Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas
ISSN: 0717-7917
editor.blacpma@usach.cl
Universidad de Santiago de Chile
Chile

TORRES-BARAJAS, Lizzeth; ROJAS-VERA, Janne; MORALES-MÉNDEZ, Antonio; ROJAS-FERMÍN, Luís; LUCENA, María; BUITRAGO, Alexis
Chemical composition and evaluation of antibacterial activity of essential oils of Ageratina jahnii and Ageratina pichinchensis collected in Mérida, Venezuela
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, vol. 12, núm. 1, enero, 2013, pp. 92-98
Universidad de Santiago de Chile
Santiago, Chile

Available in: http://www.redalyc.org/articulo.oa?id=85625709010

How to cite
Complete issue
More information about this article
Journal's homepage in redalyc.org

Scientific Information System
Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal
Non-profit academic project, developed under the open access initiative

© 2013 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 12 (1): 92 - 98
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Chemical composition and evaluation of antibacterial activity of essential oils of *Ageratina jahnii* and *Ageratina pichinchensis* collected in Mérida, Venezuela

[Composición química y evaluación de la actividad antibacteriana de los aceites esenciales de *Ageratina jahnii* y *Ageratina pichinchensis* recolectadas en Mérida,Venezuela]

Lizzeth TORRES-BARAJAS[1], Janne ROJAS-VERA*[1], Antonio MORALES-MÉNDEZ[1], Luís ROJAS-FERMÍN[1], María LUCENA[2] & Alexis BUITRAGO[1]

[1]Organic Biomolecular Research Group, Research Institute, Faculty of Pharmacy and Biomedical Sciences. University of Los Andes. Mérida, Venezuela.
[2]Clinical Bioanalysis Department, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela
Contactos | Contacts: Janne ROJAS VERA - E-mail address: janner@ula.ve

Abstract
Essential oil from leaves of *Ageratina jahnii* (B.L.Rob.) R. M. King & H. Rob. and *Ageratina pichinchensis* (Kunth) R. M. King & H. Rob (Asteraceae) collected in January 2010 were analyzed by **GC/MS**. Oils extracted by hydrodistillation yielded 0.50% and 0.43 % w/v, respectively. Fifteen and twenty five components were identified by comparison of their mass spectra with the Wiley **GC-MS** Library data and by their retention indices (**RI**). The major components identified in *A. jahnii* were *β*-myrcene (37.6 %), *α*-pinene (17.1 %), limonene (8.8 %) and pentacosane (9.2 %) while for *A. pichinchensis* 8,9-epoxythymyl isobutyrate (20.2 %), germacrene-*D* (19.8 %), thymyl isobutyrate (10.8 %), eupatoriochromene (6.5 %) and encecalol (5.9 %) were observed as main compounds. This is the first report regarding the essential oil composition and antibacterial activity of the essential oil of *A. jahnii*.

Keywords: *Ageratina jahnii, Ageratina pichinchensis*, Asteraceae, essential oil, *β*-myrcene, *α*-pinene, germacrene-*D*

Resumen
Aceites esenciales de las hojas de *Ageratina jahnii* (B.L.Rob.) R. M. King & H. Rob. y *Ageratina pichinchensis* (Kunth) R. M. King & H. Rob (Asteraceae) colectadas en enero 2010 fueron analizados por **CG/EM**. Los aceites extraídos por hidrodestilación produjeron 0,50 % y 0,43 % p/v de rendimiento, respectivamente. Quince y veinticinco compuestos fueron identificados por comparación de sus espectros de masas con la base de datos de la librería Wiley **CG/EM** y por sus índices de retención (**IR**). Los compuestos identificados como majoritarios en *A. jahnii* fueron *β*-mirceno (37,6 %), *α*-pineno (17,1 %), limoneno (8.8 %) y pentacosano (9,2 %) mientras para *A. pichinchensis* isobutirato de 8,9-epoxitimilo (20,2 %), germacreno-*D* (19,8 %), isobutirato de timilo (10,8 %), eupatoriocromeno (6,5 %) y encecalol (5,9 %) fueron observados como compuestos mayoritarios. Este es el primer reporte sobre la composición química y actividad antibacteriana del aceite esencial de *A. jahnii*.

