© 2009 The Authors
© 2009 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 8 (5), 419 - 427
BLACPMA ISSN 0717 7917

Artículo Original | Original Article

Acute toxicity against *Artemia franciscana* of essential oils isolated from plants of the genus *Lippia* and *Piper* collected in Colombia

[Toxicidad aguda contra *Artemia* franciscana de aceites esenciales aislados de plantas de los géneros *Lippia* y *Piper* colectadas en Colombia]

Jesús OLIVERO-VERBEL[1,3*], Jorge GÜETTE-FERNANDEZ [1,3], Elena STASHENKO[2, 3]

[1]*Environmental and Computational Chemistry Group. Campus of Zaragocilla. Faculty of Pharmaceutical Sciences. University of Cartagena*

[2]*CIBIMOL;* [3]*Chromatography Laboratory, Research Center of Excellence CENIVAM, Industrial University of Santander (UIS), Building 45, Bucaramanga, Colombia.*

Abstract

Thirty two essential oils, twenty from plants belonging to the genus *Lippia* (*L. alba, L. citrodora, L. dulcis, L. micromera*, and *L. origanoides*), and twelve from the genus *Piper* (*P. auritum, P. bogotense, P. brachypodom, P. bredemeyeri, P. divaricatum, P. eriopodon, P. lanceifolium, P. marginatum*, and *P. septuplinervium*), collected in different geographical sites in Colombia were isolated using microwave-assisted hydrodistillation, and their cytotoxicity measured as the medium lethal concentration (LC_{50}), tested against *Artemia franciscana*. Selected oils were also characterized by gas chromatography-mass spectrometry (GC-MS) analysis. Essential oils from *Lippia* exhibited LC_{50} values ranging between 8.72-34.90 µg/mL and 3.44-13.66 µg/mL, after 24 and 48 h exposure, respectively. Essential oils of *Piper* presented acute toxicity with LC_{50} values between 4.43-22.38 µg/mL and 2.54-12.64 µg/mL after 24 and 48 h exposures, respectively. The oils with greatest bioactivities belonged to *Lippia citriodora* and *Piper eriopodon* in their respective groups. GC-MS data revealed that main constituents of essential oils from *Lippia* species include thymol, geranial, neral, *trans*-β- caryophyllene, carvone, limonene and *p*-cymene, whereas in *Piper* species those were *trans*-β-caryophyllene, elemecin, germacrene D, *trans*-sabinene hydrate, and α-phellandrene. Most of the assayed plants are commonly used for prevention and treatment of diseases in Colombia, and the application of this screening demonstrated its usefulness to evaluate the bioactivity of these natural products at an early stage of traditional medicine development.

Keywords: Essential oils; *Artemia franciscana;* Colombia; *Lippia*; *Piper*.

Resumen

Treinta y dos aceites esenciales, veinte de plantas pertenecientes al género *Lippia* (*L. alba, L. citrodora, L. dulcis, L. micromera*, and *L. origanoides*), y doce al género *Piper* (*P. auritum, P. bogotense, P. brachypodom, P. bredemeyeri, P. divaricatum, P. eriopodon, P. lanceifolium, P. marginatum*, and *P. septuplinervium*), colectadas en diferentes sitios de Colombia, fueron aislados usando hidrodestilación asistida por microondas, y su citotoxicidad medida como la concentración letal media (CL_{50}), evaluada frente a *Artemia franciscana*. Aceites seleccionados fueron también caracterizados mediante análisis por cromatografía de gases acoplada con espectrometría de masas (CG-EM). Los aceites esenciales de *Lippias* mostraron valores de CL_{50} que variaron entre 8,72-34,90 µg/mL y 3,44-13,66 µg/mL, luego de 24 y 48 h de exposición, respectivamente. Los aceites de *Piper* presentaron toxicidad aguda con valores de CL_{50} entre 4,43-22,38 µg/mL y 2.54-12,64 µg/mL para 24 y 48 h de exposición, respectivamente. Los aceites con la bioactividad más alta fueron los de *Lippia citriodora* y *Piper eriopodon*, en sus respectivos grupos. Los datos de CG-EM revelaron que los componentes principales de los aceites esenciales de las especies de *Lippia* fueron timol, geranial, neral, *trans*-β- cariofileno, carvona, limoneno y *p*-cimeno, mientras que para las especies de *Piper*, estos fueron el *trans*-β-cariofileno, elemecina, germacreno D, hidrato de *trans*-sabineno y α-felandreno. La mayoría de las plantas evaluadas son comúnmente empleadas para la prevención y el tratamiento de enfermedades en Colombia, y la aplicación de este tamizaje demostró su utilidad en la evaluación de la bioactividad de estos productos naturales en un estado preliminar de desarrollo de la medicina tradicional.

Palabras Clave: Aceites esenciales; *Artemia franciscana;* Colombia; *Lippia*; *Piper*.

