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Chemical study and larvicidal activity against *Aedes aegypti* of essential oil of *Piper aduncum* L. (Piperaceae)

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ABSTRACT

Piper aduncum L. is used in folk medicine to treat respiratory and inflammatory diseases. The aim of this study was to analyze the essential oil from leaves of *P. aduncum* collected in the Brazilian Cerrado, North of Minas Gerais, as well as to evaluate the larvicidal activity of this oil and of its major constituent. The essential oil was analyzed by gas chromatography coupled to flame ionization detector and gas chromatography coupled to mass spectrometry that allowed characterizing 23 compounds (monoterpenes: 90.4%; sesquiterpenes: 7.0%). The major component was 1,8-cineole (53.9%). This oil showed to be very different from those obtained from the same species. Larvae of *A. aegypti* were exposed to different concentrations of the essential oil and 1,8-cineole. The mortality rate of 100% was obtained after 24h of treatment with the oil at concentrations of 500 and 1,000 ppm. After 48h of treatment, the mortality rate was 80% and 50% for concentrations of 250 and 100 ppm, respectively. The LC_{50} obtained after 24h was estimated in 289.9 ppm and after 48h was 134.1 ppm. The major compound 1,8-cineole showed no larvicidal activity.

Key words: *Piper aduncum*, *Aedes aegypti*, larvicidal activity, dengue, 1,8-cineole, GC-MS.

INTRODUCTION

Dengue is a tropical disease, characterized by acute infectious of short duration and can take severe and lethal forms than concern for medical and health authorities around the world. Dengue is transmitted by Culicidae of the genus *Aedes*, specifically, by the bite of female mosquito infected with dengue virus. The *A. aegypti*, widely distributed in tropical and

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subtropical regions, is the most important arbovirus vector of dengue in the Americas and the main vector of dengue in the world (Costa et al. 2005).

In Brazil, the *A. aegypti* program controls are focused on environmental management associated with the use of biological products, such as *Bacillus thuringiensis* Berliner, or chemicals products, such as insecticides of the pyrethroid and organophosphate classes, that act at the mosquito in the larval stage. However, the use of insecticides associated with the educational actions and environmental management have not controlled the infestations of the vector and are making the insects increasingly resistant, besides being pollutants and toxic (Carvalho et al. 2004, Santiago et al. 2005).

Currently, there is a growing search for new insecticides using products derived from plants as an alternative to control disease-transmitting mosquitoes. In this context, substances found in essential oils are distinguishing themselves in control of insects. These insecticides are important and interesting because they are biodegradable and the near absence of toxicity (Franzios et al. 1997, Isman 2000, Pimenta et al. 2006).

Piper, the largest genus in the family Piperaceae, is known to be rich in essential oils and some species of this genus such as *P. aduncum*, *P. brachystachyum*, *P. falconeri*, *P. guineense* and *P. hispidum* stand out for having insecticidal activity or as an insect repellent (Parmar et al. 1997, Pessini et al. 2003).

Piper aduncum, popularly known as "aperta-ruão", "falso-jaborandi", "aduncum", "jaborandi-do-mato" and "pimenta-de-macaco", is a widely distributed species in tropical regions, and it is considered an opportunistic plant that invades cleared areas, high hardiness and resistance to climate change. *P. aduncum* occurs spontaneously in pastures and in the edge of forests of the Southeast of Brazil, where it is considered weed (Lorenzi and Matos 2002, Sousa et al. 2008).

Phytochemical studies carried out with *P. aduncum* led to the isolation and characterization of chromenes and flavonoids, with emphasis on the 2',6'-dihydroxy-4'-methoxychalcone which showed excellent leishmanicidal effect (Moreira et al. 1998, Torres-Santos et al. 1999a, b). This plant is used in folk medicine as anti-inflammatory, to treat respiratory diseases, gynecological and intestinal disorders, as diuretic, carminative, digestive stimulant, for treatment of liver ailments and of chronic ulcers (Berg 1993, Coimbra 1994, Guadalupe-Rojas et al. 1999).

