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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]

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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 (LC₅₀), tested against *Artemia franciscana*. Selected oils were also characterized by gas chromatography-mass spectrometry (GC-MS) analysis. Essential oils from *Lippia* exhibited LC₅₀ 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 LC₅₀ 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 citrodora* 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, elemecicin, 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 hidroddestilación asistida por microondas, y su citotoxicidad medida como la concentración letal media (CL₅₀), 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 CL₅₀ 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 CL₅₀ 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 citrodora* 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, germacrene 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*.

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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-cidreirado-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; Diasdos Santos et al., 2001; Facundo et al., 2004; Rukachaisirikul et al., 2004; Lago et al., 2005; Gragasini et al., 2006; De Moraes 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 (LC₅₀) 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. organoides*) 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 in 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 specimens 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 (LC₅₀) 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 µm, 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 CH₂Cl₂) of 1.0 µL 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 LC₅₀ 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 LC₅₀ 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 LC₅₀ greater than 30 µg/mL. However, the oils considered of greater toxicity were *Lippia citrodora* (VEamW01E), *Lippia origanoides* (VEsaW02B), VEsaWCR-01 and VEsaWCR-02 which showed values of LC₅₀ lower than 10 µg/mL. 80% (16/20) of the essential oils from *Lippia alba* presented moderate toxicity, with LC₅₀ 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 LC₅₀ and 48-h LC₅₀ 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
<i>Genus Lippia</i>					
<i>Lippia alba</i>	VEmoW02E	Pronto alivio	Bolívar	Santander	484650 ^a
<i>Lippia alba</i>	VEamW02E	Pronto alivio	Armenia	Quindío	484650 ^a
<i>Lippia alba</i>	VEtoW02B	Pronto alivio	Flandes	Tolima	484650 ^a
<i>Lippia alba</i>	VEsaW15H	Pronto alivio	Suaita	Santander	517306
<i>Lippia alba</i> “Citral”	VEbgW03H	Pronto alivio	Bucaramanga	Santander	512077
<i>Lippia alba</i> “Carvona”	LAA455005	Pronto alivio	Bucaramanga	Santander	480750
<i>Lippia alba</i>	VEboW01E	Pronto alivio	Turbaco	Bolivar	516929
<i>Lippia alba</i>	VEbgW01E	Pronto alivio	Bucaramanga	Santander	480750
<i>Lippia organoides</i>	VEbyW06B2	Orégano de monte	Soatá	Boyacá	517741
<i>Lippia organoides</i>	VENaW02B	Orégano de monte	Pedregal	Nariño	520285
<i>Lippia organoides</i> (After blooming)	VEsaWCR-01	Orégano de monte	Bucaramanga	Santander	519798
<i>Lippia organoides</i> (Before blooming)	VEsaWCR-02	Orégano de monte	Bucaramanga	Santander	519798
<i>Lippia organoides</i>	VEbyW06B3	Orégano de monte	Soatá	Boyacá	517741
<i>Lippia organoides</i>	VEbgW05H	Orégano de monte	Bucaramanga	Santander	512075
<i>Lippia organoides</i>	VEsaW06H	Orégano de monte	Piedecuesta	Santander	516290
<i>Lippia organoides</i>	VEsaW04B	Orégano de monte	Los Santos	Santander	516294
<i>Lippia micromera</i>	VEceW01H	Orégano serrano	Manaure	Cesar	516924
<i>Lippia citriodora</i>	VEamW01E	Cidrón	Armenia	Quindío	484334
<i>Lippia dulcis</i>	VEbgW04H	Orozuz	Bucaramanga	Santander	512079
<i>Lippia organoides</i>	VEcdW01H	Orégano de monte	Bucaramanga	Santander	512075
<i>Genus Piper</i>					
<i>Piper brachypodom</i>	PPccW23E	Cordoncillo	Quibdó	Chocó	520011
<i>Piper var. brachypodom</i>	PPccW24E	Cordoncillo	Quibdó	Chocó	519974
<i>Piper var. brachypodom q2</i>	PPccW25E	Cordoncillo	Quibdó	Chocó	519974
<i>Piper auritum</i> Kunth	PPvcW01H	Santamaría de anís	Cali	Valle del Cauca	512209
<i>Piper cf. divaricatum</i>	PPtoW02H	Cordoncillo	Ibagué	Tolima	519593
<i>Piper bredemeyeri</i> Jacq.	PPceW04H	Cordoncillo	Pueblo Bello	Cesar	516939
<i>Piper eriopodon</i>	PPceW03A	Cordoncillo	Pueblo Bello	Cesar	516937
<i>Piper septuplinervium</i>	PPccW20E	Cordoncillo	Quibdó	Chocó	520006
<i>Piper cf. brachypodon</i>	PPccW18E	Cordoncillo	Quibdó	Chocó	519974
<i>Piper marginatum</i>	PPtoW01H	Cordoncillo	Venadillo	Tolima	519600
<i>Piper lanceifolium</i> Kunth	PPccW21E	Cordoncillo	Quibdó	Chocó	519993
<i>Piper bogotense</i>	PPnaW01H	Matico	Ipiates	Nariño	519590

a. Samples with the same voucher

Table 2. LC₅₀ of essential oils from *Lippia* and *Piper* species against *Artemia franciscana*.

