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Inga laurina **crude extract to control** *Aedes aegypti*

Extrato bruto de *Inga laurina* **no controle de** *Aedes aegypti*

Extracto crudo de *Inga laurina* **en el control de** *Aedes aegypti*

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Abstract

Aedes aegypti is the um mosquito responsible for the transmission of several diseases to humans such as dengue, chikungunya, Zika virus and urban yellow fever. The control of this culicid is done by utilizing insecticides that cause insect resistance. Therefore, natural alternatives to control *A. aegypti* have been sought. Thus, the objective of our work was to characterize the chemical composition of the crude extract of the leaves of *Inga laurina* and to evaluate the insecticidal activity of the extract on the larvae of *A. aegypti*. The crude extract was prepared form dry leaves by dynamic maceration using alcohol 96º GL as extracting solvent. The chemical identification of the compounds found in the crude extract was done by a gas chromatography coupled to mass spectrometry. The larvicidal activity was determined by larval packet test in third-stage development larvae *A. aegypti*. Fifteen compounds were identified in the leaves and the major ones were γ- sitosterol (34.39%), phytol (14.51%), squalene (8.57%) and stigmasterol (7.38%). *I. laurina* leaf crude extract presented larvicidal activity potential against *A. aegypti* larvae presenting lethal concentration of 50% (CL_{50}) of 0.98 mg/mL and 99% (CL_{99}) of 2.69 mg/mL. Thus, *I. laurina* leaf crude extract presented rich composition of phytosterols and promising insecticide activity against *A. aegypti* larvae, offering new possibilities for the application and development of products.

Keywords: *Ingá*; Dengue; Phytosterols; γ- sitosterol; Phytol.

Resumo

Aedes aegypti é um mosquito responsável pela transmissão de várias doenças para o homem como a dengue, chikungunya, Zika vírus e febre amarela urbana. O controle deste culicídeo é realizado pelo uso de inseticidas os quais causam resistência dos insetos. Assim, tem-se buscado alternativas naturais para o controle de *A. aegypti*. Deste modo, o objetivo do nosso trabalho foi caracterizar a composição química do extrato bruto das folhas de *Inga laurina* e avaliar a atividade inseticida do extrato sobre as larvas de *A. aegypti*. O extrato bruto foi preparado a partir das folhas secas pela técnica de maceração dinâmica utilizando álcool 96º GL como solvente extrator. A identificação química dos compostos presentes no extrato bruto foi realizada por cromatografia em fase gasosa acoplada à espectrometria de massas e atividade larvicida foi determinada pelo teste de imersão larval em larvas do terceiro estágio de desenvolvimento de *A. aegypti*. Nas folhas foram identificados quinze compostos tendo como majoritários γ- sitosterol (34,39%), fitol (14,51%), esqualeno (8,57%) e estigmasterol (7,38%). O extrato bruto das folhas de *I. laurina* apresentou potencial atividade larvicida contra as larvas de *A. aegypti* apresentando concentração letal de 50% (CL_{50}) de 0,98 mg/mL e 99% (CL_{99}) de 2,69 mg/mL. Portanto, o extrato bruto das folhas de *I. laurina* apresentou rica composição em fitoesteróis e promissora atividade inseticida frente às larvas do *A. aegypti* oferecendo novas possibilidades para a aplicação e desenvolvimento de produtos.

Palavras-chave: Ingá; Dengue; Fitoesteróis; γ- sitosterol; Fitol.

Resumen

Aedes aegypti es un mosquito responsable de la transmisión de diversas enfermedades al hombre como el dengue, el chikungunya, el virus Zika y la fiebre amarilla urbana. El control de este mosquito se realiza mediante el uso de insecticidas que provocan resistencia a los insectos. Así, se han buscado alternativas naturales para el control de *A. aegypti*. Así, el objetivo de nuestro trabajo fue caracterizar la composición química del extracto crudo de las hojas de *Inga laurina* y evaluar la actividad insecticida del extracto sobre las larvas de *A. aegypti*. El extracto crudo se preparó a partir de las hojas secas mediante la técnica de maceración dinámica utilizando alcohol 96º GL como disolvente de extracción. La identificación química de los compuestos presentes en el extracto crudo se realizó mediante cromatografía de gases acoplada a espectrometría de masas y la actividad larvicida se determinó mediante la prueba de inmersión larvaria en larvas del tercer estadio de desarrollo

de *A. aegypti*. Se identificaron quince compuestos en las hojas, con γ-sitosterol (34,39%), fitol (14,51%), escualeno (8,57%) y estigmasterol (7,38%) como compuestos principales. El extracto crudo de hojas de *I. laurina* mostró actividad larvicida potencial contra larvas de *A. aegypti*, con una concentración letal del 50% (CL_{50}) de 0,98 mg/mL y del 99% (CL_{99}) de 2,69 mg/mL. Por tanto, el extracto crudo de las hojas de *I. laurina* presentó una composición rica en fitoesteroles y una actividad insecticida prometedora contra las larvas de *A. aegypti*, ofreciendo nuevas posibilidades para la aplicación y desarrollo de productos.

