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Cytotoxic activity of Asteraceae and Verbenaceae family essential oils

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Thirty-six essential oils from Verbenaceae and Asteraceae family plants along with their major constituents were evaluated to determine cytotoxicity on Jurkat, HeLa, HepG2 and Vero cell lines. The plants were collected in different regions of Colombia, and their essential oils were extracted through microwave-assisted hydrodistillation and then characterized through GC/MS and GC/FID analyses. Cytotoxic activity testing was carried out using a tetrazolium-dye (MTT) assay. The essential oils from *Lippia alba* Citral and Carvone chemotypes showed the lowest IC_{50} and selectivity index (IS) > 10. The essential oils from Asteraceae family were not selective against the tumor cell lines tested. Citral chemotype *L. alba* essential oils showed values up to 27.2, and Carvone chemotype *L. alba* essential oils showed values up to 30.7. Citral, showed the highest cytotoxic activity, with an SI value of 1241, and Limonene and Linalool showed SI values of 6.97 and 10.1, respectively, on HeLa cells.

Keywords: *Lippia* sp; cytotoxicity; Verbenaceae

Introduction

Drugs currently used for treatment of cancer cause a variety of effects including cardiac, pulmonary, neurological and renal toxicity. Furthermore, some of these treatments have limited anticancer activities (1). Therefore, it is necessary to discover new anticancer agents that are more selective and less toxic than those currently used.

Plants have been an important source of anticancer drugs, considering that approximately 60% of the chemotherapeutic drugs currently approved for cancer treatment have been isolated from plants (2). They include, among others, vinca alkaloids (Vinblastine, Vincristine, Vinorelbine and Vinflunine) (2), taxanes (Taxol, Docetaxel) (3), camptotheca alkaloids (Camptothecin) (4), podophyllum lignans (Podophyllotoxin, Etoposide, Teniposide) (2) and polyphenols (Curcumin) (2). Currently, there are several plant-derived compounds that are being tested in clinical trials (5).

The essential oils from Verbenaceae and Asteraceae family plants have shown anti-inflammatory, antimicrobial and antioxidant activity (6, 7); however, little is known about their cytotoxic activity. Verbenaceae family consists of herbs, trees and shrubs; it includes the genus *Lippia*, which is distributed throughout South and Central America, and Tropical Africa (8). In traditional medicine, it has been used for treatment of gastrointestinal and respiratory disorders, and as anti-inflammatory agent (6). Previous studies carried out in our laboratory have uncovered cytotoxic activity of essential oils from *Lippia origanoides* and *Lippia alba* against Human cervix epitheloid carcinoma (HeLa) cells at concentrations of 9.1 and 3.5 μg/ml, respectively (9, 10).

Asteraceae family plants are in almost all continents, except Antarctica (11), and they have been used in traditional medicine for the treatment of infections and skin disorders (7). However, there are few studies about the cytotoxic activity of essential oils from this family. Maggy et al. (7) found cytotoxic activity of flower essential oil from *Achillea ligustica* against multiform glioblastoma (T98G), squamous carcinoma (A431), prostatic adenocarcinoma (PC-3), melanoma (B16-F1) and murine cell lines (5) at high concentrations corresponding to 376, 446, 294 and 220 μg/ml, respectively.

Taking into account the few studies that have been reported about the cytotoxic activity of essential oils from Asteraceae and Verbenaceae plants, the aim of this study was to evaluate the cytotoxic activity of essential oils from the Asteraceae and Verbenaceae families, and their main components on the HeLa, hepatocellular carcinoma cells (HepG2), Vero and Jurkat cell lines.