Recibido | Received: January 16, 2009.
Aceptado en Versión Corregida | Accepted in Corrected Version: August 7, 2009.
Publicado en Línea | Published Online: September 30, 2009.
Declaración de intereses | Declaration of interests: Authors have no competing interests.
Financiación | Funding: This work was financed by Colciencias (RC-432/2004).
This article must be cited as: Jesús Olivero-Verbel, Jorge Güette-Fernandez , Elena Stashenko. 2009. Acute toxicity against *Artemia franciscana* of essential oils isolated from plants of the genus *Lippia* and *Piper* collected in Colombia. Bol Latinoam Caribe Plant Med Aromat 8(5):419 – 427. {EPub September 30, 2009}.

*Contactos | Contacts: Email joliverov@unicartagena.edu.co; jesusolivero@yahoo.com Phone: 57-5-6698180. Fax: 57-5-6699771 http://www.reactivos.com

INTRODUCTION

Essential oils are complex mixtures of volatile compounds isolated from plant materials. Chemically, they are secondary metabolites derived from terpenes and their oxygenated compounds, and each component contributes to their biological activities (Edris, 2007). Essential oils from *Lippia* and *Piper* species have been traditionally used for diverse purposes in different parts of the world. The genus *Lippia* L. (*Verbenaceae* family) is widely distributed in tropical, subtropical, central and South America, as well as in Africa. It consists of approximately 250 species of herbs, shrubs and small trees (Jansen-Jacobs, 1988; Braga et al., 2005). In general, the genus appears to display a consistent profile of chemical composition, pharmacological activities and folk medicine uses. In most cases, the used parts are leaves and flowers (Oliveira et al., 2006). They are commonly prepared as an infusion or decoction, and administered orally. The most common use of *Lippia* species is for the treatment of respiratory and gastrointestinal disorders (Pascual et al., 2001a,b), although some have shown analgesic, antipyretic, anti-inflammatory (Abenaa et al., 2003), and antihelminthic properties (Camurca-Vasconcelos et al., 2007). It is believed that their essential oils and phenolic compounds (flavonoids) are responsible for these effects (Argyropoulou et al., 2007). *Lippia alba* (Miller) N.E. Brown is an aromatic herb with recognized importance in folk medicine, and its use is widespread in Colombia. This plant has several popular names, represented by over 25 synonymies (Portuguese) such as: “carmelitana”, “erva-cidreira-do- campo”, “falsa-melissa” (Nogueira et al., 2007); and in Spanish, it is called “pronto alivio” (Stashenko et al., 2004). The sedative, digestive, carminative, and anti-hypertensive properties of this aromatic plant have been reported in the literature (Di Stasi et al., 2002). Large variations have been observed in the composition of *L. alba* essential oils, depending on the part of the plant used in the distillation, on the plant’s state of development, geographic location, the characteristics of the soil or climate, and other local conditions (Stashenko et al., 2003, 2004).

Piperaceae is well established in tropical and subtropical regions in the world and contains several aromatic species used in traditional medicine (Constantin et al., 2001; Santos et al., 2001). The genus *Piper* is the most representative of this family, with more than 700 species, from which many have been described (Carvalho-Silva and Cavalcanti, 2002; Potzernheim et al., 2006). Those are mostly represented by herbs, shrubs and trees (Cysne et al., 2005). Aromatic fruits of *Piper* species have been used in folk medicine (Vila et al., 2001), and many have been reported with diverse biological and pharmacological properties (Boll et al., 1994; Dias-dos Santos et al., 2001; Facundo et al., 2004; Rukachaisirikul et al., 2004; Lago et al., 2005; Gragasin et al., 2006; De Morais et al., 2007; Silva and Bastos, 2007). *Piper* plants are rich in many compounds including unsaturated amides, flavonoids, lignans, aristolactams, long and short chain esters, monoterpenes, sesquiterpenes, arylpropanoids, aldehydes, ketones, propenylphenols, and alkaloids (Parmar et al., 1997, 1998; Navickiene et al., 2000; Mundina et al., 2001; Jirovetz et al., 2002; Vargas et al., 2004; Facundo et al., 2005).

One of the most popular organisms used to perform biological preliminary screening of natural products is the brine shrimp. The medium lethal concentration (LC_{50}) against brine shrimp is a simple, inexpensive, rapid and convenient method for assessment of relative biological activity (Persoone and Wells, 1987; Vanhaecke et al., 1981; Solis et al., 1993). The assay could be meaningful at an ecological level but we should be careful in not trying to infer preliminary animal pharmacological and toxicological activities of the different compounds and materials assayed. In this work, essential oils from *Lippia* and *Piper* plants have been screened for their lethal effects on *Artemia franciscana*, and selected oils have also been characterized by gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant material and isolation of essential oils

The vegetal materials of forty three plants of two different genus, twenty of the genus *Lippia* (*Verbenaceae*), that included five species (*L. alba*, *L. citrodora*, *L. dulcis*, *L. micromera*, and *L. origanoides*) and twelve of the genus *Piper* (Piperaceae), that included nine species (*P. auritum*, *P. bogotense*, *P. brachypodom*, *P. bredemeyeri*, *P. divaricatum*, *P. eriopodon*, *P. lanceifolium*, *P. marginatum*, and *P. septuplinervium.*). Samples were collected early in the morning at different departments of Colombia (Antioquia, Arauca, Bolívar, Boyacá, Choco, Cundinamarca, Nariño, Santander, and Tolima), based on information from ethnobotanical surveys. The plant material was stored in the dark and transported to the laboratory of CENIVAM at the Industrial University of Santander, Bucaramanga (Colombia), and treated to isolate the essential oils. The plant material was classified systematically by the botanist José Luis Fernández from the Institute of Natural Sciences of the National University of Colombia. Voucher specimena were deposited at the herbarium of this university under the codes showed in Table 1.