The essential oil obtained from *P. aduncum* was active against cercariae intermediate form of schistosomiasis, and as insecticidal and larvicidal against phytophagous insects and mosquitoes that transmit dengue and malaria (Lorenzi and Matos 2002, Souto 2006, Almeida et al. 2009). According to literature records, the major substance of the essential oil of *P. aduncum*, the arylpropanoid derivative dillapiole, is responsible for fungicide, larvicidal, insecticide, and molluscicide activities (Maia et al. 1998, Almeida 2004). As there are only records of the volatile composition of *P. aduncum* from woodlands of the Southeast and Northern Brazil, this study aims to investigate the chemical composition of essential oil obtained from this species that occurs spontaneously in the Cerrado ecosystem (Savanna) of Northern Minas Gerais, as well as to evaluate the larvicidal activity of this oil and of its major constituent, against the larvae of *A. aegypti*.

MATERIALS AND METHODS

Collect and Identification of Plant Material

Leaves of *Piper aduncum* L. were collected in April 2011 in the Forest of Gallery River Angico, Bocaiúva, Minas Gerais (MG), Brazil (S 16° 57.582'; W 43° 51.912'). The botanical material was identified by Dr. Elsie Franklin Guimarães and a voucher specimen was deposited in the Herbarium of the Botanic Garden of Rio de Janeiro, registered with the number RB 501.330.

Extraction and Essential Oil Analysis

The essential oil was extracted from fresh leaves (150 g) by hydrodistillation in a modified Clevenger type apparatus, in the Laboratory of Medicinal and Aromatic Plants of the Institute of Agricultural Sciences of Universidade Federal de Minas Gerais (ICA/UFMG), Montes Claros, Minas Gerais, Brazil. The yield of extraction was estimated at 0.8%.

The obtained essential oil was packed in amber vial, lightless. A sample of the oil was diluted in dichloromethane and subjected to analysis by gas chromatography coupled to flame ionization detector (GC-FID) and by gas chromatography coupled to mass spectrometry (GC-MS), in Farmanguinhos, FIOCRUZ, Rio de Janeiro.

Analysis conditions by GC-FID were: column HP-5MS (30m x 0.32 mm x 0.25 μm film thickness), temperature programming from 60°C to 240°C, with increase of 3°C.min^{-1}, using hydrogen and synthetic air as carrier gases, with a flow rate of 1.0 mL.min^{-1}. The Retention Indices (RI) were determined from the retention time of a homologous series of hydrocarbons (C8-C28), obtained by GC-FID under the same conditions of analysis of essential oils. The conditions of analysis by GC-MS were: column HP-5MS (30m x 0.32 mm x 0.25 μm), temperature programming from 60°C to 240°C, with increase of 3°C.min^{-1}, using helium as carrier gas, with a flow rate of 1.0 mL.min^{-1}.

The relative percentage of substances in the essential oil was estimated by GC-FID. The quantification of the major substance was accomplished using GC-FID with external standard 1,8-cineole (99.0%), obtained from Aldrich (Lot 1398664V), from the calibration curve made with different concentrations of standard (500 to 100 μg.mL^{-1}, $R^2 = 0.996283 \pm 0.003855$).

The substances present in the essential oil were identified by comparing their mass spectra with database registration (WILEY7n) and by comparison of Retention Index (RI) calculated with those from literature data (Adams 2001).

Biological Assays

Aedes aegypti larvae were provided by Center of Zoonoses of Montes Claros, Minas Gerais. Larvicidal activity was conducted in the Laboratory of Zoology of Faculdade de Saúde Ibituruna (FASI), Montes Claros, Minas Gerais. Initially, the larvae of *A. aegypti* were placed in a container with water and were selected according to the larval stage. Larvae in the third final instar or fourth initial were collected and separated for the test. A stereoscopic microscope it was used for identification of larvae stages. The biological assays were performed in triplicate, according to the methodology described by Oliveira et al. (2002). Different concentrations of essential oil from leaves of *P. aduncum* and pure 1,8-cineole varying from 1,000, 500, 250, 100, 50 and 10 ppm were used. The results represent mean of larvae mortality.