Experimental

Cell lines

The cell lines were obtained from the American Type Culture Collection (ATCC). HeLa cells (HeLa cell line

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ATCC CCL-2), HepG2 cells (HepG2 cell line ATCC HB-8065) and *Cercopithecus aethiops* African green monkey kidney cells (Vero cell line ATCC CCL-81) were grown in MEM, supplemented with a 10% FBS, 100 units/mL of penicillin, 100 µg/mL of streptomycin, 20 µg/mL of glutamine, 0.14% $NaHCO_3$ and 1% each of non-essential amino acids and vitamin solution. Acute T cell leukaemia human cells (Jurkat cell line ATCC TIB-152) were grown in RPMI-1640 (Sigma Chemical Co, St Louis, MI, USA), supplemented with 10% FBS, 100 units/mL of penicillin and 100 mg/mL of streptomycin. Cells were maintained at 37°C in a 5% CO_2 atmosphere.

Plant material

Stems and leaves of thirty-eight plants from the Asteraceae and Verbenaceae families (Table 1), were collected in different regions of Colombia as part of a survey conducted by CENIVAM, a Research Centre devoted to the study of aromatic and medicinal plants in Colombia. Taxonomic identification of the botanical samples was performed by Dr. Jose Luis Fernandez at the National Herbarium of Colombia (Instituto de Ciencias Naturales, Faculta de Ciencias, Universidad Nacional de Colombia-Bogota), where voucher specimens were deposited.

Essential oil extraction

The essential oils were extracted from dried stems and leaves (300 g) through microwave-assisted hydrodistillation (30 min, 250 mL water), using a Clevenger-type distillation apparatus and a Dean-Stark distillation trap in a domestic microwave oven (Kendo MO-124, 2.5 GHz, 800 W), as described previously (12). Sodium sulphate (Merck, Darmstadt, Germany) was added to the decanted essential oil as a drying agent.

Essential oil analyses

Compound identification was determined by means of GC/MS and GC/FID, as previously described (12). For GC/MS, the essential oils were analysed based on mass spectra (EI, 70 eV) obtained with a gas chromatograph (Agilent Technologies 6890 Plus, Palo Alto, CA, USA), that was equipped with a mass selective detector (Agilent Technologies MSD 5973), a split/splitless injector (250°C, 1:30 split ratio) and a data system (HP ChemStation 1.05), with WILEY 138K, NIST 2002 and QUADLIB 2004 mass spectra libraries. A DB-5MS fused-silica capillary column (J & W Scientific, Folsom, CA, USA) of 60 m (L) × 0.25 mm × 0.25 µm (d_f) was employed. The GC oven temperature was programmed from 45°C (5 min) at 4° C/min to 150°C (2 min) at 5°C/min to 250°C (5 min), and finally to 275°C (15 min) at 10°C/min. Mass spectra and reconstructed ion currents (chromatograms) were obtained through automatic scanning at 5.19 scans/s within the mass range *m*/*z* 30–300. Chromatographic peaks were checked for their homogeneity with the aid of the mass chromatograms for the characteristic fragment ions.

A gas chromatograph (HP 5890 A Series II), equipped with a flame ionization detector (FID), a split/splitless injector (250°C, 1:30 split ratio) and a data system (HP ChemStation HP Rev. A.06.03 [509]), was used for GC/FID analyses of the oils and quantification of their components. The detector and injector temperatures were set at 250°C. The same capillary column, as for the GC/MS analyses was used for GC/FID separation and detection. The oven temperature was programmed from 40°C (15 min) to 250°C (20 min) at 5° C/min. Helium was used as the carrier gas, with 152 kPa column head pressure and 35.7 cm/s linear velocity. Hydrogen and air at 30 and 300 mL/min, respectively, were utilized in the FID, with N_2 (30 mL/min) as a make-up gas. The various compounds were identified by linear programme retention indices, determined using a non-logarithmic scale on both DB-5 and DB-WAX capillary columns, and by comparison of mass spectra with those of standards and reported data (13–16).

Monoterpenes

R(−) Carvone, Citral, Nerol, Geraniol, Linalool, (−) trans-β-caryophyllene and R(+) limonene were purchased from Sigma (Chemical Company St Louis, MO, USA). Stock solutions of all essential oils and monoterpenes were prepared in DMSO.