Essential oils (Code)	LC ₅₀ (95% C.I.)* (µg/mL)		24-h LC ₅₀ /48-h LC ₅₀
	24-h	48-h	
<i>Genus Lippia</i>			
<i>Lippia citriodora</i> (VEamW01E)	8.72 (7.91-9.62)	3.44 (2.88-4.13)	2.53
<i>Lippia origanoides</i> (VEaW02B)	8.87 (8.08-9.74)	2.69 (2.21-3.28)	3.30
<i>Lippia origanoides</i> , after bloming (VEsaWCR-01)	9.04 (8.08-10.10)	4.64 (4.06-5.31)	1.95
<i>Lippia origanoides</i> , before blooming (VEsaWCR-02)	9.06 (8.14-10.08)	4.59 (4.06-5.20)	1.97
<i>Lippia dulcis</i> (VEbgW04H)	10.16 (9.39-10.99)	2.01 (1.69-2.40)	5.04
<i>Lippia alba</i> "Carvona" (LAA455005)	10.29 (9.38-11.29)	6.17 (5.24-7.26)	1.67
<i>Lippia origanoides</i> (VEbyW06B2)	10.55 (9.66-11.51)	3.50 (2.82-4.36)	3.01
<i>Lippia micromera</i> (VEceW01H)	10.57 (10.02-11.15)	6.37 (5.53-7.34)	1.66
<i>Lippia alba</i> (VEboW01E)	11.41 (10.63-12.25)	3.61 (3.15-4.14)	3.16
<i>Lippia origanoides</i> (VEbyW06B3)	12.42 (12.05-12.79)	7.90 (7.16-8.73)	1.57
<i>Lippia origanoides</i> (VEsaW06H)	14.05 (13.15-15.00)	7.97 (6.68-9.50)	1.76
<i>Lippia origanoides</i> (VEbgW05H)	15.79 (15.10-16.52)	4.63 (3.87-5.54)	3.41
<i>Lippia alba</i> (VEbgW01E)	15.85 (14.63-17.17)	5.62 (4.54-6.96)	2.82
<i>Lippia origanoides</i> (VEcdW01H)	17.45 (16.90-18.02)	7.43 (6.28-8.79)	2.35
<i>Lippia origanoides</i> (VEsaW04B)	20.13 (18.87-21.48)	9.58 (8.78-10.46)	2.10
<i>Lippia alba</i> "Citral" (VEbgW03H)	24.87 (23.54-26.27)	11.62 (10.10-13.37)	2.14
<i>Lippia alba</i> (VEmoW02E)	27.57 (25.60-29.69)	8.08 (6.86-9.53)	3.41
<i>Lippia alba</i> (VEsaW15H)	29.26 (27.53-31.09)	7.18 (5.95-8.67)	4.07
<i>Lippia alba</i> (VEtoW02B)	30.45 (28.56-32.47)	9.84 (8.12-11.92)	3.09
<i>Lippia alba</i> (VEamW02E)	34.90 (32.83-37.11)	13.66 (11.79-15.84)	2.55
<i>Genus Piper</i>			
<i>Piper eriopodon</i> (PPceW03A)	4.43 (3.91-5.02)	2.54 (2.19-2.95)	1.74
<i>Piper bredemeyeri</i> Jacq.(PPceW04H)	4.96 (4.31-5.70)	2.92 (2.40-3.55)	1.70
<i>Piper brachypodom</i> (PPccW23E)	7.15 (6.32-8.08)	2.32 (2.02-2.67)	3.08
<i>Piper auritum</i> Kunth (PPvcW01H)	8.48 (7.75-9.28)	3.84 (3.18-4.64)	2.21
<i>Piper var Brachypodom</i> (PPccW24E)	8.59 (7.78-9.49)	3.56 (2.84-4.46)	2.41
<i>Piper septuplinervium</i> (PPccW20E)	9.60 (8.84-10.42)	3.87 (3.20-4.68)	2.48
<i>Piper lanceifolium</i> Kunth (PPccW21E)	14.41 (13.34-15.57)	7.54 (6.82-8.34)	1.91
<i>Piper cf. brachypodom</i> (PPccW18E)	15.72 (13.34-18.54)	10.30 (9.51-11.15)	1.53
<i>Piper bogotense</i> (PPnaW01H)	17.24 (16.75-17.75)	10.43 (9.63-11.29)	1.65
<i>Piper cf. divaricatum</i> (PPtoW02H)	19.24 (18.12-20.43)	10.12 (9.13-11.21)	1.90
<i>Piper var brachypodom q2</i> (PPccW25E)	21.60 (19.30-24.17)	9.41 (8.39-10.56)	2.30
<i>Piper marginatum</i> (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 LC₅₀ 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 eripodon* and *Piper bredemeyeri jacq.*, with LC₅₀ values lower than 5 µg/mL. Oils that presented lowest toxicity were *Piper marginatum* and *Piper var brachypodom q2* (PPccW25E), which showed LC₅₀ 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 <i>Lippia</i>	
