Emirates Journal of Food and Agriculture. 2021. 33(8): 704-710
doi: 10.9755/ejfa.2021.v33.i8.2739
http://www.ejfa.me/

SHORT COMMUNICATION

Chemical composition and antibacterial activity of *Ageratina neriifolia* (B.L.Rob.)(Asteraeae) extracts from Mérida-Venezuela

María Eugenia Lucena de Ustáriz[1]*, Francisco Javier Ustáriz Fajardo[2], Karina Meza Briceño[2], Soto Carrero[2], Luís Beltrán Rojas-Fermín †[3], Yndra Elena Cordero de Rojas[2], Silvia Hipatia Torres Rodríguez[4], Javier Ernesto Ustáriz Lucena[5]

[1]University National of Chimborazo, Faculty of Health Sciences, Clinical Laboratory School, Riobamba 060150, Ecuador, [2]University of Los Andes, Faculty of Pharmacy and Bioanalysis, Department of Clinical Bioanalysis, Mérida 5101, Venezuela, [3]University of Los Andes, Faculty of Pharmacy and Bioanalysis, Research Institute. Natural Products Section, Mérida 5101, Venezuela, [4]National University of Chimborazo, Faculty of Engineering. Riobamba, Ecuador, [5]Pontificia University Católica de Chile, Department of Chemical Engineering and Bioprocesses, Santiago 7820436, Chile

ABSTRACT

This work's main objective was to chemically characterize and determine the antibacterial activity of the extracts from fresh leaves of *Ageratina neriifolia* (Asteraceae) collected at the La Culata area in the State of Mérida in Venezuela. The extracts obtained via cold maceration with hexane and dichloromethane were concentrated under reduced pressure on a rotary evaporator and later analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The identification of the components was carried out by comparison of their mass spectra. The evaluation of the extracts' antibacterial activity was carried out employing the agar-disk diffusion test against gram-positive and gram-negative strains. Sixteen (16) compounds were identified in each extract, corresponding to 75.03% (hexane) and 68.32% (dichloromethane) of the total of isolated compounds. The main components in the hexane and dichloromethane extracts were kaureno-19-acetate (24.48% and 18.41%, respectively) and kaurenol (10.46% and 11.22%, respectively). The results showed that the hexane extract showed not to be soluble in dimethylsulfoxide, so it was not possible to determine the antibacterial activity under the experimental conditions established in the study. While, the dichloromethane extract exhibited activity against the gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and against the gram-negative bacteria (*Pseudomona aeruginosa*), at a Minimum Inhibitory Concentration (MIC) of 156, 625, and 2.500 µg/mL, respectively. Results that show the antibacterial activity of *Ageratina neriifolia* hexane extract against gram-positive and gram-negative bacterial strains, unlike that previously described for other species of the genus *Ageratina*.

Keywords: Asteraceae, *Ageratina neriifolia*, diterpenes, kaureno-19-acetate, kaurenol, antibacterial activity.

INTRODUCTION

The Asteraceae family has a cosmopolitan distribution and consists of approximately 1,535 genera and 23,000 species, mainly in tropical mountain regions in South America, made up of grasses, shrubs, trees, where plants can either be erect, climbing, or creeping (Freire-Fierro, 2004, Gonzaga et al., 2005). *Ageratina* is a genus of flowering plants in the Asteraceae family, containing 248 species divided into 5 subgenera (Tereschuk et al., 1997); most are perennial herbaceous plants from 0.5 to 3 meters tall, and a few are shrubs (Briceño et al., 2002). In Venezuela, they are located in the States of Amazonas, Aragua, Bolívar, Federal District, Monagas, Zulia, Táchira, Mérida and Trujillo, mainly between 1000 to 3850 m.a.s.l. (Briceño and Morillo, 2002). The *Ageratina neriifolia*, also formerly known as *Eupatorium neriifolia* (BLRov) species, commonly called (Velvet), is characterized by being a small, branched shrub or tree, with rounded branches, opposite leaves, petiolate, lanceolate-oblong up to lanceolate, acuminate at both ends, of around 7-18 cm long and 1.5-5 cm wide (Benitez et al., 2010). In traditional medicine, for many years, species such as *Ageratina pichinchensis* have been used to treat superficial mycoses (Romero et al., 2009, Aguilar et al., 2009),while *Ageratina glabrata* used as an analgesic (García et al., 2011). Studies carried out with extracts and essential oils obtained