Palabras Clave: *Ageratina jahnii, Ageratina pichinchensis*, Asteraceae, essential oil, *β*-mirceno, *α*-pineno, germacreno-*D*

Recibido | Received: 22 de Mayo de 2012.
Aceptado en versión corregida | Accepted in revised form: 26 de Julio de 2012.
Publicado en línea | Published online: 30 de Enero de 2013.
Este artículo puede ser citado como / This article must be cited as: L Torres-Barajas, J Rojas-Vera, A Morales-Mendez, L Rojas-Fermín, M Lucena, A Buitrago. 2013. Chemical composition and evaluation of antibacterial activity of essential oils of *Ageratina jahnii* and *Ageratina pichinchensis* collected in Mérida-Venezuela. **Bol Latinoam Caribe Plant Med Aromat** 12(1): 92 – 98.

Abbreviations list: w/v-weight/volumen; p/v-peso/volumen; **GC/MS**-gass chromatography/mass spectrometry; **CG/EM**-cromatografía de gases/espectrometría de masas.

INTRODUCTION

The *Ageratina* Spach genus belongs to Oxylobinae subtribe, Eupatorieae tribe, Asteraceae family (Herz, 2003). Species of this genus are distributed in Colombia, Ecuador, Guatemala, México, Panamá and Perú. In Venezuela are located in Amazonas, Aragua, Bolívar, Distrito Federal, Monagas, Zulia, Táchira, Mérida and Trujillo, mainly between 1000 to 3850 m.a.s.l. (Briceño and Morillo, 2002). *Ageratina* species have been used for many years in traditional medicine for the treatment of superficial mycosis, skin infections and wounds, as well as for its analgesic activity (Romero, *et al*., 2009; Aguilar *et al*., 2009; Garcia *et al*., 2011). On the other hand, previous investigations conducted on different species of this genus have revealed anti-inflammatory (Chakravarty, 2011) antiviral (del Barrio, 2011) antibacterial (Briceño and Morillo, 2002; Del Barrio *et al*., 2011; Kurade *et al*., 2010) molluscicidal (Zou F *et al*., 2009) and larvicidal activities (Mohan and Ramaswamy, 2007).

Chemical composition of the alcoholic extracts from different *Ageratina* species have also been investigated reporting flavonoids, chromens, sesquiterpenic lactones, diterpenes, triterpenes, flavones and flavanones (Briceño and Morillo, 2002; del Barrio *et al*., 2011; Kurade *et al*., 2010; Buitrago *et al*., 2002; Rios *et al*., 2003). Essential oil composition of different *Ageratina* (Syn. *Eupatorium*) species has also been investigated, which reveal a variety of compounds type monoterpernes and sesquiterpenes. So, germacrene-*D* and *α*-phellandrene have been isolated of *E. Adenophorum* (Kurade *et al*., 2010); thymol and carvacrol for *A. ibaguensis* (Sanabria *et al*., 1999); *β*-cubebene, phellandrene, *δ*-cadinene, zingiberene and *α*-cubebene in the essential oil of *A. dendroides*.

The present investigation aim to compare the chemical composition and evaluate the antibacterial activity of essential oils of *A. jahnii* and *A. pichinchesis* collected from Mérida-Venezuela. To the best of our knowledge there are no reports for the essential oil composition and the antibacterial activity of *A. jahnni*.