<i>Lippia citriodora</i> (VEamW01E)	Geranial (18.9), neral (15.6), limonene (10.7), 1,8-cineol (5.0), spathulenol (4.7), geraniol (2.7), <i>trans</i> - β -caryophyllene (2.3), nerol (2.0), sabinene (1.9), 6-methyl-5-hepten-2-ona (1.5), geranyl acetate (1.3).
<i>Lippia organoides</i> (VEnaW02B)	Thymol (54.5), <i>p</i> -cymene (10.0), α -terpinene (1.6), γ -terpinene (5.0), thymyl acetate (4.8), β -myrcene (2.8), <i>trans</i> - β -caryophyllene (2.4), methyl thymyl ether (1.9), carvacrol (1.7).
<i>Lippia organoides</i> (VESaWCR-01)	Thymol (66), <i>p</i> -cymene (7.2), γ -terpinene (4.4), <i>trans</i> - β -caryophyllene (3.6), β -myrcene (2.2), methyl thymyl eter (2.3), α -humulene (2.4), carvacrol (1.5).
<i>Lippia dulcis</i> (VEbgW04H)	<i>trans</i> - 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), <i>trans</i> - β -farnesene (2.5), sesquisabinene (2.5), caryophyllene oxide (2.5), germacrene-D-4-ol (2.4), β -bourbonene (2.0).
<i>Lippia alba</i> "Carvona" (LAA455005)	Carvone (35.0), limonene (29.7), bicyclosesquiphellandrene (8.7), piperitenone (5.7), piperitone (3.5), β -bourbonene (2.6).
<i>Lippia alba</i> (VEboW01E)	Geranial (30.5), neral (23.6), geraniol (6.3), <i>trans</i> - β -caryophyllene (6.2), 6-methyl-5-hepten-2-ona (6.0), limonene (3.7), nerol (2.6), linalol (2.1).
<i>Lippia alba</i> "Citral" (VEbgW03H)	Geranial (23.3), neral (19.5), geraniol (9.5), <i>trans</i> - 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 <i>Piper</i>	
<i>Piper brachypodom</i> (PPccW23E)	<i>trans</i> - β -Caryophyllene (20.2), caryophyllene oxide (10.8), bicyclogermacrene (8.1), germacrene D (5.9), 9-epi- <i>trans</i> - 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), <i>trans</i> -calamenene (1.5), guaiol (1.2), α -cubebene (1.0).
<i>Piper lanceifolium</i> (PPccW21E)	<i>trans</i> - 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), <i>p</i> -cymene (1.9), <i>trans</i> -nerolidol (1.7).
<i>Piper dolichotrichum</i> (PPccW18E)	Germacrene D (16.7), <i>trans</i> - 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).
<i>Piper bogotense</i> (PPnaW01H)	<i>trans</i> -Sabinene hydrate (14,2). α -phellandrene (13,7). α -Pinene (8,7). linalol (4,7). limonene (5,3). <i>p</i> -cymene (4,4). β -phellandrene (3,4). δ -cadinene (3,4). <i>trans</i> - β -caryophyllene (3,1). α -bisabolol (2,4). β -curcumene (1,7). <i>trans</i> - β -farnesene (1,6). bicyclogermacrene (1,5). α -copaene (1,1).
<i>Piper marginatum</i> (PPtoW01H)	Elemecin (18.0), <i>trans</i> - β -caryophyllene (11.0), α -phellandrene (11.1), isoelemecine (9.2), limonene (7.5), bicyclogermacrene (4.1), β -elemene (4.0), <i>trans</i> -anetol (3.4), exalatacin (3.2), α -pinene (2.5), <i>cis</i> -methyl isoeugenol (2.4), β -phellandrene (2.2), <i>cis</i> -nerolidol (1.8), α -humulene (1.7), <i>trans</i> -methyl isoeugenol (1.7), β -pinene (1.4), <i>cis</i> -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.

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