***Corresponding author:**
María Eugenia Lucena de Ustáriz, University National of Chimborazo, Faculty of Health Sciences, Clinical Laboratory School, Riobamba 060150, Ecuador, **E-mail:** mlucena@unach.edu.ec

Received: 01 May 2021; **Accepted:** 20 July 2021

from species of the genus *Ageratina* have revealed a variety of biological activities, including antiviral activity for the *Ageratina havanensis* (Del Barrio et al., 2011), antibacterial activity for *Eupatorium (E.areolare, E.arsenei, E.glabratum and E.pulchellum)* (García et al., 2015), *Ageratina jahnii* and *Ageratina pichinchensis* (Torres et al., 2013), *Eupatorium adenophorum* (Kurade et al., 2010), *Ageratina neriifolia* (Velasco et al., 2006) and *Ageratina deltoidea* (Arciniegas et al., 2018) and cytotoxic activity for *Ageratina vacciniaefolia* (Borrego et al., 2016). Likewise, various studies from alcoholic and hexane extracts of the genus *Ageratina* species have identified the main components: Flavonoids, flavones, and flavanones (Del Barrio et al., 2011; Silva et al., 2017; Torres et al., 2019), sesquiterpene lactones, sesquiterpenes, and monoterpenes (Kurade et al., 2010), diterpenes (Buitrago et al., 2002; Arciniegas et al., 2018; Torres et al., 2019), chromenes (Rojas et al., 2015; Torres et al., 2019), linked to biological activities.

The Venezuelan Andean Region is characterized by its botanical diversity, where a wide variety of genus *Ageratina* species stand out, some of which have not been studied yet or have only been partially studied according to the available literature(Briceño and Morillo, 2002; Benitez et al., 2010). The *Ageratina neriifolia* species, native to the La Culata area in the State of Mérida -Venezuela, was selected to analyze its chemical composition and the antibacterial activity of its foliar extracts against gram-positive and gram-negative bacterial strains. This was done to contribute to the characterization of these species and generate data on possible active principles with pharmacological potential.

MATERIAL AND METHODS

Collection of plant material

Fresh leaves of *Ageratina neriifolia* were collected in March 2017 in the La Culata area (Sierra La Culata National Park) located in the Libertador Municipality of the State of Mérida in the Bolivarian Republic of Venezuela at an altitude of approximately 3,100 m.a.s.l. The Sierra de La Culata National Park is part of the Andes Mountains. It is located in the heart of the Intertropical Zone, in the north of South America, west of Venezuela and in the northeast of the State of Mérida, covering an area of over 2000 km² and extending between the 8°35'22" and 9°10'4" N and 70°34'34" and 71°27'47" W. As a characteristic feature of a tropical mountain range, the park exhibits abrupt physiography and great altitude slopes, ranging from 800 to 4,760 m.a.s.l. (Aldana and Bosque, 2008). A specimen of *Ageratina neriifolia* was deposited in the herbarium "Luis Ruiz Terán" (MERF) of the Faculty of Pharmacy and Bioanalysis of the University of Los Andes. The Forest Engineer Juan Carmona Arzola identified the plant.

Preparation of leave extracts

Fresh leaves (400 g) of *Ageratina neriifolia* were dried at 37ºC for four days and pulverized in a mortar. The material, divided into two equal parts, was macerated separately with cool hexane and dichloromethane for eight days. Subsequently, the foliar material was pressed, and its extracts were filtered. Solvents were removed by distillation under reduced pressure at 60°C, obtaining dry weights of 24.20 g and 23.63 g for the hexane and the dichloromethane extracts, respectively. An aliquot of 50.3 mg was taken from the hexane extract and dissolved into 5 mL of hexane; while 50.7 mg was taken from the dichloromethane extract and dissolved into 5 mL of acetone, both extracts were filtered with activated carbon.

Chemical composition analysis

Gas chromatography coupled to mass spectrometry (GC-MS)

GC-MS was used to analyze the *Ageratina neriifolia* extracts' volatile components with a Heweltt Packard 6890 series II gas chromatograph coupled to a Heweltt Packard 5973 mass detector, equipped with an HP automatic injector and an HP-capillary column. 5MS 30 m long x 0.25 mm diameter x 0.25 μm film thickness. The initial temperature was 150°C, with a temperature increase rate of 5°C/min and a final temperature of 300°C. Helium was used as carrier gas at a flow of 1mL/min adjusted to a linear speed of 34 m/s; ionization energy was 70 eV; scan range 40-50 amu; 3.9 scans/s. The final injection volume was 1 μL with a 100:1 split (Adams, 2007).

