdot 10^{-2}$	$0.14 \pm 2 \cdot 10^{-1}$	4.74 ± 2.00

60	Anisyl <i>n</i> -valerate	1665	-	$0.07 \pm 5 \cdot 10^{-3}$	$0.17 \pm 5 \cdot 10^{-2}$	$0.19 \pm 1 \cdot 10^{-3}$	$0.13 \pm 1 \cdot 10^{-2}$	$0.15 \pm 1 \cdot 10^{-1}$	$0.11 \pm 3 \cdot 10^{-3}$
61	<i>n</i> -Hexyl nonanoate	1668	-	$0.42 \pm 2 \cdot 10^{-1}$	$0.33 \pm 1 \cdot 10^{-1}$	$0.05 \pm 3 \cdot 10^{-3}$	$0.25 \pm 4 \cdot 10^{-3}$	$0.48 \pm 4 \cdot 10^{-1}$	$0.31 \pm 9 \cdot 10^{-2}$
62	1, E-11, Z-13-Octadecatriene	1685	-	$0.10 \pm 3 \cdot 10^{-2}$	-	-	-	-	-
63	Benzyl benzoate	1723	-	-	$0.80 \pm 8 \cdot 10^{-1}$	$0.65 \pm 4 \cdot 10^{-2}$	-	-	-
64	<i>N</i> -isobutyl-6Z,8E-decadienamide	1781	2687	$0.19 \pm 7 \cdot 10^{-2}$	$2.49 \pm 4 \cdot 10^{-1}$	$1.60 \pm 5 \cdot 10^{-2}$	-	$0.09 \pm 7 \cdot 10^{-2}$	$0.11 \pm 3 \cdot 10^{-2}$
65	Not identified	1792	-	-	$0.21 \pm 7 \cdot 10^{-2}$	$0.16 \pm 4 \cdot 10^{-3}$	-	$0.40 \pm 4 \cdot 10^{-1}$	$0.11 \pm 1 \cdot 10^{-2}$
66	Benzyl salicylate	1830	-	-	$0.24 \pm 1 \cdot 10^{-1}$	$0.81 \pm 1 \cdot 10^{-2}$	-	-	-
67	<i>N</i> -isobutyl-2E,6Z,8E-decatrienamide	1844	2834	$10.1 \pm 4 \cdot 10^{-2}$	21.4 ± 2.00	$17.1 \pm 2 \cdot 10^{-2}$	$3.09 \pm 3 \cdot 10^{-1}$	$1.37 \pm 9 \cdot 10^{-1}$	$1.50 \pm 3 \cdot 10^{-1}$
68	Nitrogenated compound	1850	-	-	-	-	$0.47 \pm 3 \cdot 10^{-2}$	-	-
69	Hexadecanoic acid	1951	-	$0.55 \pm 5 \cdot 10^{-2}$	$0.28 \pm 3 \cdot 10^{-2}$	$0.45 \pm 1 \cdot 10^{-2}$	-	-	$0.16 \pm 6 \cdot 10^{-2}$
70	Not identified	1853	-	$0.53 \pm 5 \cdot 10^{-2}$	$0.78 \pm 4 \cdot 10^{-2}$	$0.45 \pm 3 \cdot 10^{-2}$	-	$0.40 \pm 4 \cdot 10^{-1}$	$0.15 \pm 4 \cdot 10^{-2}$
71	Not identified	1861	-	$0.18 \pm 4 \cdot 10^{-2}$	$0.19 \pm 7 \cdot 10^{-2}$	$0.27 \pm 4 \cdot 10^{-3}$	-	$0.05 \pm 5 \cdot 10^{-2}$	-
72	Not identified	1887	-	$0.11 \pm 1 \cdot 10^{-2}$	$0.14 \pm 4 \cdot 10^{-2}$	$0.28 \pm 4 \cdot 10^{-2}$	-	-	-