Palabras clave: *Ingá*; Dengue; Fitoesteroles; γ-sitosterol; Fitol.

1. Introduction

Aedes aegypti (Diptera: Culicidae) is an important vector in world public health, responsible for the transmission of arboviroses such as dengue, chikungunya, Zika virus and urban yellow fever that affect millions of people yearly, mainly in Latin American countries where the weather favors the proliferation of the mosquito. Dengue has been listed among the ten main potential diseases that could cause an epidemic in 2019, and current data confirm that the number of recorded cases of the disease is the greatest in the history of Latin American countries. In the region of the Americas, 2,733,635 dengue cases were recorded (280/100.000 inhabitants) with 1,206 deaths until October 2019. Belize, El Salvador, Honduras, Nicaragua and Brazil are the countries with the highest incidence of dengue (PAHO/WHO, 2019). In Brazil, 1,544,987 probable dengue cases were notified (735.2/100.000 inhabitants) with 782 confirmed deaths in 2019. Regarding chikungunya, 132,205 probable cases (62.9/100.000 inhabitants) were recorded with 92 deaths; in the same period, 10,768 probable cases of Zika virus were notified (5.1/100.000 inhabitants) with three deaths in the state of Paraíba (Brasil, 2020). These diseases have a great impact on society due to the high morbidity levels with several complications and mortality, affecting the population's health and the economy in the health service management (PAHO/WHO, 2019; Brasil, 2020).

The main measure to avoid the transmission of these arboviroses is the elimination of the transmitter mosquito (Zara, Santos, Fernandes-Oliveira, Carvalho & Coelho, 2016). The control of this culicid is done by mechanic, chemical, biological and genetic means, but the chemical control using insecticides such as organophosphates Temephós®, Malathion®, Fenitrothion® has been the most efficient utilized method in the past twenty years to control *A. aegypti* by Public Health Programs, including interepidemic periods (Manjarres-Suarez &

Olivero-Verbel, 2013; Zara, Santos, Fernandes-Oliveira, Carvalho & Coelho, 2016). However, the utilization of these compounds contributes to environmental pollution, causes toxicity to humans and non-target organisms, and has also been reported to increase the resistance of this dipteran to these insecticides in Brazil and worldwide (Camargo et al., 1998.; Macoris et al., 2003.; Braga & Valle, 2007).

Natural insecticides are promising alternatives to control this mosquito because they are obtained from renewable resources and are quickly degradable, that is, they do not endure in the environment. The development of insect resistance to these substances, composed by the association of several active principles, it is a process that occurs very slowly, causing repellence, oviposition and feeding inhibition, development disorders, deformations, infertility and mortality (Roel, 2001; Furtado, Lima, Neto, Bezerra & Silva, 2005; Zara, Santos, Fernandes-Oliveira, Carvalho & Coelho, 2016).

Due to evolution matters, plants naturally produce compounds with insecticide action in response to attack of herbivores, phytopathogens and mollusks (Okwute, 2012) and, therefore, they become excellent contributors to the research of natural products that are efficient to control adult mosquitos and to exterminate *A. aegypti* larvae (Martins et al., 2020; Santos Júnior et al., 2020). The broad biodiversity of the Brazilian flora with approximately 46,000 recorded species makes this natural resource a source of new biomolecules (Braz-Filho, 2010).

Inga laurina, belonging to the Family Fabaceae, is a native species with broad distribution in Central and South America. In Brazil, it is distributed in phytogeographic areas of the Amazon, Caatinga, Cerrado and Atlantic Forest. It is popularly known as white *ingá*, *chinchica ingá*, monkey *ingá*, beach *ingá*, little *ingá* or *ingaí*, and is utilized in traditional medicine as a laxative and for renal diseases (Lorenzi, 2002; Macedo, Garcia, Freire & Richardson, 2007). It is a tree with 5 to 8 meters of height, petiolate leaves, flower with white petals, sessile fruits with developed sweet edible pulp, generally consumed *in natura* by people as well as animals. It blooms from September to November, and fructifies from December to January (Macedo, Garcia, Freire & Richardson, 2007; Possette & Rodrigues, 2010).

Phytochemical studies with *I. laurina* leaves showed mainly the presence of phenolic compounds such as gallic acid and derivates, flavonoids such as myricetin and quercetin, and also galoil depsids of tyrosine and ascorbic acid (Milton & Jennes, 1987; Lokvam, Clausen, Grapov, Coley & Kursar, 2007; Martins et al., 2019). Although this species is an important part of the Brazilian flora, little is known about its chemical composition (Macedo, Garcia,

Freire & Richardson, 2007). In this context, the present study aimed to characterize *I. laurina* leaf crude extract by gas chromatography coupled to mass spectrometry and evaluate the potential insecticide against *Aedes aegypti* larvae.