The essential oil was dissolved in 0.4 mL of dimethylsulfoxide (DMSO), and after with 30 mL of water in containers of 50 mL. The test was performed in triplicate and in each container were added 10 larvae. As a blank control was used water with DMSO (2%). The same procedure was used for the 1,8-cineole. The result was assessed after 24h and 48h, counting the dead larvae and observing the occurrence of some morphological deformation. For this purpose, larvae of each treatment were placed separately in a container with water to observe the mortality.

The calculation of regression analysis was used to estimate the concentrations capable of causing 50% (LC_{50}), 90% (LC_{90}) and 100% (CL_{100}) mortality of the larvae. The standard-error was determined by Probit analysis with software PROBIT GW-Basic, considering a significance level of $p<0.05$ (StatPlus 2009).

RESULTS AND DISCUSSION

Chemical Composition

The analysis of the essential oil from fresh leaves of *P. aduncum* allowed characterizing 23 different

substances (Table I), distributed in monoterpenes (90.4%) and sesquiterpenes (7.0%). The major compound was identified as 1,8-cineole (53.9%), an oxygenated monoterpene. The following monoterpenes were also identified in great amounts: α-pinene (12.7%), β-pinene (8.5%) and *trans*-ocimene (5.7%), comprising almost 27% of the mixture. Considering these four monoterpenes that represents about 80% of the volatiles, biological activity of this oil can be due to these substances.

Noteworthy is the great qualitative difference of the constituents of the volatile oil extracted from leaves of *P. aduncum* of the Savanna of North of Minas Gerais, in comparison with literature records. A study about the constituents of the essential oil from *P. aduncum* leaves, collected at different locations in the Amazon Region of Brazil, showed that the oil has excellent yield (2.5 to 3.5%) and that the major constituent is a phenylpropanoid, dillapiole, ranging from 31.5 to 97.3% in the samples (Maia et al. 1998). This substance was not identified in the analyzed sample and the monoterpene 1,8-cineole was not identified in any sample of the Amazon oil (Maia et al. 1998). The presence of dillapiole as the major substance of the *P. aducnum* essential oil was also confirmed in a study lead in Eastern of Ecuador, where this substance represents 46.0% of the sample (Guerrini et al. 2009). However, interestingly, the volatile composition of *P. aduncum* collected in Bolivia and Panama is quite similar to that found in this study (Vila et al. 2005).

Dillapiole is responsible for much of the biological activities attributed to *P. aduncum* (Almeida 2004). In the Bolivian sample, the monoterpene group was the main fraction (76.0%), with high amount of the oxygenated compound 1,8-cineole (40.5%). The sesquiterpene fraction did not reach 10.0% of the total sample and the phenylpropanoids accounted for only 13.6% of essential oil (Vila et al. 2005).

In the sample collected in Panama, the sesquiterpenes were identified as the majority (49.0%), being the main components characterized as *E*-caryophyllene (17.4%) and aromadendrene (13.2%). Monoterpernes represented about 33.0% of the volatile fraction. It is interesting to note the absence of phenylpropanoids in the sample collected in Panama. In addition, the dillapiole, considered the substance responsible for the biological activities of essential oil of *P. aduncum*, was not detected in the two samples investigated in Bolivia and Panama (Vila et al. 2005).

TABLE I
Substances identified in the essential oil of *Piper aduncum* leaves.

Substances	*RI*	RI_{lit}	*Relative Percentage*
α-pinene	**931**	**939**	**12.7**
β-pinene	**976**	**980**	**8.5**
β-mircene	985	991	2.5
limonene	1033	1031	2.4
1,8-cineole (eucalyptol)	**1034**	**1033**	**53.9/ 48.0***
***trans*-ocimene**	**1044**	**1050**	**5.7**
γ-terpinene	1055	1062	0.6
4-terpineol	1177	1177	0.6
α-terpineol	1193	1189	3.5
E-caryophyllene	1412	1418	0.7
α-humulene	1447	1454	0.2
germacrene D	1473	1480	0.9
bicyclogermacrene	1488	1494	2.1
γ-cadinene	1508	1513	0.3
cubebol	1510	1514	0.8
germacrene B	1550	1556	0.1
nerolidol	1556	1564	0.4
germacrene-D-4-ol	1568	1574	0.1
spatulenol	1573	1576	0.1
globulol	1578	1583	0.1
hinesol	1632	1638	0.3
tau-cadinol	1636	1640	0.1
α-muurolol	1644	1645	0.3
Monoterpenes			**90.4**
Sesquiterpenes			**7.0**
Total			**97.4**

RI = Retention Index, RI_{lit} = Retention Index from literature.
*Amount obtained using calibration curve with pure standard.