73	<i>N</i> -(2-methylbutyl)-2E,6Z,8E-decatrienamide	1946	2546	$0.90 \pm 1 \cdot 10^{-1}$	$1.68 \pm 1 \cdot 10^{-1}$	$1.18 \pm 5 \cdot 10^{-2}$	$0.24 \pm 4 \cdot 10^{-2}$	$0.11 \pm 5 \cdot 10^{-2}$	$0.08 \pm 4 \cdot 10^{-2}$
74	Nitrogenated compound	1978	2489	$0.05 \pm 8 \cdot 10^{-1}$	$0.27 \pm 4 \cdot 10^{-2}$	$0.23 \pm 1 \cdot 10^{-1}$	-	-	-
75	<i>N</i> -(2-phenylethyl)-2E,6Z,8E-decatrienamide	2006	2290	$0.23 \pm 1 \cdot 10^{-1}$	$1.02 \pm 1 \cdot 10^{-1}$	$1.51 \pm 1 \cdot 10^{-2}$	-	-	-
76	Nitrogenated compound	2072	-	-	-	$0.18 \pm 5 \cdot 10^{-3}$	-	-	-
77	Hydrocarbon compound, C ₂₁	2098	2127	-	$0.39 \pm 3 \cdot 10^{-1}$	$0.14 \pm 3 \cdot 10^{-2}$	-	$0.57 \pm 5 \cdot 10^{-1}$	-
78	Hydrocarbon compound, C ₃₃	2292	-	-	$0.13 \pm 2 \cdot 10^{-2}$	$1.45 \pm 6 \cdot 10^{-2}$	-	-	$0.15 \pm 3 \cdot 10^{-2}$
79	Nitrogenated compound	2351	2907	-	$0.42 \pm 2 \cdot 10^{-1}$	$0.48 \pm 2 \cdot 10^{-3}$	-	-	-
80	Hydrocarbon compound, C ₂₁	2392	-	-	-	$0.40 \pm 3 \cdot 10^{-3}$	-	-	-
81	Hydrocarbon compound, C ₃₅	2493	-	$0.06 \pm 2 \cdot 10^{-2}$	$0.09 \pm 5 \cdot 10^{-2}$	$2.82 \pm 2 \cdot 10^{-2}$	$0.14 \pm 4 \cdot 10^{-2}$	-	$0.18 \pm 2 \cdot 10^{-2}$
82	Hydrocarbon compound, C ₂₆	2590	-	$0.14 \pm 1 \cdot 10^{-2}$	$0.14 \pm 5 \cdot 10^{-2}$	$0.18 \pm 1 \cdot 10^{-3}$	-	-	-
83	Not identified	2607	-	-	-	$0.15 \pm 7 \cdot 10^{-3}$	-	-	-
84	Hydrocarbon compound, C ₂₇	2691	-	-	-	$1.68 \pm 2 \cdot 10^{-2}$	-	-	-
85	Hydrocarbon compound, C ₂₈	2790	-	-	-	$0.21 \pm 3 \cdot 10^{-3}$	-	-	-
86	Hydrocarbon compound, C ₂₉	2936	-	-	$0.14 \pm 6 \cdot 10^{-2}$	$2.84 \pm 6 \cdot 10^{-2}$	-	-	-
87	Hydrocarbon compound, C ₃₀	3077	-	-	-	$1.38 \pm 2 \cdot 10^{-3}$	-	-	-
88	Hydrocarbon compound, C ₁₂	3252	-	-	-	$1.38 \pm 2 \cdot 10^{-2}$	-	-	-
Total extracted material				52.2 ± 6.20	66.0 ± 13.31	63.2 ± 3.60	31.4 ± 1.30	23.1 ± 13.1	44.7 ± 7.00