The essential oils of collected plants were obtained from the whole plant and from some parts (total plant, stem, and combinations as leaves-flowers) by microwave-assisted hydrodistillation (MWHD) method (Table 1). For MWHD, the hydrodistillation apparatus was placed inside a domestic microwave oven (Kendo, 2.45 GHz, 800 W) with a side orifice through which an external glass condenser joined the round flask with the plant material (100 g) and water (2 L), inside the oven. The oven was operated for 30 min at full power, which caused water to boil vigorously and reflux. Essential oil was decanted from the condensate and dried with anhydrous sodium sulphate (Stashenko et al., 2004).

Toxicity test

The brine shrimp, *Artemia franciscana*, was used as test organism to measure the toxicity (LC_{50}) of essential oils, as reported elsewhere (Güette-Fernández et al., 2008). Briefly, 10 mg of cysts were hatched in Petri dishes, containing seawater filtered with cellulose nitrate fillters (mean pore diameter of 0.45 mm), with a photoperiod of 12 h L/12 h D. After hatching, nauplii with age below 24 h were transferred to vials of 10 mL with different concentrations (0.1 – 100 µg/mL) of essential oil. Four bioassays were conducted by oil, each one with three replicates for each concentration and ten larvae per replicate. Dimethyl sulfoxide (DMSO) was used as dissolvent at a maximum concentration of 1% v/v. Counting of dead organisms was carried out after 24 and 48 hour after exposure to the different concentrations of essential oil.

Gas chromatography–mass spectrometry (GC-MS) analysis

The analysis of selected essential oils were performed on an *Agilent Technologies* 6890 *Plus* equipped with a HP-5 MS capillary column (60 m x 0,25 mm i.d. x 0,25 *µ*m, d_f), coupled to an *Agilent Technologies* MSD 5973 mass-selective detector. Detection was carried out using an electron ionization system with ionization energy of 70 eV. Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 250 and 285 °C, respectively. Column temperature was initially at 50 °C, and then gradually increased to 150 °C at a 3 °C/min rate, held for 10 min, and finally raised to 250 °C at 10 °C/min. Diluted samples (1:100 v/v, in CH_2Cl_2) of 1.0 *µ*L were injected manually and in the splitless mode. Constituents' identification was based on mass spectra and comparison with those of some standard compounds, NIST, Wiley and Adams Library data of the GC-MS, and reported data (Davies, 1990; Adams, 1995).

Statistical analysis

Results of acute toxicity experiments were presented as LC_{50} values and 95% confidence intervals, calculated by the probit method (Finney, 1971).

RESULTS AND DISCUSSION

The results of the bioassays on *Artemia franciscana* are presented in Table 2. The essential oils of species from the *Lippia* genus exhibited acute toxicity with LC_{50} values between 8.72-34.90 µg/mL and 3.44-13.66 µg/mL after 24 and 48 h exposures, respectively. The oils with lower toxicity were *Lippia alba* VEamW02E, and VEtoW02B, which showed values of LC_{50} greater than 30 µg/mL. However, the oils considered of greater toxicity were *Lippia citrodora* (VEamW01E), *Lippia origanoides* (VEnaW02B), VEsaWCR-01 and VEsaWCR-02 which showed values of LC_{50} lower than 10 µg/mL. 80% (16/20) of the essential oils from *Lippia alba* presented moderate toxicity, with LC_{50} values greater than 10 µg/mL. The vulnerability index (γ) for the development of the *A. franciscana* larvae in the presence of the essential oils was calculated as the relationship between 24-h LC_{50} and 48-h LC_{50} and for *Lippia* species, those ranged from 1.57 to 5.04 (Table 2). This index revealed that 40% (8/20) showed γ values greater than 3, suggesting a developmental-dependent effect.

Table 1. Taxonomy, sampling location and vouchers of plants used for essential oil extraction.

