

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]

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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 %), eupatoriocromene (6.5 %) and enecalol (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 analizadas 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 mayoritarios en *A. jahnii* fueron β -mirreno (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 enecalol (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 -mirreno, α -pineno, germacreno-D

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Abbreviations list: w/v-weight/volumen; p/v-peso/volumen; GC/MS-gas 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 monoterpenes 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. pichinchensis* 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. jahnii*.

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 °C.

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 μ m, 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 C₈-C₂₄ *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 μ m (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 μ l 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 (6th 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 °C for 18 h. The bacterial inoculum was diluted in sterile saline solution (0.85%) to obtain turbidity visually comparable to a McFarland N° 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 °C 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%), eupatoriocromene (6.5%) and enecalol (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. jahnii*, 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. stoehadosmum* 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 (%)
<i>α</i> -Pinene	943	939	17.1	-
Sabinene	980	976	0.4	-
<i>β</i> -Pinene	984	980	4.3	-
<i>β</i> -Myrcene	997	991	37.6	-
<i>α</i> -Phellandrene	1011	1005	4.3	-
<i>p</i> -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
<i>α</i> -Copaene	1380	1376	-	0.4
Modheph-2-ene	1383	1382	-	1.8
<i>β</i> -Cubebene	1391	1390	-	0.5
<i>β</i> -Elemene	1393	1391	-	1.4
<i>α</i> -Himachalene	1454	1449	-	0.3
<i>α</i> -Humulene	1459	1454	-	0.7
<i>β</i> -Selinene	1482	1483	-	0.9
Germacrene- <i>D</i>	1489	1484	4.6	19.8
Thymyl isobutyrate	1491	1490	-	10.8
Neryl isobutanoate	1496	1491	-	4.4
Bicyclogermacrene	1504	1494	-	2.2
<i>α</i> -Muurolene	1512	1500	0.7	-
Germacrene- <i>A</i>	1512	1509	-	1.5
<i>δ</i> -Cadinene	1529	1524	2.1	1.2
Germacrene- <i>B</i>	1565	1556	0.4	-
8,9-Epoxythymyl isobutyrate	1576	-	-	20.2
Linalyl 3-methylbutanoate	1582	-	-	3.0
Spathulenol	1583	1601	0.6	-
Desmethoxy enecalol	1653	1646	-	2.3
<i>α</i> -Cadinol	1659	1653	-	0.8
Andro enecalol	1684	1675	-	2.8
Eupatoriochromene	1773	1759	-	6.5
Enecalol	1868	-	-	5.9
Ripariocromeno- <i>A</i>	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-epoxythymyl isobutyrate, germacrene-*D*, thymyl

isobutyrate, eupatoriochromene and enecalol 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 were 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		
<i>S. aureus</i> ATCC (25923)	13	15			35	49.5	104
<i>E. faecalis</i> ATCC (19433)	12	11		32		49.5	104
<i>E. coli</i> ATCC (25922)	NA	NA	25			NT	NT
<i>K. pneumoniae</i> ATCC (25955)	NA	NA	27			NT	NT
<i>P. aeruginosa</i> ATCC (27853)	NA	NA	25			NT	NT

Amk: Amikacin® (30 μ g), **Amp:** Ampicillin® (10 μ g), **Ery:** Erythromycin® (15 μ g), ***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. pneumoniae*, *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).

CONCLUSION

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 enecalol were the components observed in major proportions. To the best of our knowledge there are no reports for the essential oil composition of *A. jahnii*. 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.

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