Thus, the differences in the volatile composition of leaves from *P. aduncum* from Amazon Region, Bolivia, Ecuador, Panama and our data may suggest the existence of some chemical polymorphism that could be due to difference in altitude and climate, as well as genetic factors.

Larvicidal Activity

The essential oil of *P. aduncum* showed larvicidal activity against the larvae of *A. aegypti*. After 24h there was a mortality rate of 100% of the larvae to concentrations of 500 and 1,000 ppm of essential oil, and 40%, 30%, 20% and 10% for 250, 100, 50 and 10 ppm, respectively (Figure 1). In the concentrations of 500 and 1,000 ppm the *Larvicidal activity* was registered 10 minutes after the larvae come into contact with the essential oil.

The evaluating of the larvae after a period of 48h of exposure, it was observed that the essential oil remained active, being registered a mortality rate of 80%, 50%, 40% and 10% for concentrations of 250, 100, 50 and 10 ppm, respectively (Figure 1).

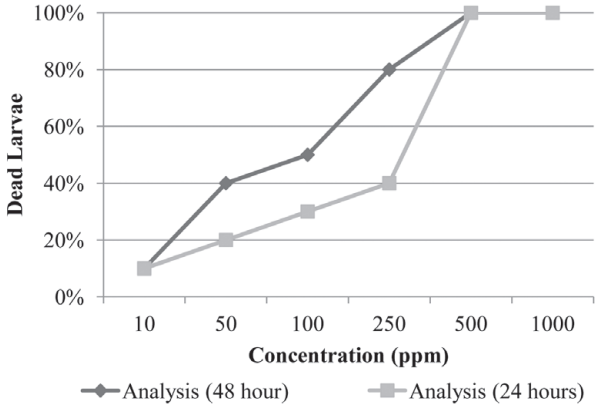

Fig. 1 - *A. aegypti* larvae mortality rate after 24h and 48h of exposure to different concentrations of essential oil of *P. aduncum*.

Based on the results obtained after 24h, the LC_{50} was calculated at 289.9 ppm, the LC_{90} was estimated at 654.9 ppm and the LC_{100} in 717.04 ppm. However, the lethal concentration values decreased after 48h: LC_{50} = 134.1 ppm, LC_{90} = 527.1 ppm, LC_{100} = 594.1 ppm. These data suggest an increase of activity after a longer exposure time, even considering the volatility of the oil. It was also shown that the activity is dose-dependent.

Studies published in the literature show that the essential oil of *P. aduncum* is very active as insecticidal and larvicidal against phytophagous insects and mosquitoes that transmit dengue and malaria (Souto 2006, Almeida et al. 2009). Pohlit et al. (2004), for example, tested the larvicidal activity of leaf extracts of *P. aduncum*, collected in the Amazonas State, against the larvae of *A. aegypti*. The extract showed larvicidal activity that was attributed to the major component of the extract, the dillapiole. The indication of this phenylpropanoid as responsible for the larvicidal activity of this species was initially proposed by Bernard et al. (1995) that compared the alcoholic extract of leaves of sixteen species of *Piper*, registering *P. aduncum* as the highest insecticidal activity against second instar larvae of *Aedes atropalpus*. In this essay, it was shown that the aqueous extract of fresh leaves of *P. aduncum* applied directly into the water at a concentration of 10 ppm, caused 50% mortality of second instar larvae of this Culicidae and the dillapiole isolated and purified presented, under the same experimental conditions, 92% efficiency in the control of larvae at a concentration of 1 ppm.