Scientific name	Essential oil code	Common name	Sampling site	Department	Voucher
Genus *Lippia*					
Lippia alba	VEmoW02E	Pronto alivio	Bolívar	Santander	484650[a]
Lippia alba	VEamW02E	Pronto alivio	Armenia	Quindío	484650[a]
Lippia alba	VEtoW02B	Pronto alivio	Flandes	Tolima	484650[a]
Lippia alba	VEsaW15H	Pronto alivio	Suaita	Santander	517306
Lippia alba “Citral”	VEbgW03H	Pronto alivio	Bucaramanga	Santander	512077
Lippia alba “Carvona”	LAA455005	Pronto alivio	Bucaramanga	Santander	480750
Lippia alba	VEboW01E	Pronto alivio	Turbaco	Bolivar	516929
Lippia alba	VEbgW01E	Pronto alivio	Bucaramanga	Santander	480750
Lippia origanoides	VEbyW06B2	Orégano de monte	Soatá	Boyacá	517741
Lippia origanoides	VEnaW02B	Orégano de monte	Pedregal	Nariño	520285
Lippia origanoides (After blooming)	VEsaWCR-01	Orégano de monte	Bucaramanga	Santander	519798
Lippia origanoides (Before blooming)	VEsaWCR-02	Orégano de monte	Bucaramanga	Santander	519798
Lippia origanoides	VEbyW06B3	Orégano de monte	Soatá	Boyacá	517741
Lippia origanoides	VEbgW05H	Orégano de monte	Bucaramanga	Santander	512075
Lippia origanoides	VEsaW06H	Orégano de monte	Piedecuesta	Santander	516290
Lippia origanoides	VEsaW04B	Orégano de monte	Los Santos	Santander	516294
Lippia micromera	VEceW01H	Orégano serrano	Manaure	Cesar	516924
Lippia citriodora	VEamW01E	Cidrón	Armenia	Quindío	484334
Lippia dulcis	VEbgW04H	Orozuz	Bucaramanga	Santander	512079
Lippia origanoides	VEcdW01H	Orégano de monte	Bucaramanga	Santander	512075
Genus *Pipper*					
Piper brachypodom	PPccW23E	Cordoncillo	Quibdó	Chocó	520011
Piper var. brachypodom	PPccW24E	Cordoncillo	Quibdó	Chocó	519974
Piper var. brachypodom q2	PPccW25E	Cordoncillo	Quibdó	Chocó	519974
Piper auritum Kunth	PPvcW01H	Santamaría de anís	Cali	Valle del Cauca	512209
Piper cf. divaricatum	PPtoW02H	Cordoncillo	Ibagué	Tolima	519593
Piper bredemeyeri Jacq.	PPceW04H	Cordoncillo	Pueblo Bello	Cesar	516939
Piper eriopodon	PPceW03A	Cordoncillo	Pueblo Bello	Cesar	516937
Piper septuplinervium	PPccW20E	Cordoncillo	Quibdó	Chocó	520006
Piper cf. brachypodon	PPccW18E	Cordoncillo	Quibdó	Chocó	519974
Piper marginatum	PPtoW01H	Cordoncillo	Venadillo	Tolima	519600
Piper lanceifolium Kunth	PPccW21E	Cordoncillo	Quibdó	Chocó	519993
Piper bogotense	PPnaW01H	Matico	Ipiales	Nariño	519590

a. Samples with the same voucher

Table 2. LC_{50} of essential oils from *Lippia* and *Piper* species against *Artemia franciscana.*

Essential oils (Code)	LC_{50} (95% C.I.)* (µg/mL)		
	24-h	48-h	24-h LC_{50}/48-h LC_{50}
Genus *Lippia*			
Lippia citriodora (VEamW01E)	8.72 (7.91-9.62)	3.44 (2.88-4.13)	2.53
Lippia origanoides (VEnaW02B)	8.87 (8.08-9.74)	2.69 (2.21-3.28)	3.30
Lippia origanoides, after bloming (VEsaWCR-01)	9.04 (8.08-10.10)	4.64 (4.06-5.31)	1.95
Lippia origanoides, before blooming (VEsaWCR-02)	9.06 (8.14-10.08)	4.59 (4.06-5.20)	1.97
Lippia dulcis (VEbgW04H)	10.16 (9.39-10.99)	2.01 (1.69-2.40)	5.04
Lippia alba “Carvona” (LAA455005)	10.29 (9.38-11.29)	6.17 (5.24-7.26)	1.67
Lippia origanoides (VEbyW06B2)	10.55 (9.66-11.51)	3.50 (2.82-4.36)	3.01
Lippia micromera (VEceW01H)	10.57 (10.02-11.15)	6.37 (5.53-7.34)	1.66
Lippia alba (VEboW01E)	11.41 (10.63-12.25)	3.61 (3.15-4.14)	3.16
Lippia origanoides (VEbyW06B3)	12.42 (12.05-12.79)	7.90 (7.16-8.73)	1.57
Lippia origanoides (VEsaW06H)	14.05 (13.15-15.00)	7.97 (6.68-9.50)	1.76
Lippia origanoides (VEbgW05H)	15.79 (15.10-16.52)	4.63 (3.87-5.54)	3.41
Lippia alba (VEbgW01E)	15.85 (14.63-17.17)	5.62 (4.54-6.96)	2.82
Lippia origanoides (VEcdW01H)	17.45 (16.90-18.02)	7.43 (6.28-8.79)	2.35
Lippia origanoides (VEsaW04B)	20.13 (18.87-21.48)	9.58 (8.78-10.46)	2.10
Lippia alba “Citral” (VEbgW03H)	24.87 (23.54-26.27)	11.62 (10.10-13.37)	2.14
Lippia alba (VEmoW02E)	27.57 (25.60-29.69)	8.08 (6.86-9.53)	3.41
Lippia alba (VEsaW15H)	29.26 (27.53-31.09)	7.18 (5.95-8.67)	4.07
Lippia alba (VEtoW02B)	30.45 (28.56-32.47)	9.84 (8.12-11.92)	3.09
Lippia alba (VEamW02E)	34.90 (32.83-37.11)	13.66 (11.79-15.84)	2.55
Genus *Piper*			
Piper eriopodon (PPceW03A)	4.43 (3.91-5.02)	2.54 (2.19-2.95)	1.74
Piper bredemeyeri Jacq.(PPceW04H)	4.96 (4.31-5.70)	2.92 (2.40-3.55)	1.70
Piper brachypodom (PPccW23E)	7.15 (6.32-8.08)	2.32 (2.02-2.67)	3.08
Piper auritum Kunth (PPvcW01H)	8.48 (7.75-9.28)	3.84 (3.18-4.64)	2.21
Piper var Brachypodom (PPccW24E)	8.59 (7.78-9.49)	3.56 (2.84-4.46)	2.41
Piper septuplinervium (PPccW20E)	9.60 (8.84-10.42)	3.87 (3.20-4.68)	2.48
Piper lanceifolium Kunth (PPccW21E)	14.41 (13.34-15.57)	7.54 (6.82-8.34)	1.91
Piper cf. brachypodon (PPccW18E)	15.72 (13.34-18.54)	10.30 (9.51-11.15)	1.53
Piper bogotense (PPnaW01H)	17.24 (16.75-17.75)	10.43 (9.63-11.29)	1.65
Piper cf. divaricatum (PPtoW02H)	19.24 (18.12-20.43)	10.12 (9.13-11.21)	1.90
Piper var brachypodom q2 (PPccW25E)	21.60 (19.30-24.17)	9.41 (8.39-10.56)	2.30
Piper marginatum (PPtoW01H)	22.38 (11.56-13.82)	12.64 (13.01-14.81)	1.77