MATERIALS AND METHODS

Plant material

Ageratina jahnii (B.L.Rob.) R. M. King & H. Rob. was collected in the páramo "Piedra Pirela", 5 km from San José de Acequia, Mérida state at 3122 m.a.s.l. *Ageratina pichinchensis* (Kunth) R. M. King & H. Rob. was collected in the páramo "Las Coloradas", Campo Elias, Mérida State at 2500 m.a.s.l.

Plants were collected in January of 2010 and their Voucher specimens (*A. jahnii*, LT02 and *A. pichinchensis*, PM 614) were deposited in the Luis Ruiz Terán Herbarium of the Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela.

Isolation of essential oil

Fresh leaves (*A. jahnii*, 1470 g) and (*A. pichinchensis*, 1700 g) were cut into small pieces and submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulfate and stored at 4 ºC.

Gas chromatography (GC)

GC analyses were performed on a Perkin-Elmer Autosystem Gas Chromatograph equipped with a Flame Ionization Detector. A capillary column of 5% phenylmethyl polysiloxane fused-silica (AT-5, Alltech Associates Inc., Deerfield, Il), 60 m x 0.25 mm, film thickness 0.25 µm, was used for the analysis. The initial oven temperature was 60 °C; then at rate of 4° C/min, was raised to 260 °C, this was maintained for 20 min. The injector and detector temperatures were 200 °C and 250 °C, respectively. The carrier gas was helium at 1.0 mL/min. The sample was injected using a split ratio of 1:100. Retention indices were calculated relative to C_8-C_{24} *n*-alkanes, and compared with values reported in the literature (Adams, 2007; Davies, 1990)

Gas chromatography-Mass spectrometry (GC-MS)

The **GC-MS** analyses were carried out on a Hewlett Packard **GC-MS** system, model 5973, fitted with a fused-silica capillary column of 5% phenylmethyl polysiloxane of 30 m x 0.25 mm, film thickness 0.25 µm (HP-5MS, Hewlett Packard, USA). The source and quadrupole temperatures were 230 °C and 150 °C, respectively. Helium was used as carrier gas, adjusted to a linear velocity of 34 m/s. The ionization energy was 70 ev, and the scan range 40-500 amu at 3.9 scans/s. The injected volume was 1.0 µl of a dilution

of oil in *n*-heptane (2%). A Hewlett-Packard ALS injector was used with a split ratio of 1:100. The identification of the components was based on a Wiley MS data library (6^{th} ed), followed by comparisons of MS data with published literature (Adams, 2007).

Antimicrobial assay

Bacterial strains

Five strains from the American Type Culture Collection (**ATCC**) were used in the present investigation: *Staphylococcus aureus* (**25923**), *Enterococcus faecalis* (**19433**), *Escherichia coli* (**25992**), *Pseudomonas aeruginosa* (**27853**) and *Klebsiella pneumoniae* (**25955**).

Antimicrobial method

The antimicrobial assay was carried out according to the disc diffusion method described by Rondón *et al*., 2005. The strains were maintained in agar at room temperature. Every bacterial inoculum (2.5 mL) was incubated in Mueller-Hinton broth at 37 ºC for 18 h. The bacterial inoculum was diluted in sterile saline solution (0.85%) to obtain turbidity visually comparable to a McFarland Nº 0.5 standard (106-8 CFU/mL). Every inoculum was spread over plates containing Mueller-Hinton agar and a filter paper disc (6 mm in diameter) saturated with 10 µL of essential oil. The plates were left for 30 min at room temperature and then incubated at 37 ºC for 24 h. The inhibitory zone around the disc was measured and expressed in mm. A positive control was also used to check the sensitivity of the tested organisms using: Amikacin® (30 µg), Ampicillin® (10 µg) and Erythromycin® (15 µg), these are reference antibiotics commonly used to treat this kind of bacteria. The minimal inhibitory concentration (**MIC**) was determined only with microorganisms that displayed inhibitory zones. **MIC** was determined by dilution of the essential oil in dimethylsulfoxide (**DMSO**) and pipetting 10 µL of each dilution onto a filter paper disc. Dilutions of the oil within a concentration range of 10-200 mg/mL were utilized. **MIC** was defined as the lowest concentration that inhibited the visible bacterial growth (CLSI document M100-S17, 2010). A negative control was also included using a filter paper disc saturated with **DMSO** to check possible activity of this solvent against the bacteria assayed. The experiments were repeated three times.