The identification of the compounds was based on the Wiley MS Data Library database and NIST 05 (Davies, 1990)

Antibacterial activity

Antibacterial activity was determined employing the paper-disk-diffusion agar method (CLSI, 2017), using the gram-positive strains: *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212; and gram-negative: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 23357and *Pseudomona aeruginosa* ATCC 27853 as reference strains (provided by the Department of Microbiology and Parasitology of the Faculty of Pharmacy and Bioanalysis of the University of Los Andes – Venezuela).

Determination of the minimum inhibitory concentration (MIC)

Once the presence of inhibition zones had been determined in the reference bacterial strain cultures against each extract of *Ageratina neriifolia*, the minimum inhibitory concentration (MIC) was determined via dilutions of the extracts in dimethylsulfoxide (DMSO) with concentrations ranging from 10,000 to 38 μg/mL (Velasco et al., 2008).

Reference antibiotics Ampicillin® (10 μg), Erythromycin® (15 μg) and Piperacillin® (100 μg) were used as positive controls, while DMSO was used as the negative control at a concentration equivalent to the maximum used in the test crops, without ever exceeding (0.8%) v/v. All tests were done in duplicate.

RESULTS AND DISCUSSION

The yield of the extracts obtained by cold maceration with hexane and dichloromethane, which concentrated under reduced pressure, was 1.81% and 1.77%, respectively. In the hexane extract, 16 compounds were identified, corresponding to 75.03% of the *Ageratina neriifolia* extract's total components. The constituents of the hexane extract are listed in Table 1. Almost all of the constituents were of the terpene type, mainly eleven (11) sesquiterpenes, four (4) Diterpenes, and one (1) Steroid. Within the terpenoid components, ten (10) corresponded to hydrocarbon sesquiterpenes, one (1) oxygenated sesquiterpenes, and four (4) oxygenated diterpenes. The major compounds identified were: kaureno-19-acetate (24.48%), kaurenol (10.46%), kaurenal (6.91%), and α-humulene (5.52%). Table 2 lists the *Ageratina neriifolia* dichloromethane extract components, where 16 compounds were identified, corresponding to 68.32% of the extract's total components. All the constituents were of the terpene type, mainly: eleven (11) sesquiterpenes and five (5) Diterpenes. Within these terpenes, ten (10) correspond to hydrocarbon sesquiterpenes, one (1) oxygenated sesquiterpene, and five (5) oxygenated diterpenes. The major compounds identified were: kaureno-19-acetate (18.41%), kaurenol (11.22%), kaurenal (5.71%), and germacrene (4.94%). In both extracts analyzed, even though fewer diterpenes were found compared to the total of components found, they represent 43.61% of the hexane extract and 40.15% of the dichloromethane extract.

According to the available literature, only one previous work has been published regarding the chemical composition of alcoholic extracts of *Ageratina neriifolia* (Buitrago et al., 2002), making it difficult to establish specific comparisons on its chemical composition. However, there are references to the chemical composition of essential oils and extracts of different species of this genus that allow a comparative analysis between species. The presence of sesquiterpenes as major components has been previously documented in several *Ageratina* species and other genera of the Asteraeae family. Pala et al. (2002) studied the chemical composition of the essential oil of *Ageratina adenophora* in the Canary Islands (Spain), determining as main components the fraction of sesquiterpenes (44.3%), followed by the fraction of monoterpenes (32.1%). These results differ from those obtained in the present study, where the hexane and dichloromethane extracts presented diterpenes as principal components and sesquiterpenes to a lesser extent.

On the other hand, Torres et al. (2013) studied in Venezuela the essential oils of *Ageratina janhii* and *Ageratina pichinchensis* determining that the major compounds were

Table 1: Identification of the components of the hexane extract of *Ageratina neriifolia* by GC-MS

#PK	Compuesto	Retention time (min)	%
1	α-copaene	6.56	2.16
3	trans- caryophyllene	7.12	2.15
4	α-humulene	7.51	5.52
5	Cadinene	7.70	1.05
6	Cadinene	7.81	2.09
7	δ-selinene	7.97	2.35
8	δ- cadinene	8.22	2.96
9	α- farnesene	8.60	1.11
10	Spatulenol	8.93	4.47
11	gamma- eudesmol	9.51	1.35
12	Kaureno	13.83	1.76
13	Kaurenal	15.86	6.91
14	Kaurenol	16.23	10.46
15	kaurene-19- acetate	17.17	24.48
16	acetoxy- progesterone	18.97	1.84
	Total compounds identified		75.03 (%)
	Hydrocarbon Sesquiterpene		28.23
	Oxygenated Sesquiterpenes		1.35
	Oxygenated diterpenes		43.61
	Steroids		1.84

