

Insecticide Activity of *Ageratina jahnii* and *Ageratina pichinchensis* (Asteraceae) against *Lutzomyia migonei* (Diptera: Psychodidae)

Abstract

Background: Insects are mostly pathogens transmitters, thus the necessity of finding effective bioinsecticides to combat them. In the present investigation, the insecticide activity of *Ageratina jahnii* and *Ageratina pichinchensis* (Asteraceae) essential oils, methanol, and aqueous extracts was evaluated against *Lutzomyia migonei* (Diptera: Psychodidae) females, *Leishmania* transmitters, a wide distributed parasitosis in Latin America. **Materials and Methods:** All extracts were prepared by maceration at room temperature, and essential oils were obtained by hydrodistillation process. Females of *L. migonei* were used in the bioassays using the adulticide test in pots. **Results:** Essential oils from both assayed plant species showed 100% of *L. migonei* mortality at 48 h of exposure at the concentration of 10 mg/ml. *A. jahnii* essential oil exhibited the following values, LD₅₀ = 0.39 mg/ml, LD₉₀ = 1.57 mg/ml, LD₉₅ = 2.31 mg/ml, and LD₉₉ = 4.80 mg/ml while for *A. pichinchensis* essential oil values were LD₅₀ = 0.31 mg/ml, LD₉₀ = 0.99 mg/ml, LD₉₅ = 1.38 mg/ml, and LD₉₉ = 2.55 mg/ml. **Conclusion:** Higher toxicity was observed with *A. pichinchensis* essential oil against *L. migonei*, comparing to *A. jahnii* oil. Two new plant species are being reported, showing bioactive properties against common tropical disease vectors such as *L. migonei*, hence, opening possibilities to a more environmental friendly control.

Keywords: *Ageratina jahnii*, *Ageratina pichinchensis*, Asteraceae, insecticide activity, *Lutzomyia migonei*

Introduction

Ageratina Spach (1841) is the biggest and most diverse genus of Oxylobiinae subtribe belonging to Asteraceae Bercht and J. Presl (1820) family. *Ageratina jahnii* (synonym *Ageratina stevioides*) is an endemic shrub of Venezuelan andes paramo, distributed in Mérida, and Tachira states, growing up in montane forest and subparamo ecosystems, located between 2600 and 3800 m.a.s.l. On the other hand, *Ageratina pichinchensis* (synonym *Ageratina ibaguensis*, *Ageratina aschenborniana* and *Ageratina bustamenta*), is a shrub well distributed in Colombia, Ecuador, Venezuela, Perú, México, Guatemala, and Panamá. In Venezuela is located in Amazonas, Aragua, Bolívar, Distrito Federal, Mérida, Monagas, Trujillo, Zulia, and Táchira states, growing up, as well, in montane forest and subparamo ecosystems, located between 1000 and 3850 m.a.s.l.^[1]

Regarding phytochemistry of these species, there have been several reports of natural

components isolated from *A. pichinchensis* aerial parts, such as, carvacrol, ageraborniol, β -sitosterol, stigmasterol, 5-acetyl-3- β -angeloxy-2,3-dihydro-2- β -(1-hidroxy-isopropyl)-1-benzofuran; 2,2-dimethyl-7-methoxy-2H-1-chromene; and 3-O- β -D-galactopyranosyl-5,4'-dihydroxy-6,7-dimethoxy flavones;^[2-7] while for *A. jahnii*, 8- β -hidroxy-13-epi-ent-labdan-15-oic acid, 16- α -hidroxy-ent-kauran-19-oic acid, 5-hidroxy-6-7-4'-trimethoxy flavone, 5-hidroxy-3,7,4'-trimethoxy flavone,^[8,9] hardwickiic acid, jhanol, and jhanidiol have also been published.^[4,10]

A. pichinchensis is also known as antifungal; some reports have indicated that hexanic extracts are effective against infections caused by *Trichophyton rubrum*,^[11-13] fungus responsible for 80% of onychomycosis and athlete's foot infections. In addition, *A. pichinchensis* and *A. jahnii* essential oils have demonstrated antimicrobial activities against Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* bacteria.^[14] Larvicidal

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activity has also been reported for acetone extracts of *A. adenophora* leaves against *Aedes aegypti* and *Culex quinquefasciatus* larvae, reporting LC₅₀ of 356.7 ppm for *A. aegypti* and 227.2 ppm for *C. quinquefasciatus*.^[15]

Lutzomyia migonei (Diptera: Psychodidae) is an anthropophilic sandfly widely distributed in the American continent, and it is well known as a transmitter of *Leishmania* parasite, responsible of leishmaniasis disease that represents a serious health problem. Furthermore, *L. migonei* is being recognized as common sandfly vector in Venezuela, Brazil, and Argentina.^[16,17] At present, there is no efficient control system available against leishmaniasis disease. Therefore, it is necessary to search for alternative control measures based on natural insecticides from plants against sandflies.^[18] The present investigation evaluates two *Ageratina* species, *A. jahnii*, and *A. pichinchensis*, as adulticides against *L. migonei* in experimental conditions.