* Confidence intervals.

The essential oils of the *Piper* species presented acute toxicity with LC_{50} values between 4.43-22.38 µg/mL and 2.54-12.64 µg/mL after 24 and 48 h exposures, respectively. The oils that showed greatest toxicity were *Piper eriopodon* and *Piper bredemeyeri jacq*, with LC_{50} values lower than 5 µg/mL. Oils that presented lowest toxicity were *Piper marginatum and Piper var brachypodom q2* (PPccW25E), which showed LC_{50} values greater than 20 µg/mL. The vulnerability index (γ) of the *A. franciscana* to the essential oils of this genus changed between 1.74 and 3.08 (Table 2), with only one oil having a γ value greater than 3 (*Piper brachypodom*), suggesting that compared to *Lippia* oils, these seem to have less effects on crustacean larvae development. Although the references on the use of *Artemia franciscana* bioassay for testing essential oils from the *Lippia* and *Piper* genus are scarce, several authors have reported its use for evaluating toxicity of extracts isolated from these plants (Bezerra et al., 2005; Ajaiyeoba et al., 2006; David et al., 2007). Results presented here have shown some cytotoxic effects of essential oils on the brine shrimp lethality assay.

The gas chromatography-mass spectrometry characterization of selected essential oils is shown in Table 3. The most frequently found components of essential oils from *Lippia* species were thymol, geranial, neral, *trans*-β-caryophyllene, carvone, limonene, and *p*-cymene. On the other hand, main constituents of oils from *Piper* species were *trans*-β-caryophyllene, elemecin, germacrene D, *trans*-sabinene hydrate, and α-phellandrene. These results revealed the great variety of chemicals can be found in these natural mixtures. Accordingly, care must be taken with the use of these oils as they could target different systems and develop biochemical interactions that should be considered during their evaluation as therapeutic agents. The results of this investigation will be useful for the preparation of official monographs of two species frequently used for medicinal purposes in Colombia.

Table 3. Major composition of selected essential oils from plants belonging to *Lippia* and *Piper* genus.