Table 2: Identification of the components of the dichloromethane extract of *Ageratina neriifolia* by GC-MS

#PK	Compound	Retention time (min)	%
1	α-copaene	6.57	1.56
2	germacrene-D	6.70	3.07
3	trans- caryophyllene	7.11	2.21
4	α-humulene	7.51	4.84
5	Cadinene	7.70	0.8
6	Germacreno	7.81	4.94
7	δ-guaiene	7.97	2.33
8	δ- cadinene	8.22	2.46
9	Spatulenol	8.93	3.34
10	γ- eudesmol	9.51	1.36
11	aromadendrene epoxide	9.76	1.26
12	Kaureno	13.83	1.38
13	Phytol	14.17	3.43
14	Kaurenol	15.86	5.71
15	Kaurenol	16.22	11.22
16	kaureno-19-acetato	17.14	18.41
	Total compounds identified		68.32 (%)
	Hydrocarbon Sesquiterpene		26.81
	Oxygenated Sesquiterpenes		1.36
	Oxygenated diterpenes		40.15

the sesquiterpenes followed by monoterpenes in *A. jahnii*; meanwhile, for *A. pichinchensis*, there were mainly esters and sesquiterpenes. However, García et al. (2014) analyzed the chemical composition of hexanolic extracts of leaves, flowers, stems, and roots of *Ageratina jocotepecana* in Mexico, determining diterpenes as principal components. These results are consistent with those obtained by Buitrago et al. (2002) in alcoholic extracts of *Ageratina neriifolia*. Torres et al. (2019), in his study of the chemical composition of the dry alcoholic extracts of the aerial parts of the *Ageratina jahnii* and *Ageratina pichinchensis* species, managed to isolate five compounds from *A. jahnii*: two (2) diterpenes called ent-kaur- 16-en-19-oico and ent-kaur-16-en-3α-ol; three (3) flavonoids identified as 5-hydroxy-7,4'-dimethoxyflavone, 5,7-dihydroxy-4'-methoxyflavone and 5,4'-dihydroxy-7-methoxyflavone, and, from *A. pichinchensis*, the 6-acetyl-7-hydroxy-2,2-dimethyl-8-methoxy-2H-1-chromene was isolated. These results reflect the presence of a wide variety of diterpenes in different *Ageratina* species, including the hexane and dichloromethane extracts of *Ageratina neriifolia* analyzed in the present study. However, the differences in the chemical composition of essential oils and extracts are attributed to the characteristics of each species, subspecies, as well as to the climatic conditions, height, sunlight intensity, and seasonality in the collection habitats (Figuereido et al., 2008).

The dichloromethane extract of *Ageratina neriifolia* showed antibacterial activity against gram-positive bacteria, *S. aureus* and *E. faecalis*, at a MIC of 156 and 625µg/mL, respectively (Table 3 and Figure 1). It also showed antibacterial activity against the gram-negative bacteria *P. aeruginosa* at a MIC of 2,500µg/mL. These results partially coincide with the only previous report of antibacterial activity for ethanol and acetone extracts of *Ageratina neriifolia* published by Velazco et al. (2002), where both extracts showed activity only against gram-positive bacteria, S. *aureus* and *E. faecalis*. However, the antibacterial activity obtained with the dichloromethane extract of *Ageratina neriifolia* showed a broader spectrum of action not only against gram- positive bacteria but also against the gram-negative bacterium *P. aeruginosa*, a fact determined in this study for the first time.

The antibacterial activity results obtained with *Ageratina neriifolia* dichloromethane extract are directly linked to its components. Diterpenes and other compounds with a kauran nucleus have been widely described in a variety of essential oils and extracts of different genera not only from the Asteraeae family, characterized by their varied biological activity (Borrego et al., 2016, Cordero et al., 2017, Da Costa et al., 2018, Hong et al., 2019, Pfeifer et al., 2019, Costa et al., 2020, Aimond et al., 2020, Çiçek et al., 2020). Most of the diterpenes present in *Ageratina neriifolia* dichloromethane extract have been linked directly or indirectly with antibacterial activity. Phytol is described as a major or minor component of essential oils and alcoholic extracts with antibacterial and antifungal activity (Troncoso