2. Methodology

2.1 Plant material and botanical identification

I. laurina leaves were collected from April 2016 to April 2017 in the Medicinal Herbarium of UNIPAR - Umuarama, in the northwestern region of the state of Paraná, Brazil, at the coordinates S23º 46.225' and WO 53º 16.730', altitude of 391 m. The species was identified and is registered in the Medicinal Herbarium of Paranaense University under the number 294 and recorded under the number A3538F2 in the System of Brazilian Genetic Heritage and Associated Traditional Knowledge (SisGen, acronym in Portuguese).

2.2 Preparation of *I. laurina* leaf crude extract

The leaves (150 g) were dried at room temperature and powered until reaching the granulometry of 850 μm. The powder was submitted to dynamic maceration with solvent renewal using ethylic alcohol 96º GL until vegetal matter depletion (Miranda et al., 2009). Next, the filtrate was concentrated under reduced pressure in a rotary evaporator (Tecnal TE-211 model) at 35 °C, until for obtaining the crude extract (CE). The CE yield was calculated by the formula: CE yield= (crude extract mass (g) x total dry mass of leaves (g) / 100.

2.3 Chemical characterization of *I. laurina* crude extract

The identification of *I. laurina* CE chemical components was done by a gas chromatographer coupled to a mass spectrometer (GC/MS), equipped with a capillary column Agilent HP-5MS UI (30 m × 0.250 mm × 0.25 μm). For the analysis, an injection volume of 1.0 μL of a solution prepared by the dissolution of 20 mg of CE in 1.0 mL de methanol. The analysis conditions were: injector temperature, 280 °C operating in *split* mode (1:2), transfer line 280°C, He as carrier gas with 1 mL/min flow and initial temperature of the column at 80 °C (1 min), with a ramp of 2 ºC/min until reaching 185 ºC, keeping this temperature for 1 min, followed by the heating of 9 ºC/min until reaching the temperature of 275 °C, keeping it

for 2 min and finishing with heating at 25 °C/min until 300 °C, keeping it for 1 min. The MS detection system was utilizing in "scan" mode with ionization by electron impact 70 eV, at the ratio of mass/charge (*m/z*) in the range of 40 - 600. The temperatures of the ionization source and quadrupole were 230 and 150 °C, respectively. The compounds were identified by comparing their mass spectra to the ones from NIST 11.0 library as well as comparing the retention indexes (RI) obtained by a homologous series of *n*-alkane standards (C7 – C26) (Adams, 2017).

2.4 Larvicidal activity against *Aedes aegypti*

The bioassays were done in triplicate using third-stage *Aedes aegypti* larvae from the Center of Vector-Transmitted Endemics Control of the Sanitary Surveillance of the city of Umuarama, PR, Brazil. *Inga laurina* leaf CE was diluted in an aqueous solution of polysorbate (80) at 2% under the concentrations of 100.00, 50.00, 25.00, 12.50, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09, 0.04, 0.02, 0.01, 0.006 at 0.003 mg/mL (w/v). Ten third-stage *A. aegypti* larvae were separated using a Pasteur pipette and placed in 250 mL flasks with 10 mL of different concentrations of CE (Costa et al., 2005). For the negative control, an aqueous solution with polysorbate 80 at 2% was used, whereas a Temephós®-based organophosphate at a concentration of 0.4 mg/mL (Camargo et al., 1998) was utilized as a positive control. The larvae were exposed to CE at different concentrations for 24 h and those that presented absence of movement and did not respond to stimuli were considered dead. The mortality was the average of three replications from the percentage of *A. aegypti* larvae.

2.5 Anticholinesterase activity

The anticholinesterase activity was determined by bioautographic method described by Marston, Kissling & Hostettmann (2002), with modifications (Yang et al., 2009). The CE was tested from an initial concentration of 0.003 to 100 mg/mL, diluted in methanol. The samples were plotted in aluminum chromatoplates (10 x 10 cm, silica gel 60 F254 with 0.2 mm of thickness); after plotting the plates were dried and an acetylcholinesterase enzyme solution (500 U) in buffer Tris (hydroxymethyl) aminomethane hydrochloridrate (0.05 M pH 7.8) solution was sprayed on the plates; next, an α-naphthyl acetate solution (0.15%) was sprayed. The plates were kept at 37 °C for 20 minutes. After this period, the chromatoplates were sprayed with Fast Blue B salt colorimetric reagent (0.05%), resulting in a purple-color

surface. Temephós was used as positive control at concentrations that ranged from 0.003 to 4 mg/mL.

2.6 Statistical analysis

The tests were carried out in triplicate. The data were submitted to analysis of variance (ANOVA) and compared by SPSS Statistics 22 program by Tukey test ($p \leq 0,05$). The lethal concentration to kill 50% (LC_{50}) and 99% (LC_{99}) of the larvae and the respective confidence intervals (95%) were calculated by analysis of Probitos.

3. Results

The analysis by GC/MS identified 15 compounds in *I. laurina* CE, representing 96.02% of the detected compounds (Table 1). The major compounds were phytosterols (48.38%), and γ-sitosterol (34.39%) was the main compound. Oxygenated diterpenes were the second most abundant class in CE, having phytol (14.51%) as the second most abundant compound in CE. The esters of fatty acids were also found in great amount (13.23%), mainly methyl ester of hexadecanoid acid (5.91%).