Materials and Methods

Collection and maintenance of sandflies

L. migonei females from a laboratory colony that begun with specimens captured in the Arenal area, at an altitude of 1360 m above sea level (m.a.s.l.), located in Ejido, Merida, Venezuela (8°35' N-71°9'0) were used in this assay. The colony is maintained with the technique described by Killick-Kendrick *et al.*^[19] in an incubator at 25°C ± 1 and 80% ±10 of relative humidity (RH), at the LAPEX - Experimental Parasitology Laboratory, University of Los Andes, Mérida, Venezuela. Females of *L. migonei* are fed by biting hamsters previously intraperitoneally anesthetized with sodium pentobarbital (0.1 g/ml). The oviposition occurs after 72 h postfeeding. Eggs are kept in plastic buckets at 24°C with 90% RH and leave it to hatching that occurs after 10 days. Larvae are fed on a diet prepared with a mixture of equal parts of rabbit, rabbit feces, and coffee leaves kept within plastic buckets using same conditions explained before, allowing pupation that is completed in approximately 30 days. Once adults have emerged, they are transferred to nylon cages, and the whole process is repeated. Groups of 120 (3–4 days old) laboratory reared *L. migonei* females were experimentally used in the adulticide test and the experiments were repeated by 6-fold.

Botanical material

Ageratina jahnii (B.L.Rob.) R. M. King and H. Rob. was collected on January 27, 2010 in páramo "Piedra Pirela," 5 km from San José de Acequia, Mérida state, Venezuela at 3122 m.a.s.l. *A. pichinchensis* (Kunth) R. M. King and H. Rob. was collected on January 29, 2011 in páramo "Las Coloradas," Campo Elias, Mérida state, Venezuela at 2500 m.a.s.l. Both species were identified by Dr. Pablo Melendez based on identification keys described by Humboldt *et al.*^[20] 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.

Extraction of essential oils

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 Pyrex glass Clevenger equipment of 5 ml capacity. The oils were dried over anhydrous sodium sulfate and stored in small amber colored covered jar at 4°C to be used in activity assays, being careful that storage time does not exceed 4 months.

Obtaining aqueous and methanolic extracts

Ground and dry botanical material (50 g) was subjected to extraction by maceration (500 ml) in methanol and water, separately, for 10 days at room temperature. These extracts were filtered through No. 1 Whatman paper, and dried in a rotary evaporator under reduced pressure at a temperature below 50°C. The concentrated extracts were stored in sealed glass vials at 4°C until its use in testing.

Adulticide test in pots

Tests were performed using the method described by Cárdenas *et al.*^[17] Circular Whatman No. 1 filter papers with 6 cm of diameter, were used. They were saturated homogeneously with 200 µl of methanolic and aqueous extract and essential oil. Saturated filter papers were placed in petri dishes and allowed to dry overnight at room temperature. Concentrations of 100, 50, and 10 mg/mL were used for the extracts and 10, 1, and 0.1 mg/mL for the essential oil. Dried filter papers were placed at the bottom of the gypsum pots, making sure that borders of the paper remained adhered to the pot. Ten females were placed in each pot with a Castro grabber. Then, a 50% w/v sucrose solution was placed on the nylon covering the gypsum pots as food for the sandflies, and they were placed in plastic bags with 80–90% RH at room temperature. A total of six replications were carried out for the extracts, as well as for the essential oil, using a total of 40 females per sample. Control groups were also included in the experiment using filter papers moistened with either methanol, distilled water or mineral oil. Mortality was recorded at 1, 24, 48 and 72 h of exposure.

Data analysis

Adjustment for mortality was made using Abbot's formula. The LD was determined at 50, 90, 95 and 99 by means of a BioStat 2008 analysis (5.2.5 edition). Variation between oils was compared using the ANOVA analysis. $P \leq 0.05$ were considered significant. Tests with mortalities >20% in control groups were repeated.