RESULTS AND DISCUSSION

Fresh leaves of *Ageratina jahnii* (**AJ**) and *Ageratina pichinchensis* (**AP**) yielded (7.3 mL, 0.50% w/v, **AJ**), and (2.5 mL, 0.43% w/v, **AP**) of essential oil, showing the presence of 15 and 25 components, respectively (Table 1). The major components identified in **AJ** were *β*-myrcene (37.6%), *α*-pinene (17.1%), limonene (8.8%) and pentacosane (9.2%), while for **AP** 8,9-epoxithymyl isobutyrate (20.2%), germacrene-*D* (19.8%), thymyl isobutyrate (10.8%), eupatoriochromene (6.5%) and encecalol (5.9%) were detected in major concentrations. Several differences might be observed in the composition of the two oils; germacrene-*D* is present in higher concentrations (19.8%) in the **AP** sample while in the **AJ** oil was detected in lower amount (4.6%). *δ*-cadinene was identified in the two oils at low concentrations (2.1% **AJ** and 1.2% **AP**). However compounds like 8,9-epoxithymyl isobutyrate (20.2%) and thymyl isobutyrate (10.8%) were only observed in the **AP** sample while *β*-myrcene (37.6%), *α*-pinene (17.1%) and limonene (8.8%) were only present in the **AJ** essential oil. According to our knowledge, there are no reports from the essential oil composition of *A. jahnni*, however several studies about others *Ageratina* sp. (Syn. *Eupatorium*) have been publicated. For *E. Adenophorum* was reported *α*-bisabolol, bornyl acetate, *β*-bisabolene, germacrene-*D* and *α*-phellandrene as major components (Kurade *et al*., 2010); thymol and carvacrol were observed for *A. ibaguensis* (Sanabria *et al*., 1999); *β*-cubebene, phellandrene, *δ*-cadinene, zingiberene and *α*-cubebene were identified in the essential oil of *A. dendroides*. The essential oil of *A. adenophora* from Canary Islands showed *p*-cymene, epi-*α*-cadinol, *α*-phellandrene, *δ*-2-carene and camphene (Palá *et al*., 2002). *E. stoechadosmum* from Vietnam showed thymohydroquinone dimethyl ether, selina-4,11-diene and *β*-caryophyllene (Duñg *et al*., 1991); while *E. coelestinum* also from Vietnam were characterized by methyl chavicol, bornyl acetate, camphene, *cis*-cadin-4-en-7-ol (Duñg *et al*., 1998). *E. triplinerve* and *E. paniculatum* from Brasil were mainly composed by *β*-caryophyllene and 2,5-dimethoxy-*p*-cymene (Maia *et al*., 1999).

Table 1
Essential oil composition of *A. jahnii and A. pichinchensis* leaves collected in Mérida-Venezuela