Table 1 – Chemical composition of *Inga laurina* leaf crude extract by GC/MS.

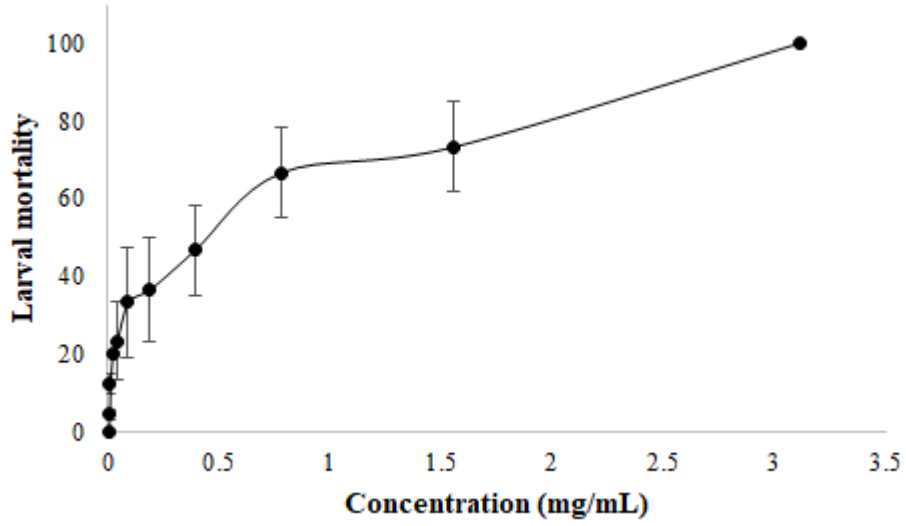
Peak	RT	Compounds	Relative area (%)	Molecular formula	Molar mass	Identification methods
1	44.462	5β,7βH,10α-Eudesm-11-en-1α-ol	2.18	$C_{15}H_{26}O$	222	a, b, c
2	48.776	Hexadecanoic acid, methyl ester	5.91	$C_{17}H_{34}O_2$	270	a, b, c
3	56.183	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.11	$C_{19}H_{32}O_2$	292	a, b, c
4	56.382	11,14-Octadecadienoic acid, methyl ester	3.87	$C_{19}H_{34}O_2$	294	a, b, c
5	56.775	Phytol	14.51	$C_{20}H_{40}O$	296	a, b, c
6	57.204	Methyl stearate	2.34	$C_{19}H_{38}O_2$	298	a, b, c
7	61.299	Vitamin E	4.17	$C_{29}H_{50}O_2$	430	a, b, c
8	64.337	Campesterol	6.61	$C_{28}H_{48}O$	400	a, b, c
9	64.884	n.i	0.63	-	-	a, b, c
10	65.204	Squalene	8.57	$C_{30}H_{50}$	410	a, b, c
11	66.297	Stigmasterol	7.38	$C_{29}H_{48}O$	412	a, b, c
12	66.742	γ-sitosterol	34.39	$C_{29}H_{50}O$	414	a, b, c
13	67.008	γ-tocopherol	2.21	$C_{28}H_{48}O_2$	416	a, b, c
14	67.477	Betulin	1.15	$C_{30}H_{50}O_2$	442	a, b, c
15	67.953	n.i.	2.67	-	-	a, b, c

16	68.225	n.i.	0.68	-	-	a, b, c
17	68.293	β-carotene	1.16	$C_{40}H_{56}$	536	a, b, c
18	68.401	Lycopene	0.46	$C_{29}H_{50}O$	536	a, b, c
		Total Identified (%)	**96.02**			
		Carotenoids	1.62			
		Fatty acid ester	13.23			
		Oxygenated sesquiterpene	2.18			
		Oxygenated diterpene	14.51			
		Phytosterols	48.38			
		Pentacyclic triterpenoid	1.15			
		Triterpene	8.57			
		Vitamin	6.38			

RT: retention time; [a] Compounds listed in elution order through the capillary column HP-5MS; [b]calculated retention index (IR) utilizing a homologous *n*-alkane series C7 to C26 in capillary column (HP-5MS); [c] identification based on the comparison of mass spectra to Nist spectrum library; Relative area (%): percentage of the area occupied by compounds within the chromatogram; n.i: non-identified (Adams, 2017). Source: Authors.

The larvicidal activity of *I. laurina* leaf CE against *A. aegypti* was evaluated, and the results are described in Figure 1. The third-stage *A. aegypti* larvae presented 100% of death when immersed in aqueous solution containing from 100 to 3.12 mg/mL of *I. laurina* CE (Figure 1). The extract, even ranging from 0.02 to 0.01 mg/mL, killed from 20.0 to 12.3% of larvae, respectively, making the toxicity for *A. aegypti* evident. The negative control did not cause larval mortality whereas the positive control killed 100% of the larvae.