^a Experimentally determined Kováts indexes.

^b Percentages were calculated from peak areas determined on the DB-1 column and were averages of three independent extractions for each part of the plant material.

25 ml min⁻¹ (NPD). The injection volume was 0.5 μ l.

A HP 5890A Series II gas chromatograph interfaced to an HP 5972 mass-selective detector with an HP MS ChemStation Data system was used for MS identification of the GC components. The column used was a DB-1 (J&W Scientific) cross-linked fused-silica capillary column (30 m \times 0.25 mm I.D.) coated with polydimethylsiloxane (0.25 μ m phase thickness). The oven was programmed from 50°C (5

min hold) at 3.5 C° min⁻¹ to 250°C. The helium inlet pressure was 78 kPa, with linear velocity 38 cm min⁻¹ (split 10 ml min⁻¹). The injector temperature was kept at 250°C and the volume injected was 0.5 μ l. The temperatures of the ionization chamber and of the transfer line were 180°C and 285°C, respectively. The electron beam energy was 70 eV. Mass spectra and reconstructed chromatograms were obtained by automatic scanning in the mass range m/z : 50–400 at 2.2 scan s⁻¹. Chromatographic peaks

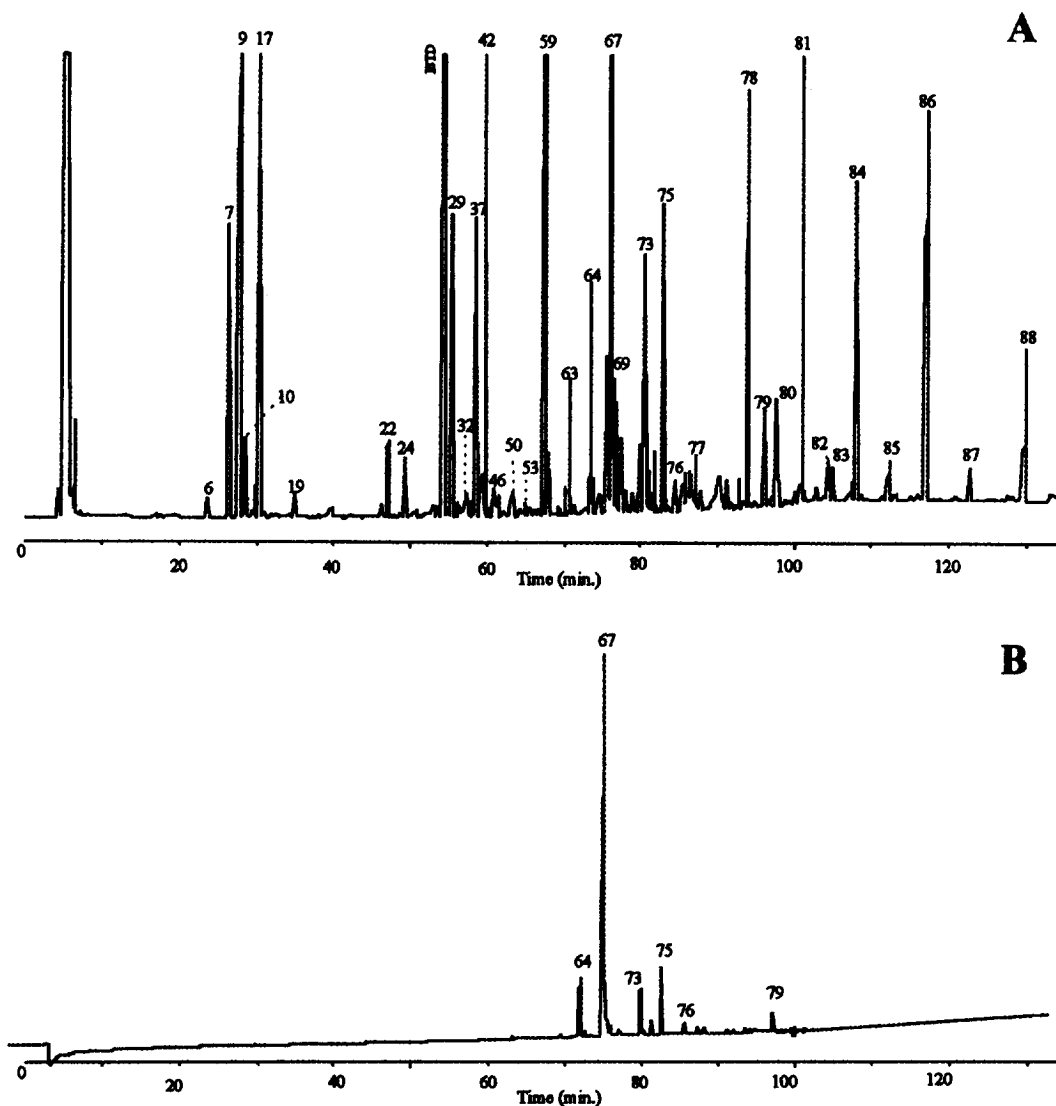


Fig. 2. Typical gas chromatograms, obtained on the DB-1 (60 m) column, fitted with (A) FID (Attn. 0) and (B) NPD (Attn. 5), of the extracts isolated from *S. americana* flowers by SFE.