Assessment of percentage of inhibition in cell growth by MTT assay

Measurement of cell proliferation inhibition on Vero, HeLa, HepG2 and Jurkat cells was carried out using tetrazolium-dye (MTT), according to the protocol previously described, with a few modifications (17). Vero, HepG2 and HeLa cells were trypsinized and washed with phosphate-buffered saline, and then plated at 1.35×10^4, 1.8×10^4 and 1.25×10^4 cells per well, respectively, in a 96-well flat-bottomed plate. After 24 hours of incubation, each diluted essential oil or monoterpene was added to the appropriate wells at final concentrations between 3.125 and 200 µg/mL, and the plates were incubated for further 48 hours at 37°C in a humidified incubator with 5% CO_2. Jurkat cells were centrifuged to remove medium, and they were again suspended in culture medium and plated at 3×10^4 cells per well in a 96-well round-bottomed plate, and

Table 1. Geographic origin and voucher number of Verbenaceae and Asteraceae plants from which essential oils were obtained.

Plant name	Plant code	Place of collection	Voucher specimen
Lippia alba Citral chemotype	A01	Bucaramanga, Santander	512077a
	A02	Colorado, Bolivar	512272
	A03	Bucaramanga, Santander	512077a
	A04	Bucaramanga, Santander	512077a
	A05	Bucaramanga, Santander	512077a
	A06	Bucaramanga, Santander	512077a
	A07	Bucaramanga, Santander	512077a
Lippia alba Palmira	C01	Palmira, Valle del Cauca	512077
Lantana camara	C02	Quibdo, Choco	520293
Lippia alba	C03	Cali, Valle del Cauca	512078
Lippia alba	C04	San Jeronimo, Tolima	484650
Lippia alba Carvona chemotype	B01	Bucaramanga, Santander	512078b
	B02	Bucaramanga, Santander	512078b
	B03	Bucaramanga, Santander	512078b
	B04	Bucaramanga, Santander	480750
	B05	Bucaramanga, Santander	512078b
Lippia alba	D01	Suaita, Santander	517306
Lippia citriodora	D02	Armenia, Quindío	480749
Lippia origanoides	D03	Soata, Boyacá	517741
Lippia alba	D04	Anolaima, Cundinamarca	484650
Lippia micromera Schauer	D05	Manaure, Cesar	516924
Lippia origanoides	D06	Bucaramanga, Santander	512075
Lippia alba Agroince	D07	San Martin, Cesar	480750
Lippia alba	D08	Puerto Berrio, Antioquia	512082
Lippia alba	D09	Flandes, Tolima	484350
Lippia alba	D10	Anolaima, Cundinamarca	484650
Lippia alba	D11	Flandes, Tolima	484350
Lippia alba	D12	Anolaima, Cundinamarca	484650
Lippia alba	D13	Flandes, Tolima	484650
Lippia alba	D14	Cachipai, Cundinamarca	484650
Conyza bonariensis	T01	Manaure, Cesar	517011
Montanoa ovalifolia	T02	Tibasosa, Boyacá	517718
Condylidium iresinoides	T03	Paz del Río, Boyacá	517735
Stevia aff. lucida Lag	T04	Molagavita, Santander	517758
Ambrosia arborescens	T05	Ipiales, Nariño	519599
Achyrocline alata	T06	Potosí, Nariño	519601

diluted compounds were added to plates and incubated for 48 hours at 37°C.

Samples were dissolved initially in DMSO, and they were further diluted in medium for cell culture experiments, resulting in a final concentration of 0.05% DMSO in biological assays. Cell controls with DMSO at 0.05% were used. Finally, spectrophotometric reading was carried out at 570 nm to determine the concentration for each compound that inhibits 50% of growth (IC_{50}).

Statistical analyses

IC_{50} was calculated from dose–effect curves through linear regression methods, and values were presented as the mean ± standard deviation of at least four dilutions by quadruplicate. To define which compounds were more selective to cancerous cells than to non-cancerous cells, a selectivity index (SI) was calculated defined as Vero IC_{50} over Jurkat, HepG2 or HeLa IC_{50}. A compound with a SI $\geqslant$ 10 was considered active.