Results

A. jahnii and *A. pichinchensis* aqueous and methanol extracts, as well as their essential oils, were assayed to establish the insecticide activity using a total of

960 *L. migonei* females under laboratory conditions. For *A. jahnii* essential oil, a 100% of sandflies mortality was observed at 48 h of exposure to 10 mg/mL concentration while 1 mg/mL of essential oil caused 100% of mortality at 72 h and 0.1 mg/mL only 17.5% of mortality [Figure 1]. Essential oil of *A. pichinchensis* showed similar results causing a mortality of 100% at 48 h of exposure to 10 mg/mL while 1 mg/mL of concentration showed mortality of 100% at 72 h of exposure and 0.1 mg/mL just caused 20% of mortality [Figure 1]. Effectiveness in both essential oils is dose-dependent since mortality increased according to concentration and time of exposure. Methanol and aqueous extracts showed no activity at any doses assayed in relation to the control.

A major insecticide activity was observed for *A. pichinchensis* essential oil at 1 mg/mL (80% mortality) and 10 mg/mL (87.5% mortality) after 24 h of exposure while 97.5% and 100% was observed at 48 h. Comparing these results to *A. jahnii* essential oil at same concentrations only 30% and 55% of mortality was observed at 24 h while 80% and 100% of mortality was observed at 48 h of exposure. Statistical analysis showed significant

differences at 24 h for both samples ($P = 0.0001 < 0.05$) and 48 h ($P = 0.0106 < 0.05$) at 1 mg/mL. After 72 h of assay, both essential oils showed 100% of mortality just at the highest dose assayed (10 mg/mL) while at 0.1 mg/mL no toxicity was observed against *L. migonei* [Figure 1].

Lethal doses values for both samples at 48 h of exposure were also determined. *A. jahnii* essential oil showed values of $LD_{50} = 0.39$ mg/mL, $LD_{90} = 1.57$ mg/mL, $LD_{95} = 2.31$ mg/mL and $LD_{99} = 4.80$ mg/mL, while for *A. pichinchensis* essential oil were $LD_{50} = 0.31$ mg/mL, $LD_{90} = 0.99$ mg/mL, $LD_{95} = 1.38$ mg/mL and $LD_{99} = 2.55$ mg/mL [Table 1]. These results indicate a major toxicity of *A. pichinchensis* essential oil against *L. migonei*.

Discussion

Commercial insecticides are mainly composed by chemicals such as organochloride, organophosphate, carbamate and synthetic pyrethroids, however, sandflies have developed resistance to these and have also caused a negative environmental impact,^[21] thus, the necessity of searching for natural insecticides obtained from plants that are less harmful to nature and represent a more efficient alternative to the biological control of *Leishmania* vectors.^[22] Essential oils extracted from *Pseudognaphalium caeruleocanum* (Asteraceae) and *Cinnamomun zeylanicum* (Lauraceae), have been reported as possible natural repellents against *L. migonei* stings.^[23] In addition, *Monticalia greenmaniana* (Asteraceae) essential oil demonstrated a high efficacy as adulticide with LD_{50} values of 0.005 mg/mL and LD_{98} of 0.0066 mg/mL at 1 h of exposure.^[17] *Antonia ovata* (Loganiaceae) and *Derris amazonica* (Papilionaceae) aqueous extracts showed activity against *Lutzomyia longipalpis* with LD_{50} of 233 mg/mL and 212 mg/mL after 48 h of exposure.^[24] Furthermore, *Tagetes minuta* (Asteraceae), *Acalypha fruticosa* (Euphorbiaceae), and *Tarconanthus camphoratus* (Compositae) methanolic extracts showed LD_{50} values of 1.6 mg/mL, 8.95 mg/mL, and 49.9 mg/mL, respectively, against adult females of *Phlebotomus duboscqui*.^[25]

The present results have revealed *A. jahnii* and *A. pichinchensis* (Asteraceae) as plants with potential