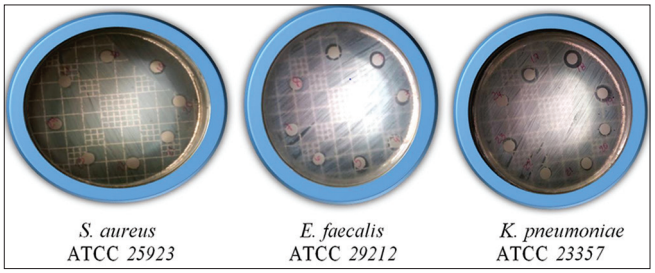

Fig 1. Antibacterial activity of *A. neriifolia* dichloromethane extract against gram positive and gram negative bacteria

Table 3: Antibacterial activity of the dichloromethane extract of *Ageratina neriifolia*

Dichloromethane extract *Ageratina neriifolia*											
Average Halos of Inhibition (mm) X ± SD											
Concentration (µg/mL)											
										Control (+)	Control (-)
Microorganisms	**10000**	**5000**	**2500**	**1250**	**625**	**312**	**156**	**78**	**38**	**AM ERI PRL**	**DMSO**
S. aureus ATCC *25923*	10,00±0,00	9,10±0,14	9,15±0,21	9,05±0,07	9,15±0,21	8,00±0,00	7,95±0,07	-	-	32	0
E. faecalis ATCC *29212*	9,00±0,00	9,05±0,07	8,95±0,07	9,00±0,00	9,10±0,14	-	-	-	-	32	0
E. coli ATCC *25922*	-	-	-	-	-	-	-	-	-	27	0
P. aeruginosa ATCC *27853*	11,00±0,00	18,85±0,21	9,00±0,00	-	-	-	-	-	-	27	0
K. pneumoniae ATCC *23357*	-	-	-	-	-	-	-	-	-	27	0

Legend: AM: ampicillin® (10 µg), PRL: piperacillin® (100µg), ERI: erythromycin® (15µg), DMSO: dimethyl sulfoxide.

et al., 2015, Vitola and Pérez, 2016, Jaramillo et al., 2020); Kaurenol with antibacterial activity (Cordero et al., 2017); Kaurenal with antifungal activity (Zimmermam et al.,2013); Spathulenol with antibacterial activity (Sánchez et al., 2014). Minor components, such as germacrene D, play an essential role as a precursor to sesquiterpenes, like cadinenes and selenanes, characterized by their antibacterial activity (Bülow and Konig, 2000). Similarly, germacrene D, present in essential oils and extracts from various families and botanical species, has shown a broad range of biological activity, highlighting its antibacterial activity (Rincón et al., 2012; Lima et al., 2015, Bruzual et al., 2015), antifungal activity (Lima et al., 2015, Bruzual et al., 2015, Ingaroca et al., 2019), and insecticidal activity against mosquitoes and repellent (Andrade et al., 2017; Valdez et al., 2018). Likewise, other minor compounds present in the dichloromethane extract of *Ageratina neriifolia* are part of essential oils and extracts of the Asteraceae family members, showing to be biologically active (Grande et al., 2016). Standing out are α-humulene related to antibacterial and antifungal activity (Ingaroca et al., 2019, Aparicio et al., 2019), adulticidal and insecticidal activity (Andrade et al., 2017, Valdez et al., 2018); δ- cadinene related to antifungal activity (Ingaroca et al., 2019), and spathulenol to antibacterial activity (Rincón et al., 2012; Lucena et al., 2019). On the other hand, the hexane extract of *Ageratina neriifolia* showed not to be soluble in DMSO, so it was not possible to carry out antibacterial activity tests under the experimental conditions established in the study.

The results obtained in this study can constitute a starting point for subsequent antimicrobial trials. Also, they can be used to carry out the elucidation of the main compounds with bioactivity that will allow for the synthesis or hemisynthesis of antimicrobial drugs.

CONCLUSIONS

The dichloromethane extract of *Ageratina neriifolia* is mainly made up of oxygenated diterpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes, exhibiting activity against the gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis)* and against the gram-negative bacteria *Pseudomona aeruginosa*, unlike that previously described for other species of the genus *Ageratina*.

Authors' contributions

María Eugenia Lucena de Ustáriz: Laboratory methodological consultants and determination of antibacterial activity. Francisco Javier Ustáriz Fajardo: Design, coordination of research and writing. Karina Meza Briceño and Verónica Soto Carrero: Determination of antibacterial activity. Luís Beltrán Rojas-Fermín†: Chemical analysis by gas chromatography coupled to mass spectrometry. Yndra Elena Cordero de Rojas and Silvia Hipatia Torres Rodríguez: Data análisis. Javier Ernesto Ustáriz Lucena: Data análisis

Conflict of interest

The authors do not incur conflicts of interest.