Components	RI*	RI**	*AJ* (%)	*AP* (%)
***α*-Pinene**	943	939	17.1	-
Sabinene	980	976	0.4	-
***β*-Pinene**	984	980	4.3	-
***β*-Myrcene**	997	991	37.6	-
***α*-Phellandrene**	1011	1005	4.3	-
***p*-Cymene**	1032	1026	3.6	-
Limonene	1037	1030	8.8	-
Terpinolene	1097	1088	0.6	-
4-Terpineol	1185	1177	0.3	-
Nerol	1234	1228	-	0.6
Bornyl acetate	1297	1285	-	0.4
Thymol	1303	1290	-	0.7
Silphinene	1352	1345	-	0.3
***α*-Copaene**	1380	1376	-	0.4
Modheph-2-ene	1383	1382	-	1.8
***β*-Cubebene**	1391	1390	-	0.5
***β*-Elemene**	1393	1391	-	1.4
α-Himachalene	1454	1449	-	0.3
α-Humulene	1459	1454	-	0.7
***β*-Selinene**	1482	1483	-	0.9
Germacrene-*D*	1489	1484	4.6	19.8
Thymyl isobutyrate	1491	1490	-	10.8
Neryl isobutanoate	1496	1491	-	4.4
Bicyclogermacrene	1504	1494	-	2.2
***α*-Muurolene**	1512	1500	0.7	-
Germacrene-*A*	1512	1509	-	1.5
***δ*-Cadinene**	1529	1524	2.1	1.2
Germacrene-*B*	1565	1556	0.4	-
8,9-Epoxythymyl isobutyrate	1576	-	-	20.2
Linalyl 3-methylbutanoate	1582	-	-	3.0
Spathulenol	1583	1601	0.6	-
Desmethoxy encecalin	1653	1646	-	2.3
***α*-Cadinol**	1659	1653	-	0.8
Andro encecalinol	1684	1675	-	2.8
Eupatoriochromene	1773	1759	-	6.5
Encecalol	1868	-	-	5.9
Ripariocromeno-A	1939	-	-	2.2
Pentacosane	2485	2500	9.2	-

Ageratina jahnii (**AJ**) and *Ageratina pichinchensis* (**AP**). The composition of the essential oil was determined by comparison of the MS of each component with Wiley GC/MS library data and also from its retention index (**RI**). * RI values calculated for this investigation, **RI values from reference (Adams, 2007).

A. pichinchensis essential oil from Ecuador was constituted by *α*-pinene, camphene, borneol, gurjunene, copaene and bourbonene (Aguilera, 2010). Comparing the components identified in the **AP** sample with those of the same species collected from Ecuador a high difference is noticed since the mayor compounds in the **AP** in the present investigation are 8,9-epoxithymyl isobutyrate, germacrene-*D*, thymyl

isobutyrate, eupatoriochromene and encecalol while in the *A. pichinchensis* collected from Ecuador the major components are *β*-cubebene, *α*-bergamotene, precocene and pentanal (Aguilera, 2010). Only copaene and *δ*-cadinene where observed similarly on the two *A. pichinchensis* samples. Several reports have pointed a variety of reasons that might explain these divergences in the composition of essential oils; climatic, seasonal, geographic conditions, harvest period, distillation techniques (Mouhssen, 2004), plant organ, age and vegetative cycle stage are among the possibilities presented (Angioni *et al*., 2006; Tripathi *et al*., 2009).

Antibacterial activity of the essential oils were also evaluated against Gram-positive (*Staphylococcus aureus* **ATCC 25923**), *Enterococcus faecalis* **ATCC 19433** and Gram-negative (*Escherichia coli* **ATCC 25922**, *Pseudomonas aeruginosa* **ATCC 27853** and *Klebsiella pneumoniae* **ATCC 25955**) bacteria using the disc diffusion agar method (Table 2) showing activity against *S. aureus* and *E. faecalis* with **MIC** values of 49.5 mg/mL for **AJ** and 104 mg/mL for **AP**. Now days the study of antibacterial agents has become an important issue, due to the constant development of resistance mechanisms, from microorganisms to conventional antimicrobials. Consequently, search for new agents, those of plant origin must be emphasized, thus, the results observed in this investigation might be of interest for the natural products research.