Results and discussion

In vitro essential oils obtained from plants have shown different kinds of biological activity, including antioxidant, antimicrobial (18, 19) and cytotoxic (20). Essential oils from Verbenaceae and Asteraceae family plants have been used in traditional medicine for treatment of different diseases (6–8) and they have shown cytotoxic activity *in vitro* (7, 9, 10). In this study, the effect on proliferation of thirty-six essential oils from plants of these families, and their main components against Vero, HeLa, HepG2 and Jurkat cell lines was evaluated using MTT assays.

According to the National Cancer Institute (USA), a crude extract is cytotoxic when its IC_{50} on normal cells is below 30 μg/mL (6); therefore, none of the tested essential oils was cytotoxic, excepting the essential oil from *Ambrosia arborescens* (T06), which showed a IC_{50} value of 15.7 ± 3.4 μg/mL (20) and was not active against the tested tumor cell lines (Table 2).

Table 2. Inhibitory concentration 50 (IC_{50}) of essential oils against Vero, HeLa, HepG2 and Jurkat cell lines.

	Cytotoxic activity									
	Vero		HeLa			HepG2			Jurkat	
Essential oil	$IC_{50a} \pm DS_c$	$R2_b$	$IC_{50} \pm DS$	R2	IS_d	$IC_{50} \pm DS$	R2	IS	$IC_{50} \pm DS$	IS
Verbenaceae family										
A01	110.6 ± 2.5	1	80.5 ± 8.5	0.95	1.3	39.4 ± 7.1	0.73	2.8	23.5 ± 6.7	0.8
A02	50.5 ± 9.2	0.78	28.9 ± 1.3	0.99	1.7	38.6 ± 4	0.91	1.3	16.6 ± 2.9	0.85
A03	119.4 ± 27.8	0.7	39.3 ± 4.4	0.89	3.0	⩾200	–	–	⩾31.6	–
A04	68 ± 9.3	0.91	6.1 ± 1.7	0.76	11.1	67.9 ± 13.9	0.79	1	28.6 ± 3.9	0.9
A05	118 ± 6.6	0.97	7.5 ± 1.1	0.89	15.7	140.6 ± 21	0.81	0.8	⩾31.6	–
A06	136.2 ± 9.2	0.96	5 ± 1.5	0.73	27.2	77.7 ± 16.2	0.79	1.7	22.3 ± 1.5	0.9
A07	133.3 ± 9.3	0.96	12.7 ± 2.5	0.78	10.4	115.9 ± 10.7	0.93	1.1	⩾31.6	–
B01	35.6 ± 11.2	0.75	33.8 ± 3.3	0.92	1.0	⩾200	–	–	⩾31.6	–
B02	144 ± 7.4	0.98	32.2 ± 8.3	0.85	4.4	121.1 ± 7.7	0.97	1.1	25 ± 3.3	0.9
B03	34.9 ± 5.1	0.82	>200	–	–	21.5 ± 10.2	0.71	–	⩾31.6	–
B04	⩾200	–	6.5 ± 1.5	0.82	30.7	⩾200	–	–	⩾31.6	–
B05	129.2 ± 15.3	0.88	30.4 ± 4.8	0.79	4.2	89.2 ± 6	0.98	1.4	⩾31.6	–
C01	35.9 ± 5	0.84	>200	–	–	67.7 ± 10.2	0.89	0.5	⩾31.6	–
C02	100.2 ± 9.6	0.94	115.2 ± 18.8	0.79	0.8	⩾200	–[e]	–	⩾31.6	–
C03	103.3 ± 4	0.99	>200	–	–	135.7 ± 23.8	0.74	0.7	⩾31.6	–
C04	12.3 ± 2.9	0.77	122.1 ± 28.1	0.76	0.1	30.9 ± 4.3	0.84	0.3	NE	
D01	39 ± 2.9	0.95	>200	–	–	>200	–	–	⩾31.6	–
D02	116.9 ± 6.6	0.97	40.7 ± 6	0.82	2.87	>200	–	–	18.2 ± 1.8	0.95
D03	52.3 ± 11.5	0.8	106.7 ± 9	0.95		24.5 ± 4.4	0.79	2.1	⩾31.6	–
D04	99.9 ± 3.8	0.99	>200	–	–	97.8 ± 18.7	0.77	1	⩾31.6	–
D05	129.1 ± 20	0.8	>200	–	–	17.5 ± 4.2	0.72	7.3	⩾31.6	–
D06	34.4 ± 5.9	0.76	48.8 ± 10.8	0.81	0.7	130.4 ± 2.2	0.76	0.2	⩾31.6	–
D07	>200	–	>200	–	–	>200	–	–	⩾31.6	–
D08	>200	–	>200	–	–	94.3 ± 12.7	0.89	2.1	⩾31.6	–
D09	⩾200	–	46.3 ± 11.4	0.78	4.3	⩾200	–	–	⩾31.6	–
D10	36.2 ± 6.1	0.76	12.5 ± 2.6	0.72	2.8	39.5 ± 1.8	0.98	0.9	⩾31.6	–
D11	36.2 ± 6.4	0.74	>200	–	–	34.2 ± 5.9	0.76	1	⩾31.6	–
D12	126.5 ± 12.6	0.91	>200	–	–	35.1 ± 6	0.76	–	⩾31.6	–
D13	116.7 ± 12.9	0.9	>200	–	–	>200	–	–	NE	
D14	32.8 ± 3.6	0.89	>200	–	–	134.9 ± 19.5	0.82	0.24	⩾31.6	–
Asteraceae family										
T01	70 ± 16.1	0.73	96.4 ± 17.6	0.76	0.7	117.4 ± 18.6	0.8	0.59	⩾31.6	–
T02	30.2 ± 5.4	0.76	73.8 ± 14.7	0.8	0.4	>200	–	–	⩾31.6	–
T03	>200	–	111 ± 5.7	0.98	1.8	>200	–	–	⩾31.6	–
T04	94.7 ± 7	0.93	31.6 ± 5.8	0.74	2.9	>200	–	–	⩾31.6	–
T05*	15.7 ± 3.4	0.78	10.9 ± 2.5	0.74	1.4	104.5 ± 16.4	0.82	0.1	13.5 ± 6.2	0.9
T06	73.8 ± 17.3	0.82	14 ± 2	0.83	5.2	75.7 ± 18.8	0.76	0.9	⩾31.6	–