Still, an essential oil of *P. aduncum* containing 86.9% of dillapiole and a purified fraction, containing between 95.0 and 98.9% of dillapiole, were tested as larvicidal against the larvae of mosquistos *Anopheles marajoara* e *A. aegypti* (vectors of malaria and dengue, respectively) using five different concentrations (from 20 to 100 ppm) for 24h and 48h, separately. It was observed a mortality rate of 96% for the oil and 100% for the dillapiole, after the exposure time. For both larvae the LC_{50} was calculated as 52.7 ppm and 42.9 ppm for 24h and 48h respectively (Almeida et al. 2009).

Tests for the larvicidal activity of the oxygenated monoterpene 1,8-cineole (eucalyptol) against the larvae of *A. aegypti*, in this study, it was not observed mortality for any of the concentrations, excluding, thus, the major compound (53.9%) of oil as responsible for

this activity. The other identified monoterpenes, in lower percentage, α-pinene (12.7%), β-pinene (8.5%) and *trans*-ocimene (5.7%), but in combination, may be responsible for this activity. In a study with essential oils from the leaves of *Pinus caribaea* and *Pinus tropicalis*, endemic species of Cuba, it was observed larvicidal activity against larvae of *A. aegypti*. Both essential oils showed monoterpenes in their chemical composition, mainly α-tuyen, α-pinene, sabinene, β-pinene and myrcene. The compound β-pinene represented 26.5% of *Pinus caribaea* and 23.6% of *Pinus tropicalis* essential oils (Leyva et al. 2009). In another search for larvicidal activity it was observed for pine resin (turpentine), rich in α-pinene and β-pinene, a great activity against *A. aegypti*. The isolated pinenes were tested and also showed larvicidal activity (Lucia et al. 2007).

Other studies have shown a potential larvicidal activity for species rich in monoterpenes. Leyva et al. (2009) studied the larvicidal potential of essential oil of *Piper racemosa* against the larvae of *A. aegypti* and observed a positive result, noting that this activity can be attributed to the presence of monoterpenes 4-terpineol and 1,8-cineole, which together represent 41.1% of the total composition of the oil. The monoterpene 4-terpineol was identified in the studied *P. aduncum* essential oil (Table I), but at low percentage (0.6%). It is reasonable that 4-terpineol can be responsible for the activity registered in the *P. racemosa* essential oil since we not registered any activity for 1,8-cineole.

In addition, in a survey performed with essential oils of species of other families, such as *Hyptis martiusii* (Lamiaceae) and *Lippia sidoides* (Verbenaceae), Costa et al. (2005) described the larvicidal activity of essential oils against larvae of *A. aegypti* and of *Culex quinquefasciatus*. It was observed in this study the presence of mono and sesquiterpenes in the chemical constitutions. The major constituents were monoterpenes 1,8-cineole (*H. martiusii*) and thymol (*L. sidoides*). Thus, the authors state that these constituents are, probably, responsible for this activity, in isolated forms or in synergistic action with other constituents.

The main difference between our results and others published is the fact that the specimen collected in the Savanna of Northern of Minas Gerais State does not exhibit dillapiole as constituent of the volatile fraction. The major constituent is an oxygenated monoterpene, 1,8-cineole (53.9%). Therefore, the larvicidal activity of essential oil for the studied specimen cannot be attributed to dillapiole nor to its major constituent, consequently, can not be attributed in a general way the biological activity of an essential oil to its major component, being the dillapiole, or any other substance.

Morphological Characteristics of Larvae

Besides mortality, there was a morphological change of the larvae exposed to concentrations of 250, 500 and 1,000 ppm of essential oil of *P. aduncum*, both 24h and 48h. Several dead larvae showed a blackish color on their edges and curved body (Figure 2A); characteristic not observed before exposure to oil (Figure 2B).

The darkening of the larvae and the curved body were also observed in other studies (Barreto et al. 2006, Abed et al. 2007, Aciole 2009). Abed et al. (2007) investigated the morphological changes of the larvae of *A. aegypti* caused by essential oils from *Copaifera reticulata* (Fabaceae). The authors described in this study that this darkening of the edges of the larvae, possibly, occur due to the overlap of cuticle of abdominal segments.