Figure 1 – Mortality percentage of de mortalidade das larvas de *Aedes aegypti* larvae submitted to larval packet test in *I. laurina* leaf crude extract.



Source: Authors.

With the mortality percentage data (Figure 1), the lethal concentrations of *I. laurina* CE needed to kill 50% (LC_{50}) and 99% (LC_{99}) of larvae were calculated. The LC_{50} and LC_{99} of *I. laurina* leaf CE were 0.98 and 2.69 mg/mL, respectively (Table 2). The mortality of the positive control (Temephós 0.4 mg/mL) was 100% and the negative control (aqueous solution of polysorbate 80 at 2.0%) was zero.

Table 2 – Lethal concentrations (mg/mL) of *Inga laurina* leaf crude extract needed to kill 50% (LC_{50}) and 99% (LC_{99}) of *Aedes aegypti* larvae.

LC_{50} (CI)	**LC_{99} (CI)**
0.98 ± 0.04	2.69 ± 0.05
(0.95 – 1.02)	(2.65 – 2.74)

LC_{50}: lethal concentration to kill 50% of larvae; LC_{99}: lethal concentration to kill 99% of larvae; CI: confidence interval. Source: Authors.

In order to investigate the effect of *I. laurina* leaf CE on the nervous system of *A. aegypti* larvae and propose the possible action mechanism, the inhibitory activity on the acetylcholinesterase enzyme was evaluated. As shown in Table 3, *I. laurina* CE inhibited acetylcholinesterase enzyme at the concentration from 0.006 to 100 mg/mL (Table 3). These results corroborated the ones found for the larvicidal activity against *A. aegypti*, suggesting that the action mode of *I. laurina* leaf CE be by the acetylcholinesterase enzyme inhibition in the synaptic transmission.

Table 3 – Evaluation of *Inga laurina* leaf crude extract activity on acetylcholinesterase enzyme.

Concentration (mg/mL)	*Inga laurina* **CE**	**Positive Control**
100.00	+	+
50.00	+	+
25.00	+	+
12.50	+	+
6.25	+	+
3.12	+	+
1.56	+	+
0.78	+	+
0.39	+	+
0.19	+	+
0.09	+	+
0.04	+	+
0.02	+	+
0.01	+	+
0.006	+	+
0.003	-	+

Positive control: Temephós®; (+): acetylcholinesterase enzyme inhibition; (-): absence of acetylcholinesterase enzyme inhibition. Source: Authors.

4. Discussion

The increase in the number of diseases transmitted by *A. aegypti* mosquito causes a great impact in public health, mostly in Latin American countries. The main measure to avoid the transmission of these diseases is the elimination of the vector, but this is still a challenge because there is a lack of efficient control mechanisms to eradicate the mosquito (Zara, Santos, Fernandes-Oliveira, Carvalho & Coelho, 2016; PAHO/WHO, 2019).

Inga laurina leaf CE presented potential use with larvicidal activity against *A. aegypti* (LC_{50} and LC_{99} = 0.98 and 2.69 mg/mL, respectively). This activity is likely to be associated to the chemical compounds found in *ingá* leaves. By GC/MS, the presence of phytosterols, terpenes, esters of fatty acids, carotenoids and vitamins were detected in *I. laurina* leaf CE. The phytosterols were the compounds found in greater concentration of *I. laurina* leaf CE. In plants, the phytosterols are fond in cell membranes and are responsible to keep fluidity and permeability of the cell membrane, involved in the adaptation process to the temperature and metabolic ones, are also precursors of growth factors associated to the processes of plant growth, cell proliferation and differentiation, and have action against phytopathogens (Piironen, Lindsay, Miettinen, Toivo & Lampi, 2000; Dufourc, 2008;

Wang, Senthil-Kumar, Ryu, Kang & Mysore, 2012). Sitosterol, stigmasterol and campsterol are among the most abundant phytosterols found in plants, and present chemical structure more similar to cholesterol, differing only in the lateral chain of the molecule (Piironen, Lindsay, Miettinen, Toivo & Lampi, 2000). In mosquitos, the ingestion of toxic phytosterols can cause larval mortality as well as the inhibition of the sterol carrier protein (SCP) enzyme activity, resulting in development deformities that lead to death (Ghosh, 2013).

Sitosterol is the main phytosterol found in plants and in this study, γ-sitosterol (34.39%) was the major compound of *I. laurina* CE. The larvicidal activity of γ-sitosterol against *A. aegypti* has not been reported in the literature, but γ-sitosterol is a β-sitosterol isomer. Rahuman, Gopalakrishnan, Venkatesan & Geetha (2008) isolated β- sitosterol from *Abutilon indicu* leaves and evaluated its larvicidal activity against 3 species of mosquitos; the results showed that β-sitosterol presented potential larvicidal activity against *Aedes aegypti* (LC_{50}= 11.49 mg/L), *Anopheles stephensi* (LC_{50}= 3.58 mg/L) and *Culex quinquefasciatus* (LC_{50}= 26.67 mg/L). Amin et al. (2012) tested β-sitosterol glucoside isolated from *Acanthus montanus* aerial parts against *A. aegypti* adult females at the concentration of 1.25 µg/mg. β-sitosterol caused 100% of mortality of female adults. These studies make evident that β-sitosterol is toxic for *A. aegypti* larvae and adults.