(*Continued*)

Table 2. (*Continued*)

	Cytotoxic activity									
	Vero		HeLa			HepG2			Jurkat	
Essential oil	$IC_{50a} \pm DS_c$	$R2_b$	$IC_{50} \pm DS$	R2	IS_d	$IC_{50} \pm DS$	R2	IS	$IC_{50} \pm DS$	IS
Monoterpenes										
Citral*	124.1 ± 12.2	0.92	<0.1	0.87	1241	35.2 ± 1.2	0.99	3.5	25.1 ± 0.5	0.99
R (+) limonene**	154.1 ± 14.4	0.93	22.1 ± 1.2	0.98	6.97	⩾200	–	–	⩾31.6	–
Linalool	>200	–	>200	–	–	>200	–	–	19.8 ± 2.8	0.9

Notes: HeLa, human cervix epitheloid adenocarcinoma cells ATCC CCL-2; HepG2, hepatocellular carcinoma cells ATCC HB-8065; Jurkat, Acute T cell leukaemia human cells Vero, *Cercopithecus aethiops* African green monkey kidney cells ATCC CCL-81. [a]Concentration of compounds that induces 50% growth inhibition in 48 hours. [b]Coefficient of determination of linear regression. [c]Standard deviation. [d]Selectivity index is defined as VERO IC_{50} over either HepG2, Jurkat or HeLa IC_{50}. [e]Not calculated. *Previously report by Mesa-Arango et al. (10). **Previously report by Zapata et al. (9).