According with Aciole (2009), morphological and behavioral changes in larvae subjected to treatments with substances isolated from plants are very important, because it will probably lead to a full explanation of the toxic action of essential oils on larvae of Culicidae. These changes provide indications of the way of action of plant substances in the body of the larvae. Thus, through of more studies could potentiate their effects and produce a highly effective insecticide and that at the same

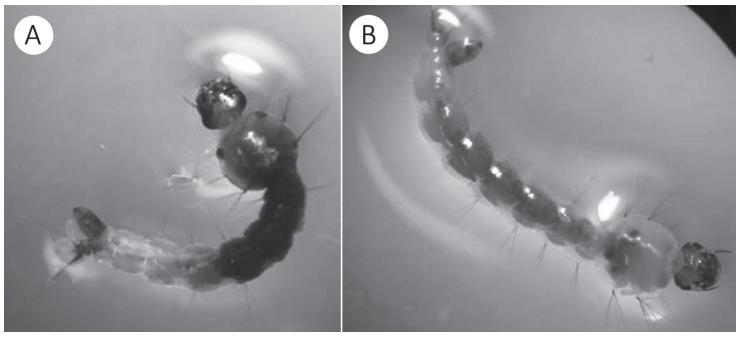

Fig. 2 - Larvae dead of *A. aegypti* with blackened color and curved body **(A)**; larvae alive and healthy, before the exposure to the essential oil **(B)**.

time do not interfere in other organisms nor to environment that are being exposed.

In conclusion, the volatile chemical composition of this specimen was very different of the specimens from the Amazon region, and similar to specimens from Bolivia and Panama. These data suggest for *P. aduncum* the occurrence of chemotypes, a hypothesis that should be investigated. This essential oil showed great larvicidal activity against *A. aegypty*, unlike its major component, which showed no activity. The potential lethality of the essential oil against *A. aegypti*, even after 48h, suggesting a residual effect, coupled with excellent oil yield (0.8%), may represent a new possibility for obtaining an active natural product to combat the mosquito that transmits yellow fever and dengue.

RESUMO

Piper aduncum L. é utilizada na medicina popular para o tratamento de doenças respiratórias e inflamatórias. O objetivo desse estudo foi analisar o óleo essencial das folhas de *P. aduncum* coletadas no Cerrado brasileiro, Norte de Minas Gerais, assim como avaliar a atividade larvicida desse óleo e de sua substância majoritária. O óleo essencial foi analisado por cromatografia em fase gasosa acoplada à espectrometria de massas e por cromatografia em fase gasosa acoplada a detector por ionização de chamas, que permitiu a caracterização de 23 substâncias (monoterpenos: 90,4%; sesquiterpenos: 7,0%). O componente majoritário foi o 1,8-cineol (53,9%). O óleo essencial desse exemplar mostrou ser muito diferente do óleo da mesma espécie. Larvas de *Aedes aegypti* foram expostas a diferentes concentrações do óleo essencial e do 1,8-cineol. Após 24h do tratamento com o óleo registrou-se uma taxa de mortalidade de 100% das larvas nas concentrações de 500 e 1,000 ppm. Após 48h a taxa de mortalidade foi de 80% e 50% para as concentrações de 250 e 100 ppm, respectivamente. A CL_{50} obtida após 24h foi estimada em 289,9 ppm e após 48h foi de 134,1 ppm. A substância majoritária 1,8-cineol não apresentou atividade larvicida.

Palavras-chave: *Piper aduncum*, *Aedes aegypti*, atividade larvicida, dengue, 1,8-cineol, CG-EM.

REFERENCES

Abed RA, Cavasin GM, Silva HHG, Geris R and Silva IG. 2007. Alterações morfohistológicas em larvas de *Aedes aegypti* (Linnaeu, 1762) (Diptera, Culicidae) causadas pela atividade larvicida do óleo-resina da planta medicinal *Copaifera reticulata* Ducke (Leguminosae). Rev Pat Trop 36: 75-86.

Aciole SDG. 2009. Avaliação da Atividade Inseticida dos Óleos Essenciais das Plantas Amazônicas Annonaceae, Boraginaceae e de Mata Atlântica Myrtaceae como Alternativa de Controle às Larvas de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). Dissertação, Universidade de Lisboa, Faculdade de Ciências, Lisboa, 86 p. (Unpublished).

Adams RP. 2001. Identification of essencial oil components by gas chromatography/mass spectrometry. Allure Pub. Corp., Carol Stream, 804 p.