Phytol (14.51%) was the second most abundant compound of *I. laurina* leaf CE. Studies carried out by Mahesh Babu et al. (2016) revealed that phytol presented antifeedant activity (100%) at the concentration of 2.5 mg/L and larvicidal activity with LC_{50} of 24.51 mg/L, against *Spodoptera litura* caterpillar. However, the larvicidal activity of phytol against *A. aegypti* has not yet been reported. Among the major compounds found in *I. laurina* leaf CE, stigmasterol presented larvicidal activity against *Culex quinquefasciatus* (CL_{50}= 70.84 mg/L) mosquito and against *Chironomus riparius* fly. Gade et al. (2016) observed that stigmasterol was less active against *A. aegypti* when compared to other species, and that stigmasterol showed neurotoxic effect on mosquito larvae (*C. quinquefasciatus* and *A. aegypti*) by inhibiting acetylcholinesterase enzyme.

Insecticides, specially organophosphates and carbamates, promote acetylcholinesterase inhibition, causing the interruption of the nervous transmission by accumulation of acetylcholine in the nervous system and causing the insect death (Fukuto, 1990; Alout et al., 2012). In the nervous system, acetylcholine is the neurotransmitter responsible for cholinergic transmission of nervous impulses. During the nervous impulse, acetylcholine is released by pre-synaptic neuron in the synaptic cleft to link to chemical receptors in the post-synaptic neuron membrane to propagate the nervous system. The

acetylcholinesterase enzyme is a regulating agent of the nervous transmission, degrading acetylcholine in acetate and choline. The inhibition of this enzyme causes the accumulation of acetylcholine in the synaptic cleft and neuromuscular joint, resulting in hyperexcitability due to the continuous uncontrolled transmission of nervous impulses causing shakings, sizures and, consequently, collapse of the central nervous system and death (Alout et al., 2012; Čolović, Krstić, Lazarević-pašti, Bondžić & Vasić, 2013; Gullan, 2017).

Inga laurina leaf CE presented inhibitory effect on acetylcholinesterase enzyme up to the concentration of 0.006 mg/mL and at this concentration by larval packet test, *ingá* CE also caused larval mortality (4.33%). At the next concentration of 0.003 mg/mL, the CE did not kill larvae or inhibit acetylcholinesterase enzyme. Therefore, that suggests that *I. laurina* leaf CE interfered in the normal functioning of cholinergic neuronal transmission of *A. aegypti* larvae by inhibiting acetylcholinesterase enzyme.

Species of genus *Inga* were evaluated regarding the larvicidal potential against *A. aegypti*. The values of LC_{100} found by Calderón et al. (2006) for the extracts of *Inga jefensis*, *Inga multijuga*, *Inga mucuna* and *Inga sapindoides* plants are above 500 mg/L. Therefore, *I. laurina* CE in the present study showed potential larvicidal activity (LC_{50}= 0.68 mg/mL and LC_{99} = 2.69 mg/mL) when compared to other studies reported in the literature for the genus *Inga*.

5. Final Considerations

Inga laurina leaf crude extract presented terpenes, fatty acid esters, carotenoids and vitamins in its composition, and the larvicidal activity against *A. aegypti* was modulated by the inhibitory activity of acetylcholinesterase enzyme. The larval control of *A. aegypti* larvae by *I. laurina* leaf crude extract is promising considering that in this development stage they are more vulnerable and are found in a more limited area when compared to an adult mosquito, demanding smaller amounts of insecticide and making the control safer and more economic. Thus, this study offers new possibilities for the application and development of *I. laurina* crude extract products to control *A. aegypti* mosquito.

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References

Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/mass spectrometry*. Allured: Carol Stream.

Alout, H., Labbe, P., Berthomieu, A., Djogbenou, L., Leonetti, J. P., Fort, P. & Weill, M. (2012). Novel AChE inhibitors for sustainable insecticide resistance management. *PLoS One*, *7*(10), e47125, 2012. doi: 10.1371/journal.pone.0047125

Amin, E., Radwan, M. M., El-Hawary, S. S., Fathy, M. M., Mohammed, R., Becnel, J. J. & Khan, I. (2012). Potent insecticidal secondary metabolites from the medicinal plant *Acanthus montanus*. *Records of Natural Products*, *6*(3), 301-305.

Braga, I. M., & Valle, D. (2007). *Aedes aegypti:* histórico do controle no Brasil. *Epidemiologia e Serviço de Saúde*, *16*, 113-118. doi:10.5123/S1679-49742007000200006

Brasil. (2020). *Monitoramento dos casos de arboviroses urbanas transmitidas pelo Aedes (dengue, chikungunya e Zika), Semanas Epidemiológicas 01 a 52*. Boletim Epidemiológico da Secretaria de Vigilância em Saúde do Ministério da Saúde.