ACKNOWLEDGMENTS

The authors are grateful to Direct Support Program for Research Groups (ADG in Spanish) of the Council for Scientific, Humanistic, Technological and Arts Development of the Universidad de Los Andes, Mérida, Bolivarian Republic of Venezuela. Research Group: Natural Products and Medicinal Chemistry; Research Institute, Natural Products Section, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida, the Bolivarian Republic of Venezuela (No Code or reference number assigned).

REFERENCES

Adams, R. 2007. In: A. P. Corporation, (Ed.), Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy. 4th ed. Allured Publishing, Carol Stream, Illinois, USA.

Aguilar, B., V. Navarro, I. León and M. Rios. 2009. Active compounds against tinea pedis dermatophytes from *Ageratina pichinchensis* var. bustamenta. Nat. Prod. Res. 23(16): 1559-1565.

Aimond, A., K. Calabro, C. Audoin, E. Olivier, M. Dutot, P. Buron, P. Rat, O. Laprévote, S. Prado, E. Roulland, O. Thomas and G. Genta. 2020. Cytotoxic and anti-inflammatory effects of ent-kaurane derivatives isolated from the Alpine plant *Sideritis hyssopifolia*. Molecules. 25: 589.

Aldana, A. and J. Bosque. 2008. Evaluación de la zonificación de uso del Parque nacional sierra de La Culata, Mérida-Venezuela. Rev. Forest. Latinoam. 23(1): 9-34.

Arciniegas, A., A. Pérez, M. Meléndez, J. Ávila, A. García, J. Villaseñor and A. Romo. 2018. Chemical composition and antimicrobial activity of *Ageratina deltoidea*. Chem. Biodivers. 15(3): e1700529.

Andrade, S., L. Sánchez, G. Nevárez, A. Camacho and B. Nogueda. 2017. Aceites esenciales y sus componentes como una alternativa en el control de mosquitos vectores de enfermedades. Biomédica. 37(S2): 224-243.

Aparicio, R., R. Velasco. R. R. Paredes and L. Rojas. 2019. Caracterización química y actividad antibacteriana del aceite esencial de *Mangifera indica* L. de tres regiones de Venezuela. Rev. Colomb. Quím. 48(3): 13-18.

Benitez, C., F. Leal and L. Schnee. 2010. El Manual de Plantas Comunes de Venezuela de Ludw Schnee. 3rd ed. Universidad Central de Venezuela, Caracas.

Borrego, P., L. Rojas, J. Robles, S. Fiorentino, J. Hernández, L. Orduz and L. Pombo. 2016. Evaluación de la actividad citotóxica de extractos y fracciones de las especies *Conyza trihecatactis* y *Ageratina vacciniaefolia*. Rev. Facul. Cien. Básic. 12(2): 212-227.

Briceño, B. and G. Morillo. 2002. Catálogo abreviado de las plantas

con flores de los páramos de Venezuela. Parte I. Dicotiledóneas (Magnoliopsida). Acta Bot. Venez. 25(1): 1-75.

Bruzual, H., W. Henríquez, O. Crescent and J. Lanza. 2015. Aceite esencial de *Wedelia calycina* (ASTERACEAE): Composición química, actividad antibacteriana y antifúngica. Saber. 27(1): 87-93.

Buitrago, D., A. Morales, J. Rojas, M. Rondón and A. Bahsas. 2002. Diterpenoids from *Ageratina neriifolia* (B. Robinson) R. King and H Robinson. Rev. Latinoamer Quím. 30(2): 49-51.

Bülow, N. and W. Konig. 2000. The role of germacrene D as a precursor in sesquiterpene biosynthesis: Investigations of acid catalyzed, photochemically and thermally induced rearrangements. Phytochemistry. 55(2): 141-68.

Çiçek, S., A. Wenzel, U. Girreser and D. Tasdemir. 2020. Biological activities of two major copaiba diterpenoids and their semi-synthetic derivatives. Rev. Bras. Farmacogn. 30: 18-27.

CLSI. 2017. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI, United State.

Cordero, Y., M. Lucena, L. Araujo, A. Usubillaga, L. Rojas and L. Moujir. 2017. Actividad antibacteriana de diterpenos del kaurano aislados de *Coespeletia moritziana* (Sch. Bip. ex Wedd.) Cuatrec. Rev. Facult. Farm. 59: 3-7.