Almeida RRP. 2004. Isolamento, purificação e isomerização do dilapiol, componente majoritário do óleo essencial de *P. aduncum* para comprovação de sua atividade biológica. Dissertação, Universidade Federal do Pará, Belém. (Unpublished).

Almeida RRP, Souto RNP, Bastos CN, Silva MHL and Maia JGS. 2009. Chemical Variation in *Piper aduncum* and Biological Properties of Its Dillapiole-Rich Essential Oil. Chem and Biod 6: 1427-1434.

BARRETO CF, CAVASIN GM, SILVA H-HG AND SILVA IG. 2006. Estudo das alterações morfohistológicas em larvas de *Aedes aegypti* (Diptera, Culicidae) submetidas ao extrato bruto etanólico de *Sapindus saponária* Lin. (Sapindaceae). Rev Pat Trop 35: 37-57.

BERG MEVD. 1993. Plantas medicinais na Amazônia: Contribuição ao seu conhecimento sistemático. 2ª ed., Belém: Museu Paraense Emílio Goeldi, 223 p.

BERNARD CB, KRISHNAMURTY HG, CHAURET D, DURST T, PHILOGÈNE NJR, SÀNCHEZ-VINDAS P, HASBUN C, POVEDA L, ROMÁN LS AND AMASON JT. 1995. Insecticidal defenses of Piperaceae from the neotropics. J Chem Ecol 21: 801-814.

CARVALHO MSL, CALDAS ED, DEGALLIER N, VILARINHOS PTR, SOUZA LCK, YOSHIZAWA MAC, KNOX MB AND OLIVEIRA C. 2004. Susceptibilidade de larvas de *Aedes aegypti* ao inseticida Temefós no Distrito Federal. Rev Saúde Públ 38: 623-629.

COIMBRA R. 1994. Manual de fitoterapia. 2ª ed., Belém: CEJUP, 196 p.

COSTA JGM, RODRIGUES FFG, ANGÉLICO EC, SILVA MR, MOTA ML, SANTO NKA, CARDOSO ALH AND LEMOS TLG. 2005. Estudo químico-biológico dos óleos essenciais de *Hyptis martiusii*, *Lippia sidoides* e *Syzigium aromaticum* frente às larvas do *Aedes aegypti*. Rev Bras Farmacogn 15: 304-309.

FRANZIOS G, MIROTSOU M, HATZIAPOSTOLOU E, KRAL J, SCOURAS ZG AND MAVRAGANITSIPIDOU P. 1997. Insecticidal and genotoxic activities of mint essential oils. J Agric Food Chem 45: 2690-2694.

GUADALUPE-ROJAS M, MOREIRA DL, PEREIRA NA AND KAPLAN MAC. 1999. Atividade antiinflamatória de extratos de *Piper aduncum* L. (Piperaceae). Rev Bras Farm 80: 5-6.

GUERRINI A, SACCHETTI G, ROSSI D, PAGANETTO G, MUZZOLI M, ADREOTTI E, TOGNOLINI M, MALDONADO ME AND BRUNI R. 2009. Bioactivities of *Piper aduncum* L. and *Piper obliquum* Ruiz & Pavon (Piperaceae) essential oils from Eastern Ecuador. Environ Toxicol Pharmacol 27: 39-48.

ISMAN MB. 2000. Plant essential oil for pest and disease management. Crop protection 19: 603-608.

LEYVA M, MARQUETTI MDC, TACORONTE JE, SCULL R, TIOMNO O, MESA A AND MONTADA D. 2009. Actividad larvicida de aceites esenciales de plantas contra *Aedes aegypti* (L.) (Diptera: Culicidae). Rev Biomed 20: 5-13.

LORENZI H AND MATOS FJA. 2002. Plantas Medicinais no Brasil: nativas e exóticas. Nova Odessa, SP: Instituto Plantarum, 512 p.

LUCIA A, GONZALEZ AP, SACCACINI E, LICASTRO S, ZERBA E AND MASUH H. 2007. Larvicidal effect of *Eucalyptus grandis* essential oil and turpentine and their major components on *A. Aegypti* larvae. J Am Mosq Control Assoc 23: 293-303.