In a previous study of antifungal activity carried out in our laboratory, we evaluated the cytotoxic activity of essential oils from Asteraceae family plants against a Vero non-tumour cell line. The essential oils of *Conyza bonariensis* (T01), *Montanoa ovalifolia* (T02), *Condylidium iresinoides* (T03), *Stevia aff. lucida Lag.* (T04), *Achyrocline alata* (T06) (21) and *Lippia citriodora* (D02) (22) showed IC_{50} values of 70 ± 16.1 μg/ml, 30.2 ± 5.4 μg/ml, ⩾200 μg/ml, 108.6 ± 5.5 μg/ml, 94.7 ± 7 μg/ml, 73.8 ± 17.3 μg/ml and 116.9 ± 6.6 μg/ml, respectively. None of these essential oils evaluated for cytotoxic activity on the tumour cell lines in this study showed values of IS ⩾ 10 against Jurkat, HeLa or HepG2 cells (Table 3).

The cytotoxic activity of essential oils from Asteraceae family has been demonstrated in a study carried out by Maggy et al. (7), which found that essential oil from *A. ligustica* (Asteraceae) was cytotoxic against several tumour cell lines, but its lowest IC_{50} value was of 220 μg/ml against the B16-F1 cell line; furthermore, the selectivity of its cytotoxic activity to tumour and non-tumour cell lines was not evaluated. In contrast, the lowest IC_{50} value found in this study with essential oils from the Asteraceae family was 10.9 μg/ml, shown by *A. arborescens* (T05) against HeLa cells; however, this activity was not selective against tumour cell lines.

About the Verbenaceae family, none of tested essential oils showed a SI ⩾ 10 against Jurkat or HepG2 cells (Table 2), but all the essential oils produced dose-dependent inhibition on the growth of HeLa cells with R2 (coefficient of determination) > 0.7. Essential oils from *L. alba* (C01, C03, D01, D04, B03, D07, D08, D11, D12, D13, D14) and *Lippia micromera Schauer* (D05) showed SI ⩽ 10 against HeLa cells. Only five of thirty-two samples of the Verbenaceae family were active against HeLa cells, corresponding to four essential oils of the *L. alba* Citral chemotype (A04, A05, A06, A07) and one oil of the *L. alba* Carvone chemotype (B04); they showed the lowest IC_{50} values and the highest SI on HeLa cells, corresponding to 11.1, 15.7, 27.2, 10.4 and 30.7, respectively (Table 2). Therefore, the essential oils from genus *Lippia* were active only against HeLa cells, which could be due to the presence of a target on HeLa that is not present on HepG2 and Jurkat cells.

Although the use of *Lippia* genus plants is known in traditional medicine (8), there have been few studies on their cytotoxic activity. Previous studies conducted by us demonstrated the cytotoxic activity of the *L. alba* citral chemotype and *L. origanoides* against HeLa cells, with IC_{50} values of 3.5 ± 0.7 and 9.1 μg/ml (9, 10), respectively. These results are similar to those found in this study.

The chromatographic analyses of active essential oils from the Verbenaceae family were carried out in a

Table 3. Essential oil composition (%) of *Lippia alba* oils active against HeLa cells.