Costa, L., T. Souza, A. Colmanette, M. Ferreira, H. Pereira, G. Hollaender, L. de Lima, J. Kenupp, N. Jacometti, R. Tame, R. Sola, L. Guidi and S. Ambrósio. 2020. Schistosomicidal activity of kaurane, labdane and clerodane-type diterpenes obtained by fungal transformation. Proc. Biochem. 98: 34-40.

Da Costa, R., J. Bastos, M. Costa, M. Ferreira, C. Mizuno, G. Caramori, G. Nagurniak, M. Simão, R. Dos Santos, R. Veneziani, S. Ambrósio and R. Parreira. 2018. *In vitro* cytotoxicity and structure-activity relationship approaches of ent-kaurenoic acid derivatives against human breast carcinoma cell line. Phytochemistry. 156: 214-223.

Davies N. 1990. Gas chromatographic retention indices of monoterpenes and on methyl silicone and Carbowax 20 M phases. J. Chromatogr. 503: 24.

Del Barrio, G., I. Spengler, T. García, A. Roque, Á. Álvarez, J. Calderón and F. Parra. 2011. Antiviral activity of *Ageratina havanensis* and major chemical compounds from the most active fraction. Rev. Bras. Farmacogn. 21(5): 915-920.

Freire-Fierro, A. 2004. Botánica Sistemática Ecuatoriana. 2nd ed. Missouri Botanical Garden Press, St. Louis, p. 96-97.

García, E., C. Ramírez, R. Martínez, A. Flores, R. del Río and M. Martínez. 2015. Antibacterial activity of some medicinal Eupatorium species against antibiotic resistant pathogenic bacteria. Polibotánica. 39: 91-101.

García, E., C. Ramírez, A. Talavera, A. León, R. Martínez, M. Martínez, M. Gómez, C. Cerda, P. Joseph and R. del Río. 2014. Absolute configuration of (13R)-and (13S)-labdane diterpenes coexisting in *Ageratina jocotepecana*. J. Natl. Prod. 77(4): 1005-1012.

García, G., R. del Río, R. Guzmán, M. Martínez and T. Scior. 2011. Estudios preliminares sobre el efecto analgesic del extracto de hojas de *Ageratina glabrata* en dos modelos térmicos de dolor agudo. Rev. Mex. Cien. Farm. 42(1): 45-51.

Gonzaga, L., I. Costa and M. Pizzolatti. 2005. The *Baccharis genus* (*Asteraceae*): Chemical, economic and biological aspects. Quim. Nova. 28(1): 85-94.

Grande, C., C. Chaves, M. Viuda, A. Serio, J. Delgado, J. Perez, N. Ospina, S. Tora, S. Palmieri and A. Paparella. 2016. Sub-lethal concentrations of Colombian *Austroeupatorium inulifolium* (H.B.K.) essential oil and its effect on fungal growth and the production of enzymes. Ind. Crops Prod. 87: 315-323.

Hong, R., K. Soo, G. Muk, M. Yeom, B. Lee, S. Lee, K. Sung, H. Sook, H. Park and D. Hahm. 2019. Continentalic acid rather than kaurenoic acid is responsible for the anti-arthritic activity of Manchurian Spikenard *in vitro* and *in vivo*. Int. J. Mol. Sci. 20: 5488.

Ingaroca, S., A. Castro and N. Ramosa. 2019. Composición química y ensayos de actividad antioxidante y del efecto fungistático sobre *Candida albicans* del aceite esencial de *Piper aduncum* L. "MATICO". Rev. Soc. Quím. Perú. 85(2): 268-279.

Jaramillo, C. 2020. Actividad antifúngica y antibacteriana in vitro del extracto etanólico de *Usnea laevis* frente a *Candida albicans*, *Staphylococcus aureus* y *Pseudomonas aeruginosa*. Rev. Med. Hered. 31: 169-174.

Kurade, N., V. Jaitak, V. Kaul and O. Sharma. 2010. Chemical composition and antibacterial activity of essential oils of *Lantana camara*, *Ageratum houstonianum* and *Eupatorium adenophorum*. Pharm. Biol. 48(5): 539-544.

Lima, P., A. Lucchese, R. Gambari, R. Piva, L. Penolazzi, M. Di Ciano, A. Trovatti, F. Silva and M. Avila. 2015. Essential oils and isolated compounds from *Lippia alba* leaves and flowers: Antimicrobial activity and osteoclast apoptosis. Int. J. Mol. Med. 35: 211-217.