Essential oils (Code)	Composition (%)
Genus *Lippia*	
Lippia citriodora (VEamW01E)	Geranial (18.9), neral (15.6), limonene (10.7), 1,8-cineol (5.0), spathulenol (4.7), geraniol (2.7), *trans*-β-caryophyllene (2.3), nerol (2.0), sabinene (1.9), 6-methyl-5-hepten-2-ona (1.5), geranyl acetate (1.3).
Lippia origanoides (VEnaW02B)	Thymol (54.5), p-cymene (10.0), α-terpinene (1.6), γ-terpinene (5.0), thymyl acetate (4.8), β-myrcene (2.8), *trans*-β-caryophyllene (2.4), methyl thymyl ether (1.9), carvacrol (1.7).
Lippia origanoides (VEsaWCR-01)	Thymol (66), *p*-cymene (7.2), γ-terpinene (4.4), *trans*-β-caryophyllene (3.6), β-myrcene (2.2), methyl thymyl eter (2.3), α-humulene (2.4), carvacrol (1.5).
Lippia dulcis (VEbgW04H)	*trans*-β-caryophyllene (10.4), δ-cadinene (8.8), α-copaene (8.4), α–bisabolol (8.2), 6-methyl-5-hepten-2-ona (4.0), 3-methyl-2-cyclohexen-2-ona (3.3), β-bisabolene (2.6), *trans*-β-farnesene (2.5), sesquisabinene (2.5), caryophyllene oxide (2.5), germacrene-D-4-ol (2.4), β-bourbonene (2.0).
Lippia alba “Carvona” (LAA455005)	Carvone (35.0), limonene (29.7), bicyclosesquiphellandrene (8.7), piperitenone (5.7), piperitone (3.5), β-bourbonene (2.6).
Lippia alba (VEboW01E)	Geranial (30.5), neral (23.6), geraniol (6.3), *trans*-β-caryophyllene (6.2), 6-methyl-5-hepten-2-ona (6.0), limonene (3.7), nerol (2.6), linalol (2.1).
Lippia alba “Citral” (VEbgW03H)	Geranial (23.3), neral (19.5), geraniol (9.5), *trans*-β-caryophyllene (6.6), geranyl acetate (3.6), limonene (2.9), 6-methyl-5-hepten-2-ona (2.6), nerol (2.4), bicyclosesquiphellandrene (3.0), linalol (1.5).

Table 3. Major composition of selected essential oils from plants belonging to *Lippia* and *Piper* genus (continued…).

Essential oils (Code)	Composition (%)
Genus *Piper*	
Piper brachypodom (PPccW23E)	*trans*-β-Caryophyllene (20.2), caryophyllene oxide (10.8), bicyclogermacrene (8.1), germacrene D (5.9), 9-epi-*trans*- caryophyllene (5.8), spathulenol (5.7), α-copaene (4.0), δ-cadinene (3.1), β-cubebene (3.1), α-humulene (3.0), aromadendrene (2.1), γ-muurolene (1.5), valencene (1.5), γ-cadineno (1.1), *trans*-calamenene (1.5), guaiol (1.2), α-cubebene (1.0).
Piper lanceifolium (PPccW21E)	*trans*-β-Caryophyllene (11.6), germacrene D (10.7), β-selinene (7.8), δ-cadinene (6.1), caryophyllene oxide (5.9), β-pinene (5.4), α-cubebene (4.3), γ-muurolene (4.0), α-copaene (3.8), α-pinene (3.6), α-humulene (2.6), elemecin (2.5), β-elemene (2.5), spathulenol (2.4), p-cymene (1.9), *trans*-nerolidol (1.7).
Piper dolichotrichum (PPccW18E)	Germacrene D (16.7), *trans*-β- caryophyllene (9.8), β-elemene (6.4), bicyclogermacrene (6.2), α-guaiene (5.9), α-humulene (4.9), β-selinene (3.8), δ-cadinene (3.4), α-copaene (3.1), β-bourbonene (2.8), γ-muurolene (2.5), β-eudesmol (2.4), β-copaene (2.2), cadinol (1.8), spathulenol (1.8), cadinene (1.5), γ-elemol (1.4), phytol (1.4), neo-intermedol (1.2), aromadendrene (1.0).
Piper bogotense (PPnaW01H)	*trans*-Sabinene hydrate (14,2). α-phellandrene (13,7). α-Pinene (8,7). linalol (4,7). limonene (5,3). *p*-cymene (4,4). β-phellandrene (3,4). δ-cadinene (3,4). *trans*-β-caryophyllene (3,1). α-bisabolol (2,4). β-curcumene (1,7). *trans*-β-farnesene (1,6). bicyclogermacrene (1,5). α-copaene (1,1).
Piper marginatum (PPtoW01H)	Elemecin (18.0), *trans*-β-caryophyllene (11.0), α-phellandrene (11.1), isoelemecine (9.2), limonene (7.5), bicyclogermacrene (4.1), β-elemene (4.0), *trans*-anetol (3.4), exalatacin (3.2), α-pinene (2.5), *cis*-methyl isoeugenol (2.4), β-phellandrene (2.2), *cis*-nerolidol (1.8), α-humulene (1.7), *trans*-methyl isoeugenol (1.7), β-pinene (1.4), *cis*-asarone (1.3), β-myrcene (1.1).

CONCLUSION

Essential oils isolated from genus *Lippia* and *Piper* collected in Colombia exhibited a wide range of toxicities against *Artemia franciscana*, with activities highly dependent on the sampling site. The results of this screening, as well as the GC/MS characterization, indicates that these species are important sources of diverse natural products with potential pharmacological properties.

REFERENCES

Abenaa AA, Diatewaa M, Gakossoa G, Gbeassorc M, Hondi-Assaha TH, Ouambab JM. 2003. Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. Fitoterapia 74:231-236.

Adams RP. 1995. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation: Carol Stream, USA, 469 p.

Ajaiyeoba EO, Abiodun OO, Falade MO, Ogbole NO, Ashidi JS, Happi CT, Akinboye DO. 2006. *In vitro* cytotoxicity studies of 20 plants used in Nigerian antimalarial ethnomedicine. Phytomedicine 13:295-298.