Table 2
Antibacterial activity of essential oils of *A. jahnii* and *A. pichinchensis*

Microorganism	Inhibition zone (mm)					MIC	
	AJ	AP	Positive control			AJ	AP
			Amk	Amp	Ery		
S. aureus ATCC (25923)	13	15			35	49.5	104
E. faecalis ATCC (19433)	12	11		32		49.5	104
E. coli ATCC (25922)	NA	NA	25			NT	NT
K. pneumoniae ATCC (25955)	NA	NA	27			NT	NT
P. aeruginosa ATCC (27853)	NA	NA	25			NT	NT

Amk: Amikacin® (30 μg), **Amp**: Ampicillin® (10 μg), **Ery**: Erythromycin® (15 μg), ***Inhibition zone**, diameter measured in mm, disc diameter 6 mm. **MIC**: Minimal inhibitory concentration (concentration range: 10-200 mg/mL). **AJ**: *A. jahnii*, **AP**: *A. pichinchensis*, **NA:** none active, **NT:** None tested.

Previous investigations have reported antibacterial activity for *E. adenophorum* essential oil against *Escherichia coli, Micrococcus luteus* and *Staphylococcus aureus* (Kurade *et al*., 2010). Thymol and carvacrol isolated from *Ageratina ibaguensis* showed antibacterial and antifungal activities against *S. typhi*, *K. pneumoniae*, *P. aeruginosa*, *E.coli*, *S. aureus*, *A. niger*, *F. oxysporum* and *C. albicans* (Sanabria *et al*., 1999). On the other hand, solvent extracts from leaves of *E. glandulosum* from India exhibited antibacterial activity against *B. subtilis*, *S. aureus*, *S. pyogenes*, *E. coli*, *K. pneumonie*, *P. aeruginosa* and *S. typhi* (Sasikumar *et al*., 2005). Acetonic and ethanolic extracts of *Ageratina neriifolia* from Venezuela also revealed antibacterial activity against *S. aureus* and *E. faecalis* (Velasco *et al*., 2006).

CONCLUSSION

In the present investigation chemical composition and antibacterial activity of essential oils of fresh leaves of *A. jahnii* and *A. pichinchensis* were evaluated. *β*-

myrcene, *α*-pinene, limonene, germacrene-*D*, isobutyrate de 8,9-epoxitimilo, isobutyrate de timilo, eupatoriochromene and encecalol were the components observed in major proportions. To the best of our knowledge there are no reports for the essential oil composition of *A. jahnni*. With reference to *A. pichinchensis* oil composition, a large difference may be notice comparing to the sample analyzed in Ecuador for the same species, however, climatic, geographic conditions and vegetative cycle stage are among the reasons that could explain such differences.

ACKNOWLEDGMENTS

Authors are grateful to Dr. Alfredo Usubillaga and Dr. Rosa Aparicio, Faculty of Pharmacy and Biomedical Sciences, University of Los Andes for helping in performing the **GC/MS** chromatogram.

REFERENCES

Adams R. 2007. **Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry.** 4th ed. Allured Publishing Corporation, Carol Stream IL, USA.

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

Aguilera R. 2010. **Determinación de propiedades físico-químicas de los aceites esenciales de cuatro especies de la familia asteraceae: Ageratina dendroides, *Ageratina pichinchensis*, *Coreopsis venusta* Kunth y *Gynoxys verrucosa* Weed de la provincia de Loja.** Tesis de Ingeniería Industrial Agropecurias, Universidad Técnica Particular de Loja, Ecuador.

Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. 2006. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. Stoechas essential oils from stem/leaves and flowers**. J Agr Food Chem** 54: 4364 - 4370.

Briceño B, Morillo G. 2002. Catálogo abreviado de las plantas con flores de los páramos de Venezuela. Parte I. Dicotiledóneas (*Magnoliopsida*). **Acta Bot Venez** 25: 1 - 75.

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

Chakravarty A, Mazumder T, Chatterjee S. 2011. Anti-inflammatory potential of ethanolic leaf extract of *Eupatorium adenophorum* Spreng. through alteration in production of TNF-*α*, ROS and expression of certain genes. **Evid Based Complement Alternat Med** 2011: 1 - 10.