No	Compound	RI_a	RI_b	B04	Relative amount (%) A04	A05*	A06*	A07*
1	α-pinene	939	932	0.1	0.1	–[c]	–	–
2	Camphene	954	946	0.2	0.1	–	–	–
3	1-octen-3-ol	979	974	0.1	0.2	–	–	–
4	6-methyl-5-hepten-2-one	986	986	–	4.3	–	–	–
5	β-myrcene	991	988	0.7	0.1	–	–	–
6	Isopentyl isobutanoate	1015	1013	0.1	0.1	–	0.05	0.05
7	Limonene	1037	1024	23.1	3.7	2.0	2.2	1.9
8	*trans*-β-ocimene	1048	1044	0.7	0.4	0.3	0.4	0.2
9	Linalool	1100	1095	0.5	1.9	1.5	1.4	1.4
10	Citronellal	1154	1148	–	0.8	0.9	0.7	0.9
11	*cis*-crisantenol	1162	1159	–	0.5	–	–	–
12	Rose furan epoxide	1171	1173	–	0.4	–	–	–
13	Borneol	1180	1165	0.9	0.9	–	–	–
14	*cis*-dihydrocarvone	1203	1191	0.3	–	–	–	–
15	*trans*-dihydrocarvone	1212	1200	0.3	–	–	–	–
16	*trans*-carveol	1230	1215	0.6	–	–	–	–
17	Nerol	1231	1227	–	3.9	1.4	1.3	2.1
18	Neral	1236	1235	–	22.6	23.1	22.1	20.0
19	Geraniol	1248	1249	–	5.4	8.0	8.7	9.4
20	Geranial	1252	1264	–	28.8	29.3	27.3	24.5
21	Carvone	1245	1239	36.7	–	–	–	–
22	Piperitone	1255	1249	2.7	–	–	–	–
23	Piperitenone	1349	1356	5.0	–	–	–	–
24	Eugenol	1357	1340	–	0.4	–	–	–
25	Geranyl acetate	1379	1379	–	1.6	–	–	–
26	β-bourbonene	13	1419	2.7	–	–	–	–
27	β-ylangene	1432	1417	0.3	–	–	–	–
28	*trans*-β-caryophyllene	1420	1431	0.2	7.6	5.9	6.3	5.30
29	β-gurjunene	1435	1437	0.3	–	–	–	–
30	α-guaiene	1443	1387	–	1.8	1.2	1.3	1.3
31	*trans*-β-farnesene	1456	1454	1.2	0.1	–	–	–
32	α-humulene	1452	1452	–	2.4	1.6	1.8	1.6
33	γ-gurjunene	1475	1475	0.3	–	–	–	–
34	γ-muurolene	1479	1478	0.4	–	–	–	–
35	Bicyclosesquiphellandrene	1488	1487	11.1	3.1	1.8	2.2	1.2
36	Bicyclogermacrene	1509	1500	0.8	0.7	–	–	–
37	α-bulnesene	1515	1509	–	1.2	0.5	0.5	0.5
38	Cubebol	1518	1514	–	0.3	–	–	–
39	*trans*-γ-bisabolene	1528	1529	0.1	0.4	0.5	0.8	1.2
40	Germacrene-4-ol	1580	1574	0.3	0.1	–	–	–
41	Caryophyllene oxide	1584	1582	–	1.9	1.0	0.7	2.8

Notes: [a]Experimentally determined retention indices on the DB-5MS column. [b]Literature retention indices on the DB-5MS column (16). [c]Not found.*Previously reported by Agudelo-Gómez et al. (23).

previous study (23) through GC/MS and GC/FID analyses. In this study, the chromatographic analyses of two essential oils, corresponding to *L. alba* (A04) and *L. alba* Carvone chemotype (B04), were carried out. The major compounds of *L. alba* Citral chemotype essential oils were Neral and Geranial. The percentages of Neral found in *L. alba* oils A04, A05, A06 and A07 were 22.6, 23.1, 22.1, 20% and the percentages of Geranial were 28.8, 29.3, 27.3, 24.5, respectively (Table 3). Other components found in an essential oil of *L. alba* (A04) were Limonene, Geraniol, Nerol and Linalool (Figure 1).

The chromatographic analyses of *L. alba* Carvone chemotype (B04) revealed that its major compounds were Limonene (23.17%) and Bicyclosesquiphellandrene (11.1%) (Table 3 and Figure 2).

About monoterpenes, the highest SI was shown by Citral and Limonene against HeLa cells (9, 10), and Linalool against Jurkat cells, corresponding to > 1241, 6.97 and 10.1 (Table 2), respectively. These results suggest that the cytotoxic activity of *L. alba* Citral chemotype oils demonstrated in this study could be due to their main components. The cytotoxic activity of Citral, Limonene and Linalool has been demonstrated