Argyropoulou C, Daferera D, Tarantilis PA, Fasseas C, Polissiou M. 2007. Chemical composition of the essential oil from leaves of *Lippia citriodora* H.B.K. (Verbenaceae) at two developmental stages. Biochem Syst Ecol 35:831-837.

Bezerra DP, Pessoa C, de Moraes MO, Silveira ER, Lima MA, Elmiro FJ, Costa-Lotufo LV. 2005. Antiproliferative effects of two amides, piperine and piplartine, from *Piper* species. Z. Naturforsch [C] 60:539-543.

Boll PM, Parmar VS, Tyagi OD, Prasad A, Wengel J, Olseng CE. 1994. Some recent isolation studies from potential insecticidal *Piper* species. Pure Appl Chem 66:2339-2342.

Braga MEM, Ehlert PAD, Ming LC, Meireles MAA. 2005. Supercritical fluid extraction from *Lippia alba*: global yields, kinetic data, and extract chemical composition. J Supercrit Fluid 34:149-156.

Camurca-Vasconcelos ALF, Bevilaqua CML, Morais SM, Maciel MV, Costa CTC, Macedo ITF, Oliveira LMB, Braga RR, Silva RA, Vieira LS. 2007. Anthelmintic activity of Croton zehntneri and *Lippia* sidoides essential oils. Vet Parasitol 148:288-294.

Carvalho-Silva M, Cavalcanti TB. 2002. Piperaceae, PP 93-124. In Cavalcanti TB, Ramos AE: Flora do Distrito Federal, Brasil. Vol. 2. Embrapa-Cenargen, Brasilia, Brasil.

Constantin MB, Sartorelli P, Limberger R, Henrlques AT, Steppe M, Ferreira MJP, Ohara MT, Emerenciano VP, Kato MJ. 2001. Essential oils from *Piper* cernuum and *Piper* regnellli: Antimicrobial activities and analysis by GC/MS and ^{13}C-NMR. Planta Med. 67:771-773.

Cysne JB, Canutoa KM, Pessoa ODL, Nunesb EP, Silveira ER. 2005. Leaf Essential Oils of Four *Piper* Species from the State of Ceará - Northeast of Brazil. J Braz. Chem Soc 16:1378-1381.

David JP, Meira M, David JM, Brandão HN, Branco A, de Fátima Agra M, Barbosa MR, de Queiroz LP, Giulietti AM. 2007. Radical scavenging, antioxidant and cytotoxic activity of Brazilian Caatinga plants. Fitoterapia 78:215-218.

Davies NW. 1990. Gas chromatographic retention indices of monoterpenes and *sesquiterpenes* on methyl silicone and Carbowax 20M phases. J Chromatogr 503:1-24.

De Morais SM, Facundo VA, Bertini LM, Barreira-Cavalcanti ES, Dos Anjos-Junior JF, Ferreira SA, De Brito ES, De Souza-Neto MA. 2007. Chemical composition and larvicidal activity of essential oils from *Piper* species. Biochem. Syst Ecol 35:670-675.

Di Stasi LC, Oliveira GP, Carvalhaes MA, Queiroz M Jr, Tien OS, Kakinami SH, Reis MS. 2002. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia 73:69-91.

Dias-dos Santos PR, De Lima-Moreiraa D, Guimaraesb EF, Coelho-Kaplana MA. 2001. Essential oil analysis of 10 *Piperaceae* species from the Brazilian Atlantic forest. Phytochemistry 58:547-551.

Edris AE. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 21:308-323.

Facundo VA, Sá AL, Silva SAF, Morais SM, Matos CRR, Braz-Filho R. 2004. Three New Natural Cyclopentenedione Derivatives from *Piper carniconnectivum*. J Braz Chem Soc 15:140-145.

Facundo VA, Silveira ASP, Morais SM. 2005. Constituents of *Piper alatabacum* Trel & Yuncker (*Piperaceae*). Biochem Syst Ecol 33:753-756.

Finney D. 1971. Probit analysis. , 3rd ed. Cambridge Univ. Press, Cambridge, UK. pp. 76-80.

Gragasin MCB, Wy AM, Roderos BP, Acda MA, Solsoloy AD. 2006. Insecticidal activities of essential oils from *Piper* betle Linn. against storage insect pests. Philippine Agr Sci 89:212-216.

Güette-Fernández J, Olivero-Verbel J, O`Byrne-Hoyos I, Jaramillo B and Stashenko E. 2008. Chemical Composition and Toxicity Against *Artemia franciscana* of the Essential Oil of *Callistemon Speciosus* (Sims) DC. Collected in Bogota (Colombia). J Essent Oil Res 20:1-4.

Jansen-Jacobs MJ. 1988. *Verbenaceae*, p. 116. In Rijn, ARA: Flora of the Guianas, Series A: Phanerogams, Fascicle 4 (148). Gorts-Van Hoetz Scientific Books, Koenigstein, Germany.