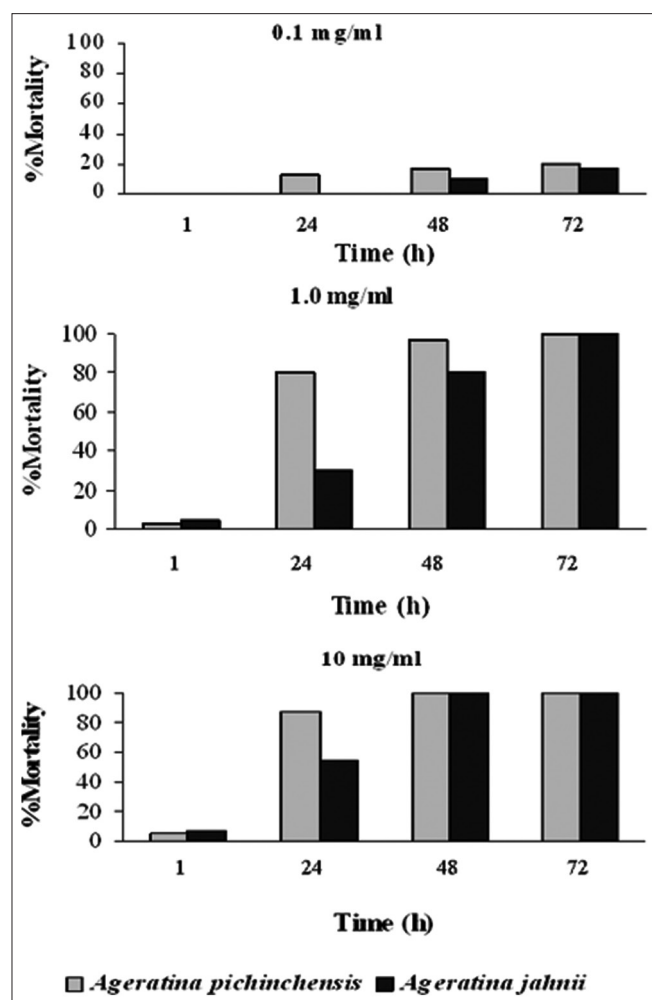


Figure 1: Insecticide activity of *Ageratina jahnii* and *Ageratina pichinchensis* essential oils at different concentrations

Table 1: Lethal dose of *A. jahnii* and *A. pichinchensis* essential oil against *L. migonei* at 48 h exposure

Parameter	<i>A. jahnii</i>	<i>A. pichinchensis</i>
LD_{50}	0.39 mg/ml	0.31 mg/ml
SE	0.19	0.13
LD_{90}	1.57 mg/ml	0.99 mg/ml
SE	1.39	0.71
LD_{95}	2.31 mg/ml	1.38 mg/ml
SE	2.58	1.19
LD_{99}	4.80 mg/ml	2.55 mg/ml
SE	7.99	3.08

LD_{50} : Lethal dose-50, LD_{90} : Lethal dose-90, LD_{95} : Lethal dose-95, LD_{99} : Lethal dose-99, SE: Standard error

adulticide effect against *L. migonei*. Hydrodistilled essential oils from these two species showed 100% of sandflies mortality at concentration of 10 mg/mL in 48 h. It is very difficult to correlate the adulticide effect observed in this investigation to a single compound, since extracts and essential oils are composed by several chemical components that might act synergistically, however, it is interesting to mention several differences on the chemical composition of these essential oils, since *A. pichinchensis* essential oil exhibited a higher insecticide activity comparing to *A. jahnii* essential oil.

Previous investigation has reported that *A. pichinchensis* is mainly composed by monoterpene, sesquiterpene and chromene type components being 8,9-epoxythymyl isobutyrate (20.2%), germacrene D (19.8%), thymyl isobutyrate (10.8%), eupatoriocromene (6.5%), enecalol (5.9%), and neryl isobutyrate (4.4%) in major concentrations.^[14] Chromene derivatives are known to have insecticide activity; 6-acetyl-2,2-dimethyl-7-methoxy-2H-1-chromene is active against *Oncopeltus fasciatus* while 2,2-dimethyl-7-methoxy-2H-1-chromene and 2,2-dimethyl-6,7-dimethoxy-2H-1-chromene have been reported for its inhibitory activity of insect hormones.^[26] On the other hand, *A. jahnii* essential oil hold a different composition represented mainly by monoterpenes, being β -myrcene (37.6%), α -pinene (17.1%), pentacosane (9.2%), limonene (8.8%) and germacrene D (4.6%) the main components.^[14] Insecticide activity have been reported for limonene against fourth instar larvae *C. quinquefasciatus* with LC₅₀ of 32.52 ppm at 48 h of exposure, as well as myrcene topically applied over *Dendroctonus rufipennis* adults at concentration of 20 ppm showed over 60% of toxicity.^[27,28]

In case of essential oils, mechanism of action is difficult to establish due to the complex mixture of compounds, however, toxicity by contact might be a reasonable way of action. Although organochloride insecticides perform a similar mechanism, natural insecticides offer a more environmental friendly solution for plague control.^[22] Finally, it is important to mention that present results are showing two new botanical species with bioactive properties against insects, opening possibilities to a more suitable control of vectors that causes different diseases.

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Conflicts of interest

There are no conflicts of interest.

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