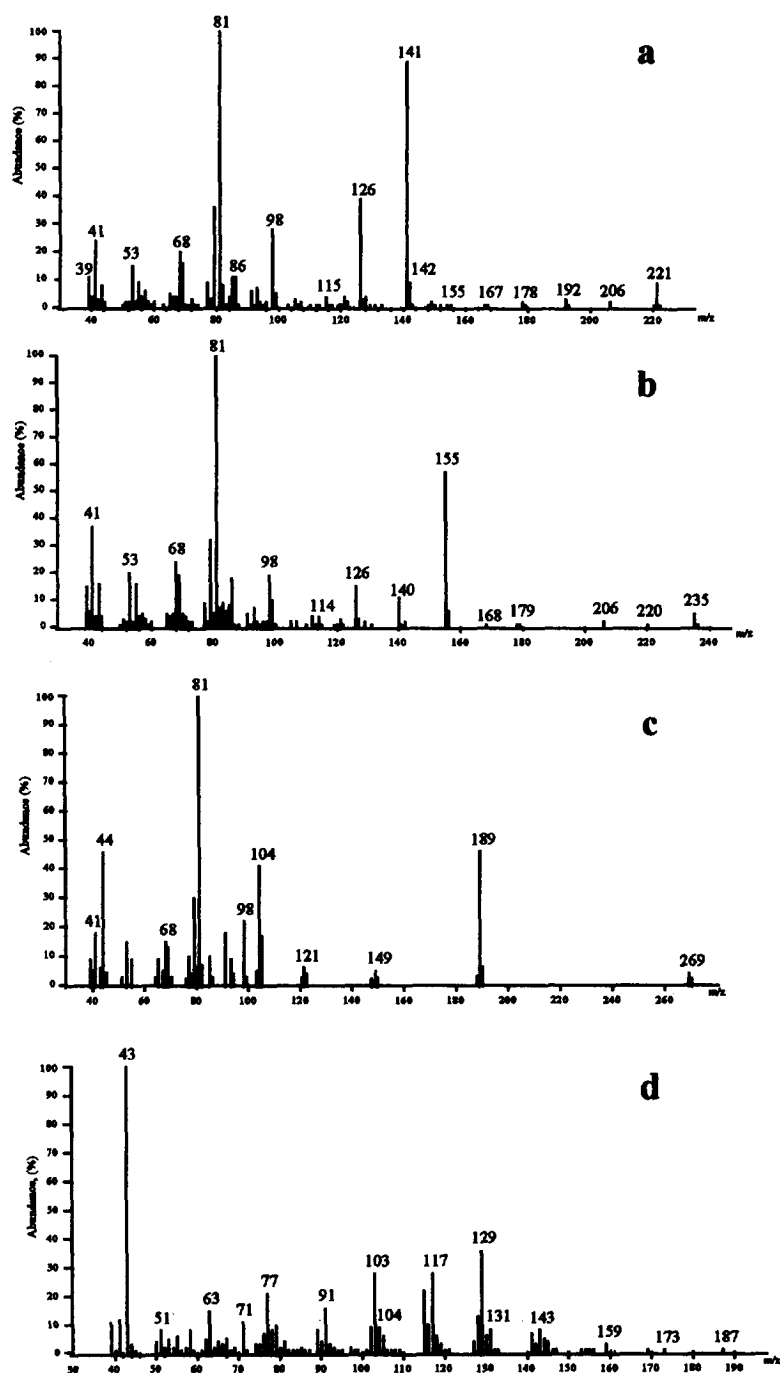


Fig. 3. Mass spectra (EI, 70 eV): (a) N-isobutyl-2E,6Z,8E-decatrienamide (spilanthol); (b) N-(2-methylbutyl)-2E,6Z,8E-decatrienamide; (c) N-(2-phenylethyl)-2E,6Z,8E-decatrienamide and (d) 5-phenyl-2,4-pentadienyl acetate.

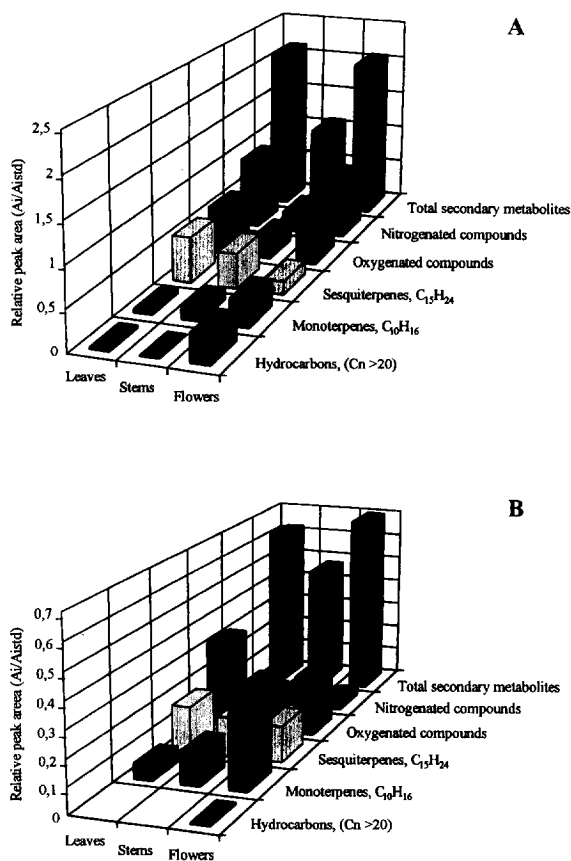


Fig. 4. Compositional variation of Colombian *Spilanthes americana* extracts obtained by (A) SFE and (B) SDE from different parts of the plant.

were checked for homogeneity with the aid of the mass chromatograms for the characteristic fragment ions. NBS75K and WILEY138 Data bases were used for automatic identification of GC peaks.

3. Results and discussion

Fig. 1 shows typical chromatographic profiles of the volatile secondary metabolites isolated by SDE or SFE from different parts of Colombian *S. americana*. The various compounds were identified using both chromatographic (Kováts indexes, DB-1 and DB WAX 60 m columns) [10,11] and spectroscopic (mass spectra, EI, 70 eV) criteria [12]. While only 46, 48 and 50 compounds were detected at concentrations above 100 ppb in the SDE extracts from

flowers, leaves and stems, respectively, the secondary metabolite mixtures isolated by SFE from these parts contained 67, 51 and 53 compounds. A total of 80 out of 88 different secondary metabolites detected in the Colombian *S. americana* at concentrations higher than 100 ppb were positively identified by means of HRGC using FID, NPD or MS. Table 1 contains the chemical composition found for the various extracts under study.