Braz-Filho, R. (2010). Phytochemical contribution to development of a emergent country *Química Nova*, *33*(1), 229-239.

Calderón, A. I., Romero, L. I., Ortega-Barría, E., Brun, R., Correa, M. D. & Gupta, M. P. (2006). Evaluation of larvicidal and *in vitro.* antiparasitic activities of plants in a biodiversity plot in the Altos de Campana National Park, Panama. *Pharmaceutical Biology*, *44*(7), 487-498. doi: 10.1080/13880200600878361

Camargo, M. F., Santos, A. H., Oliveira, A. W. S., Abrão, N., Alves, R. B. N. & Isac, E. (1998). Evaluation of the residual action of the larvicide Temephós over *Aedes aegypti* (Díptera-Culicidae) in different types of containers. *Revista de Patologia Tropical*, *27*, 65-70. doi:10.1590/S0102-311X2002000600005

Čolović, M. B., Krstić, D. Z., Lazarević-Pašti, T. D., Bondžić, A. M., & Vasić, V. M. (2013). Acetylcholinesterase inhibitors: pharmacology and toxicology. *Current Neuropharmacology, 11*(3), 315-335. doi:10.2174/1570159X11311030006

Costa, J. G. M., Rodrigues, F. F. G., Angélico, E. C., Silva, M. R., Mota, M. L., Santos, N. K. A., Cardoso, A. L. H., & Lemos, T. L. G. (2005). Chemical-biological study of the essential oils of *Hyptis martiusii*, *Lippia sidoides* and *Syzigium aromaticum* against larvae of *Aedes aegypti* and *Culex quinquefasciatus*. *Brazilian Journal of Pharmacognosy*, *15*(4), 304-309. doi:10.1590/S0102-695X2005000400008

Dufourc, E. J. (2008). The role of phytosterols in plant adaptation to temperature. *Plant Signaling & Behavior, 3*(2), 133-134. 10.4161/psb.3.2.5051

Fukuto, T. R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. *Environmental Health Perspectives*, *87*, 245-254. doi:10.1289/ehp.9087245

Furtado, R. F., Lima, M. G. A., Neto, M. A., Bezerra, J. N. S. & Silva, M. G. V. (2005). Larvicidal activity of essential oils against *Aedes aegypti* L. (Diptera: Culicidae) *Neutropical Entomology, 34*(5), 843-847. doi: 10.1590/S1519-566X2005000500018

Gade, S., Rajamanikyam, M., Vadlapudi, V., Nukala, K. M., Aluvala, R., Giddigari, C., Karanam, N. J., Barua, N. C., Pandey, R., Upadhyayula, V. S. V., Sripadi, P., Amanchy, R. & Upadhyayula, S. M. (2017). Acetylcholinesterase inhibitory activity of stigmasterol & hexacosanol is responsible for larvicidal and repellent properties of *Chromolaena odorata*. *Biochimica et Biophysica Acta*, *1861*(3), 541-550. doi:10.1016/j.bbagen.2016.11.044

Ghosh, A. (2013). Efficacy of phytosterol as mosquito larvicide. *Asian Pacific Journal of Tropical Disease, 3*(3), 252. 10.1016/S2222-1808(13)60050-X

Gullan, P. J. (2017). *Insetos: fundamentos da entomologia.* Gullan, P. J., Cranston P. S., Com ilustrações de Karina H. McInnes; Tradução e Revisão Técnica Eduardo da Silva Alves dos Santos, Sonia Maria Marques Hoenen, 5. ed. Rio de Janeiro: Roca.

Lokvam, J., Clausen, T. P., Grapov, D., Coley, P. D. & Kursar, T. A. (2007). Galloyl depsides of tyrosine from young leaves of *Inga laurina*. *Journal of Natural Products*, *70*(1), 134-136. doi: 10.1021/np060491m

Lorenzi, H. (2002). *Árvores brasileiras: Manual de identificação e cultivo de plantas arbóreas nativas do Brasil*. 4 ed. Instituto Plantarum de Estudos da Flora Ltda: Nova Odessa, 384.

Macedo, M. L. R., Garcia, V. A., Freire, M. G. M. & Richardson, M. (2007). Characterization of a kunitz trypsin inhibitor with a single disulfide bridge from seeds of *Inga laurina* (SW). Willd. *Phytochemistry*, *68*(8), 1104-1111. 10.1016/j.phytochem.2007.01.024

Macoris, M. L. G., Andrighetti, M. T. M., Takaku, L., Glasser, C. L., Garbeloto, V. C. & Bracco, J. E. (2003). Resistance of *Aedes aegypti* from the state of São Paulo, Brazil, to organophosphates insecticides. *Memórias do Instituto Oswaldo Cruz***,** *98*, 703-708. doi:10.1590/S0074-02762003000500020

Mahesh Babu, S., Baranitharan, M., Dhanasekaran, S., Thushimenan, S., Kovendan, K. & Jeyasankar, A. (2016). Chemical compositions, antifeedant and larvicidal activity of *Pongamia pinnata* (L.) against polyphagous field pest, *Spodoptera litura*. *International Journal of Zoological Investigations*, *2*(1), 48-57.