Clinical & Laboratory Standards Institute. 2010. performance standards for antimicrobial susceptibility testing; twentieth informational supplement. **CLSI** document M100-S17.

Davies N. 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and carbowax 20 M. phases. **J Chromatogr A** 503: 1 - 24.

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

Duñg N, Cu L, Khien P, Muselli A, Casanova J, Barthel A, Leclerq P. 1998. Volatile constituents of the stem and leaf oils of *Eupatorium coelestinum* L. from Vietnam. **J Essent Oil Res** 10: 478 - 482.

Duñg N, Tam N, Kruk C, Leclerq P. 1991. Composition of the Oil of *Eupatorium stoechadosmum* Hance from Vietnam. **J Essent Oil Res** 3: 115 - 116.

Duñg N, Tam N, Kruk C, Leclerq P. 1991. Composition of the Oil of *Eupatorium stoechadosmum* Hance from Vietnam. **J Essent Oil Res**. 3: 115-116.

García G, Del Rio E, Guzman R, Martinez M, Scior T. 2011. Estudios preliminares sobre el efecto analgesic del extracto de hojas de *Ageratina glabrata* en dos modelos térmicos de dolor agudo. **Rev Mex Cienc Farm** 42: 45 - 51.

Herz, W. 2003. Chemistry of the Oxylobinae. **Biochem Syst Ecol** 31: 963 - 993.

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

Maia J, Zoghbi M, Da Silva H, Andrade E. 1999. Essential oils of *Eupatorium triplinerve* Vahl and *E. paniculatum* Poepp. et Endl. **J Essent Oil Res** 11: 541 - 544.

Mohan D, Ramaswamy M. 2007. Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora*, against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus.* **Afr J Biotechnol** 6: 631 - 638.

Mouhssen L. 2004. Methods to study the phytochemistry and bioactivity of essential oils. **Phytother Res** 18: 435 - 448.

Palá J, Pérez M, Velasco A, Sanz J. 2002. Analysis by gas chromatography-mass spectrometry of the volatile components of *Ageratina adenophora* Spreng. Growing in the Canary islands. **J Chromatography A** 947: 327 - 331.

Rios M, Aguilar A, Navarro V. 2003. Two new benzofuranes from *Eupatorium aschenbornianum* and their antimicrobial activity. **Planta Med** 69: 967 - 970.

Romero Ofelia, Román Rubén, Zamilpa Alejandro, Jiménez J, Rojas Gabriela, Tortoriello Jaime. 2009. Clinical trial to compare the effectiveness of two concentrations of the *Ageratina pichinchensis* extract in the topical treatment of onychomycosis. **J Ethnopharmacol** 126: 74 - 78.

Rondón M, Velasco J, Morales A, Rojas J, Carmona J, Gualtieri M, Hernández V. 2005. Composition and antibacterial activity of the essential oil of *Salvia leucantha* Cav. cultivated in Venezuela Andes. **Rev Latinoamer Quím** 33: 55 - 59.

Sanabria A, Ariciniegas N. 1999. Timol y carvacrol: sustancias responsables de la actividad antimicrobiana de *Ageratina ibaguensis*. **Rev Colomb Cienc Quim Farm** 28: 83 - 88.

Sasikumar J, Pichai A, Doss A. 2005. Antibacterial activity of *Eupatorium glandulosum* leaves. **Fitoterapia**. 76: 240 - 243.

Tripathi A, Upadhyay S, Bhuiyan M, Bhattacharya P. 2009. A review on prospects of essential oils as biopesticide in insect-pest management. **J Pharmacognosy Phytother** 1: 52 - 63.

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

Zou F, Duan G, Xie Y, Zhou Y, Dong G, Lin R, Zhu X. 2009. Molluscicidal activityof the plant *Eupatorium adenophorum* against *Oncomelania hupensis* the intermediate host snail od *Schistosoma japonicum*. **Ann Trop Med Parasitol** 103: 549 - 553.