Fig. 2 shows chromatographic profiles of the SFE extract from flowers, on the DB-1 (60 m) column fitted with (A) FID (Attn. 0) and (B) NPD (Attn. 5). Mass spectra of the main nitrogenated compounds (a) N-(isobutyl)-2*E*,6*Z*,8*E*-decatrienamide (*spilanthol*), (b) N-(2-methylbutyl)-2*E*,6*Z*,8*E*-decatrienamide, and (c) N-(2-phenylethyl)-2*E*,6*Z*,8*E*-decatrienamide) are shown in Fig. 3. One of the principal compounds, detected at concentrations of 13.9%, 8.8% and 3.2% in the SDE extracts, and of 7.3%, 4.7% and 2.6% in the SFE extracts from leaves, flowers and stems, respectively, was identified tentatively as 5-phenyl-2,4-pentadienyl acetate, C₁₃H₁₄O₂ (Fig. 2, peak 59), from its mass spectrum, *M*_r 202, *M*⁺ (-), [M-CH₃]⁻ *m/z* 187 (3), [M-CH₃CO]⁺ *m/z* 159 (4), [M-OCOCH₃]⁺ *m/z* 143 (9), CH₃CO⁺ *m/z* 43 (100) and aromatic fragments *m/z* 77 (19), 91 (16), 103 (31), 115 (13), 117 (30) and 129 (38) (Fig. 3d).

The extraction method (SDE, SFE) and the source of the plant material (flowers, leaves, stems) affected the composition of the extracts as has been shown previously for *Cananga odorata* [13,14] and *Ruta graveolens* [15]. The extracts isolated by SDE or SFE from different parts of *S. americana* differed both quantitatively and qualitatively. In order to appreciate these variations, the results from Table 1 were grouped in Fig. 4 into compound families: monoterpenes, C₁₀H₁₆; sesquiterpenes, C₁₅H₂₄; oxygenated compounds (alcohols, aldehydes, esters); nitrogen containing compounds (amides) and hydrocarbons (C_n>20).

SFE extracts from stems were rich (>40%) in sesquiterpenes (α - and β -bisabolenes, caryophyllene and cadinenes), while those from leaves and flowers were abundant in nitrogenated (43 and 27%) and oxygenated (36 and 23%) compounds. *Spilanthol* was the main compound in the SFE extracts from flowers (17.03%) and leaves (21.4%).

SDE extracts from stems, leaves, and flowers of *S.*

americana contained sesquiterpene levels of 32, 28 and 20%, and a generally higher proportion of oxygenated compounds (28, 52 and 32%) and monoterpenes (27, 10 and 42%) than their SFE counterparts. Only trace-level contents of nitrogenated compounds were found using NPD in SDE extracts. Some heavy hydrocarbons ($C_n > 20$) originating from flower pigments and waxes were isolated by SFE, but not by SDE. SFE was both selective and highly efficient in the isolation of sesquiterpenes, heavy hydrocarbons and nitrogenated compounds (amides). The total amount of secondary metabolites obtained by SFE was between 1.5 and 2 times larger than that obtained by SDE for each type of plant material.

4. Conclusions

The volatile secondary metabolites from flowers, leaves and stems of the Colombian *S. americana* were obtained by SDE and SFE, using a J&W Scientific high pressure soxhlet extractor. The chemical composition of the extracts obtained by different techniques varied both quantitatively and qualitatively. SDE extracts possessed a higher content of oxygenated compounds and monoterpenes. The SFE method was more selective and particularly efficient for the isolation of sesquiterpenes and nitrogen containing compounds (amides) and afforded a higher yield (1.5–2 times) of total secondary metabolites than SDE. The use of these extraction methods combined with HRGC analysis employing different detection systems (FID, NPD or MS), permitted a thorough description of the secondary metabolite profile of *S. americana*. This methodology can be applied to many other aromatic plant studies.

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