Jirovetz L, Buchbauer G, Ngassoum MB, Geissler M. 2002. Aroma compound analysis of *Piper* nigrum and

Piper guineense essential oils from Cameroon using solid-phase microextraction -gas chromatography, solid-phase microextraction -gas chromatography -mass spectrometry and olfactometry. J Chromatogr A 976:265-275.

Lago JHG, Tanizaki TM, Young MCM, Guimarães EF, Kato MJ. 2005. Antifungal Piperolides from *Piper malacophyllum* (Prels) C. DC. J Braz Chem Soc 16:153-156.

Mundina M, Vila R, Tomi F, Tomàs X, Cicció JF, Adzet T, Casnova J, Cañigueral S. 2001. Composition and chemical polymorphism of the essential oils from *Piper lanceaefolium*. Biochem. Syst Ecol 29:739-748.

Navickiene HMD, Alecio AC, Kato MJ, Bolzani VS, Young MCM, Cavalheiro AJ, Furlan M. 2000. Antifungal amides from *Piper hispidum* and *Piper tuberculatum*. Phytochemistry 55:621-626.

Nogueira MA, Diaz G, Sakumo L. 2007. Caracterização química e atividade biológica do óleo essencial de *Lippia alba* cultivada no Paraná. Rev Ciênc Farm Básica Apl 28:273-278.

Oliveira DR, Leitao GG, Santos SS, Bizzo HR, Lopes D, Alviano CS, Alviano DS, Leitao SG. 2006. Ethnopharmacological study of two *Lippia* species from Oriximina, Braz J Ethnopharmacol 108:103-108.

Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen C, Boll PM. 1997. Phytochemistry of the genus *Piper*. Phytochemistry 46:597-673.

Parmar VS, Jain SC, Gupta S, Talwar S, Rajwanshi VK, Kumar R, Azim A, Malhotra S, Kumar N, Jain R, Sharma NK, Tyagi OD, Lawrie SJ, Errington W, Howarth OW, Olsen CE, Singh SK, Wengel J. 1998. Polyphenols and alkaloids from *Piper* species. Phytochemistry 49:1069-1078.

Pascual ME, Slowing K, Carretero E, Sanchez-Mata D, Villar A. 2001a. *Lippia*: traditional uses, chemistry and pharmacology: a review. J Ethnopharmacol 76(3):201-214.

Pascual ME, Slowing K, Carreter ME, Villar A. 2001b. Antiulcerogenic activity of *Lippia alba* (Mill.) N. E. Brown (Verbenaceae). II Farmaco 56:501-504.

Persoone G, Wells PG. 1987. *Artemia* in aquatic toxicology: a review, pp.249-275. In Sorgeloos P et al.: *Artemia* research and its applications: 1. Morphology, genetics, strain characterization, toxicology. Universa Press, Welteren Belgium.

Potzernheim M, Costa AF, Bizzo HR, Carvalho-Silva M, Vieira RF. 2006. Essential Oil of *Piper xylosteoides* (Kunth) Steud. from Federal District, Brazil. J Essent Oil Res 18:523-524.

Rukachaisirikul T, Siriwattanakit P, Sukcharoenphol K, Wongvein C, Ruttanaweang P, Wongwattanavuch P, Suksamrarn A. 2004. Chemical constituents and bioactivity of *Piper sarmentosum.* J Ethnopharmacol 93:173-176.

Santos PRD, Moreira DL, Guimaraes EF, Kaplan MAC. 2001. Essential oil analysis of 10 *Piperaceae* species from the Brazilian Atlantic Forest. Phytochemistry 58:547-551.

Silva DMMH, Bastos CN. 2007. Antifungal activity of essentials oils of *Piper* species against *Crinipellis perniciosa*, *Phytophthora palmivora* and *Phytophthora capsici.* Fitopatol Brasileira 32:143-145.

Solis PN, Wright CW, Anderson M, Gupta MP, Phillipson JD. 1993. A microwell cytotoxicitiy assay using *Artemia salina* (brine shrimp). Planta Med 59:250-252.

Stashenko EE, Jaramillo BE, Martınez JR. 2004. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Colombia, and evaluation of its in vitro antioxidant activity. J Chromatogr A 1025:93-103.

Stashenko EE, Jaramillo BE, Martinez JR. 2003. Comparación de la composición química y de la actividad antioxidante *in vitro* de los metabolitos secundarios volátiles de plantas de la familia *Verbenaceae.* Rev Acad Colombiana Ciencias. 27:579-597.

Vanhaecke P, Persoone G, Claus C, Sorgeloos P. 1981. Proposal for a short-term toxicity test with *Artemia nauplii*. Ecotoxicol Environ Saf 5:382-387.

Vargas L, Velasco-Negueruela A, Perez-Alonso MJ, Pala-Paul J, Vallejo MCG. 2004. Essential oil composition of the leaves and spikes of *Piper carpunya* Ruiz et Pavon (Piperaceae) from Peru. J Essent Oil Res 16:122-123.

Vila R, Milo B, Tomi F, Casanova J, Ferro EA, Cañigueral S. 2001. Chemical composition of the essential oil from the leaves of *Piper fulvescens*, a plant traditionally used in Paraguay. J Ethnopharmacol 76:105-107.