Lucena, M., C. Escalante, M. González, L. Rojas, Y. Cordero, F. Ustáriz, J. Carmona and S. Torres. 2019. Composición y actividad antibacteriana del aceite esencial de *Austroeupatorium inulifolium* (Kunth) King and Robinson (*Asteraceae*). Rev. Cubana Farm. 52(4): 1-16.

Pala, J., Pérez, M., Velasco, A and Sanz, J. 2002. Analysis by gas chromatography-mass spectrometry of the volatile components of *Ageratina adenophora* Spreng., growing in the Canary Islands. J. Chromatogr. A. 947: 327-331.

Pfeifer, A., A. Wenzel, J. Barbosa, C. Zidorna, C. Peifer, D. Tasdemir and S. Çiçek. 2019. Antimicrobial and cytotoxic effects of the *Copaifera reticulata* oleoresin and its main diterpene acids. J. Ethnopharmacol. 233: 94-100.

Rincón, C., J. Castaño and E. Ríos. 2012. Actividad biológica de los aceites esenciales de *Acmella ciliata* (Kunth) Cass. Rev. Cubana Plant Med. 17(2): 160-171.

Rojas, A., J. Pardo, R. del Río, M. Gómez, D. Limón, F. Luna and I. Martínez. 2015. Determinación del efecto analgésico del extracto hexánicode flores de *Eupatorium arsenei* en un modelo de dolor agudo en rata. Rev. Mex. Cien. Farm. 46(1): 64-69.

Romero, O., R. Román, A. Zamilpa, J. Jiménez, G. Rojas and J. Tortoriello. 2009. Clinical trial to compare the effectiveness of two concentrations of the *Ageratina pichinchensis* extract in the topical treatment of onychomycosis. J. Ethnopharmacol. 126(1): 74-78.

Sánchez, Y., T. Correa, Y. Abreu, L. Cotilla, G. Berroa and O. Pino. 2014. Composición química del aceite esencial de *Piper hispidum* Sw. y actividad antimicrobiana sobre *Xanthomonas albilineans* (Ashby) Dowson y *Xanthomonas campestris* pv. campestris (Pammel) Dowson. Rev. Protección Veg. 29(3): 185-191.

Silva, B., M. Gómez, L. Granados, R. del Río and G. Rodríguez. 2017. Caracterización espectroscópica de flavonoides de *Ageratina brevipes*. Cien. Nicol. 70: 94-104.

Tereschuk, M., M. Riera, G. Castro and L. Abdala. 1997. Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. J. Ethnopharmacol. 56(3): 227-232.

Torres, L., J. Rojas, A. Morales, L. Rojas, M. Lucena and A. Buitrago.

2013. Composición química y evaluación de la actividad antibacteriana de los aceites esenciales de *Ageratina jahnii* y *Ageratina pichinchensis* recolectadas en Mérida, Venezuela. Bolet. Latinoam. Caribe Plantas Med. Aromát. 12(1): 92-98.

Troncoso, N., R. Saavedra, A. Olivares, J. Farías, S. San-Martín, H. Urrutia and C. Agurto. 2015. Identificación de compuestos antibacterianos en macroalgas presentes en la Región del Biobío, Chile. Rev. Biol. Mar. Oceanog. 50(S1): 199-204.

Valdez, A., E. Delgado and J. Ramírez. 2018. Actividad adulticida y composición química del aceite esencial de hojas de *Lantana camara* sobre *Drosophila melanogaster*. MASKANA. 9(1): 21-30.

Velasco, J., S. Barreto, D. Buitrago and R. Vivas. 2006. Antimicrobial activity of extracs from *Ageratina neriifolia* (*Asteraceae*). CIENCIA. 14(4): 411-414.

Velasco, J., M. Araque, E. Araujo, A. Longa, B. Nieves, A. Ramírez, K. Sánchez and E. Velazco. 2008. Manual práctico de Bacteriología Clínica. Colección Temas Universitarios. Vicerrectorado Académico, Universidad de los Andes, Mérida, Venezuela, p. 27-28.

Vitola, D. and A. Pérez. 2016. Bioactividad de extracto de Annona muricata y aceites esenciales de *Citrus aurantium* contra *Phytophthora cinnamomi*. Rev. Colombiana Cien. Anim. 8(S): 325-334.

Zimmermam, D., E. Bolutari, H. Polonini, A. do Carmo, M. Chaves and N. Raposo. 2013. Antifungal activity of *Copaifera langsdorffii* desf oleoresin against dermatophytes. Molecules. 18: 12561-12570.