Manjarres-Suarez, A. & Olivero-Verbel, J. (2013). Chemical control of *Aedes aegypti*: a historical perspective. *Revista Costarricense de Salud Pública*, *22*(1), 68-75.

Marston, A., Kissling, J. & Hostettmann, K. A. (2002). A rapid TLC bioautography method for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. *Phytochemical Analysis*, *13*, 51-54. doi:10.1002/pca.623

Martins, C. M., Morais, S. A. L., Martins, M. M., Cunha, L. C. S., Silva, C. V., Martins, C. H. G., Leandro, L. F., Oliveira, A., Aquino, F. J. T., Nascimento, E. A. & Chang, R. (2019). Chemical composition, antifungal, and cytotoxicity activities of *Inga laurina* (Sw.) Willd leaves. *Scientific World Journal*, 9423658. doi: 10.1155/2019/9423658

Martins, T. G. T., Everton, G. O., Rosa, P. V. S., Arruda, M. O., Souto, L. A. da S., Fonseca, D., Silva, I. S., Costa, A. T., Souza, L. dos S., Souza, L. dos S., Araújo Neto, A. P. de, & Mouchrek Filho, V. E. (2020). Larvicidal activity of the essential oil of *Pimenta dioica* Lindl. front larvae of the *Aedes aegypti* mosquito. *Research, Society and Development*, *9*(8), e151985518. https://doi.org/10.33448/rsd-v9i8.5518

Milton, K., & Jenness, R. (1987). Ascorbic acid content of neotropical plant parts available to wild monkey and bats. *Experientia*, *43*, 339-342. doi:10.1007/BF01945577

Miranda, C. G., Arantes, M. C. B., Rezende, M. H., Oliveira, L. M. G., Freitas, M. R. F., Nogueira, J. C. M., Paula, J. R., & Bara, M. T. F. (2009). Pharmacognostic characterization of leaves and seeds of *Albizia lebbeck* (L.) Benth. (Fabaceae). *Revista Brasileira de Farmacognosia*, *19*(2), 537-544. doi:10.1590/S0102-695X2009000400005

Okwute, S. K. (2012). *Plants as Potential Sources of Pesticidal Agents: A Review*. In: Soundararajan, R. P. Pesticides - Advances in Chemical and Botanical Pesticides .IntechOpen. doi:10.5772/46225

Pan American Health Organization / World Health Organization (PAHO/WHO). (2019) *Epidemiological Update: Dengue*. 11 November 2019, Washington, D.C.

Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J. & Lampi, A-M. (2000). Plant sterols: biosynthesis, biological function and their importance to human nutrition. *Journal of the Science of Food and Agriculture*, 80, 939-966. 10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C

Possette, R. F. S. & Rodrigues, W. A. (2010). O gênero *Inga* Mill (Leguminosae – Mimosoideae) no estado do Paraná. *Acta Botanica Brasilica*, *24*(2), 354-368.doi:10.1590/S0102-33062010000200006

Rahuman, A. A., Gopalakrishnan, G., Venkatesan, P. & Geetha, K. (2008). Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. *Parasitology Research*, *102*, 981-988. doi:10.1007/s00436-007-0864-5

Roel, A. R. (2001). Utilização de plantas com propriedades inseticidas: uma contribuição para o Desenvolvimento Rural Sustentável. *Revista Internacional de Desenvolvimento Local*, *1*(2), 43-50.

Santos Júnior, P. S., Everton, G. O., Rosa, P. V. S., Souza, L. dos S., Conceição, F. O. V. de A., Soares, L. B. C., Farias, W. K. dos S., Souza, L. dos S., Batista, C. L. C., Pinheiro, F. S., Araújo Neto, A. P. de, & Mouchrek Filho, V. E. (2020). Larvicidal activity of *Alpinia zerumbet* essential oil in front of the larvae of the *Aedes aegypti* mosquito. *Research, Society and Development*, *9*(8), e194985578. https://doi.org/10.33448/rsd-v9i8.5578

Wang, K., Senthil-kumar, M., Ryu, C-M., Kang, L., & Mysore, K. S. (2012). Phytosterols play a key role in plant innate immunity against bacterial pathogens by regulating nutrient efflux into the apoplast. *Plant Physiology*, *158*, 1789-1802.

Yang, Z. D., Zhang, X., Duan, D. Z., Song, Z., Yang, M. J. & Li, S. (2009). Modifiel TLC bioautographie method for screening acetylcholinesterase inhibitors from plant extracts. *Journal of Separation Science*, 32, 3257-3259. doi:10.1002/jssc.200900266

Zara, A. L. S. A., Santos, S. M., Fernandes-Oliveira, E. S., Carvalho, R. G., Coelho, G. E. (2016). *Aedes aegypti* control strategies: a review. *Epidemiologia e Serviços de Saúde*, 25(2), 391-404. doi:10.5123/s1679-49742016000200017

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