MAIA JGS, ZOHHBI MGB, ANDRADE EHA, SANTOS AS, SILVA MHL, LUZ AIR AND BASTOS CN. 1998. Constituents of the essential oil of *Piper aduncum* L. growing wild in the Amazon region. Flav and Frag Jour 13: 269-272.

MOREIRA DL, GUIMARAES EF AND KAPLAN MAC. 1998. A chromene from *Piper aduncum*. Phytochemistry 48: 1075-1077.

OLIVEIRA MF, LEMOS TLG, MATTOS MC, SEGUNDO TA, SANTIAGO GMP AND BRAZ-FILHO R. 2002. New enamine derivatives of lapachol and biological activity. An Acad Bras Cienc 74: 211-221.

PARMAR VS, JAIN SC, BISHT KS, JAIN R AND TANEJA PA. 1997. Phytochemistry of the genus *Piper*. Phytochemistry 46: 597-673.

PIMENTA ATA, GILVANDETE MP, SANTIAGO GMP, ARRIAGA AMC, MENEZES GHA AND BEZERRA SB. 2006. Estudo fi toquímico e avaliação da atividade larvicida de *Pterodon polygalaefl orus* Benth (Leguminosae) sobre *Aedes aegypti*. Rev Bras Farmacogn 16: 501-505.

PESSINI GL, ALBIERO ALM, MOURÃO KSM, NAKAMURA CV, DIAS FILHO BP AND CORTEZ DAG. 2003. Análise Farmacognóstica de *Piper regnellii* (Miq.) C. DC. Var. *pallescens* (C. DC.) Yunck: Aspectos Botânicos e Enfoque Físico-químico Preliminar. Lat Am J Pharm 22: 209-216.

POHLIT AM ET AL. 2004. Screening of plants found in the State of Amazonas, Brazil for larvicial activity against *Aedes aegypti* larvae. Acta Amaz 34: 97-105.

SANTIAGO GMP, VIANA FA, PESSOA ODL, SANTOS RP, POULIQUEN YBM, ARRIAGA AMC, ANDRADE-NETO M AND BRAZ-FILHO R. 2005. Avaliação da atividade larvicida de saponinas triperpênicas isoladas de P*entaclethra macroloba* (Willd.) Kuntze (Fabacea) e *Cordia piauhiensis* Fresen (Boraginaceae) sobre *Aedes aegypti*. Rev Bras Farmacogn 15: 187-190.

SOUSA PJC, BARROS CAL, ROCHA JCS, LIRA DS, MONTEIRO GM AND MAIA JGS. 2008. Avaliação toxicológica do óleo essencial de *Piper aduncum* L. Rev Bras Farmacogn 18: 217-221.

SOUTO RNP. 2006. Avaliação das atividades repelente e inseticida de óleos essenciais de *Piper* da Amazônia em *Anopheles marajoara*, *Stegomyia aegypti* e *Solenopsis saevissima*. Tese, Universidade Federal do Pará e Museu Paraense Emílio Goeldi, Belém, 221 p. (Unpublished).

STATPLUS DE ANALYSTSOFT INC. 2009. Programa de Análise Estatística [homepage on the Internet]. [updated 2011 Jun11]. Available from: http://www.analystsoft.com/br/.

TORRES-SANTOS EC, MOREIRA DL, RODRIGUES JM, KAPLAN MAC AND BERGMANN BR. 1999a. Improvement of *in vitro* and *in vivo* antileishmanial activity of 2',6'-dihydroxy-4'-methoxychalcone in poly(D,L-Lactide) nanoparticles. Antimicrob Ag Chem 43: 1776-1778.

TORRES-SANTOS EC, MOREIRA DL, KAPLAN MAC, BERGMANN BR AND MEIRELLES MN. 1999b. Selective effect of 2',6'-dihydroxy-4'-methoxychalcone isolated from *Piper aduncum* on *Leishmania amazonensis*. Antimicrob Ag Chem 43: 1234-1241.

VILA R ET AL. 2005. Unusual composition of the essential oils from the leaves of *Piper aduncum*. Flavour Frag J 20: 67-69.