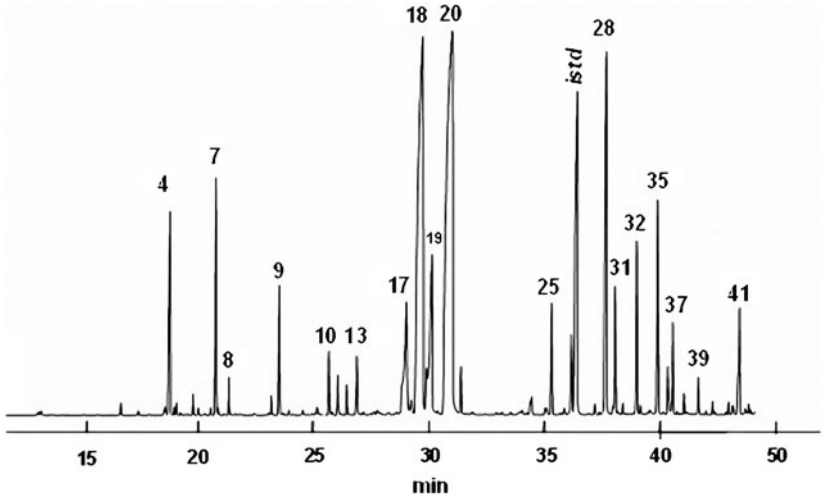

Figure 1. Typical chromatogram of the *Lippia alba* essential oil citral chemotype (A06), obtained by MWHD. Column DB-5MS (60 m). MSD (EI. 70 eV). Identification appears in Table 3.

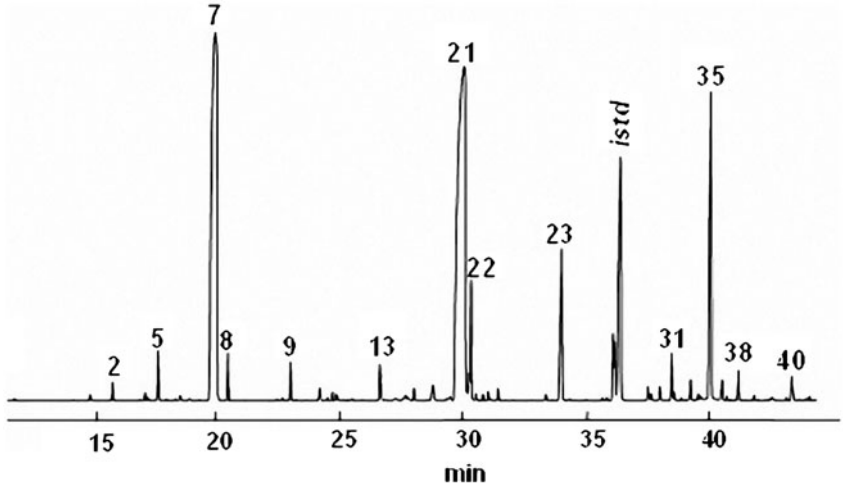

Figure 2. Typical chromatogram of the *Lippia alba* essential oil carvone chemotype (B04), obtained by MWHD. Column DB-5MS (60 m). MSD (EI. 70 eV). Identification appears in Table 3.

in other studies (24, 25). Limonene has shown activity against human cancer prostate cells (DU-145) with a IC_{50} value of 2.8 mM (20); Citral was cytotoxic against mouse lymphoma cell lines BS-24-1 and RL-12, Human leukaemic monocyte lymphoma cell line U937, Human promyelocytic leukaemia cells (HL60) at a concentration of 45 µg/mL (24) and Linalool showed activity against renal carcinoma (ACHN) and melanoma cell lines (C32) at concentrations of 23.16 and 23.77 µg/mL (25).

Carvone was not cytotoxic against Vero cells which showed an IC_{50} value of $\geqslant 200$ µg/mL; however, it did not show cytotoxic activity against the cell lines evaluated in this study and it has shown antiproliferative activity against HL-60 cells with IC_{50} of 115.6 µM (26).

Considering that an SI value of 14.3 on cell lines is indicative of potential antitumor activity for biopharmaceutical use (27) and an IC_{50} value on normal cells above 30 µg/mL indicates that a compound is not cytotoxic (6), *L. alba* Carvone and Citral chemotype essential oils could be promising anticancer agents; however, other studies *in vivo* will be necessary to determine their potential to be used as anticancer agents.

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