ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. X:X (2017) XX-XX

records of natural products

Bioactivity of *Licaria puchury-major* Essential Oil Against *Aedes aegypti*, *Tetranychus urticae* and *Cerataphis lataniae*

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(Received May 22, 2017; Revised September 29, 2017; Accepted October 01, 2017)

Abstract: The present study was carried out to evaluate an alternative controlling agent for greenhouse pests and the yellow fever mosquito larvae. The potential bioactivity of *Licaria puchury-major* (Mart.) Kosterm. ("puxuri") was evaluated here against three most common pests in tropical and subtropical countries: *Aedes aegypti* Linn. Larvae, *Tetranychus urticae* Koch. mites and *Cerataphis lataniae* Boisd. aphids. The essential oil from seeds was analyzed by GC-FID and GC-MS. The major compounds were safrole (38.8%) and eucalyptol (21.7%). Phenylpropanes (51.7%) was the main group of compounds and oxygenated monoterpenes represented 28.8% of the total oil. The essential oil has shown no inhibition of acetylcholinesterase (AChE) in the tested concentrations. However, potential antioxidant activities were evaluated by different methods [DPPH: LD_{50} = (27.8 ± 1.0) µg/.mL; ABTS: (977.3 ± 25.2) µM TEs/g (Trolox Equivalents); FRAP: (548.2 ± 29.0) µM Fe(II)/g]. A significant larvicidal potential for 24 h of exposure was observed with LD_{50} = 98.9 µg/mL, being an indicative that the larval mortality may occur by ingestion or contact due to the no inhibition against AChE. Volatile phase effects were evaluated against *T. urticae* Koch. and *C. lataniae* Boisd. and LD_{50} were found about 30.8 and 13.5 µg/mL, respectively. These results are consistent with an octopaminergic effect, since some phenylpropanoids (such as Safrole, identified as the major compound in this work) can block octopamine, a multi-functional, naturally occurring biogenic amine. Then, this study clearly illustrated the efficacy of the investigated seeds, which encourages the development of a new potential natural controlling agent against these common pests due to the abundance of these seeds in the Amazon region and to the high essential oil yield.

Keywords: Puxuri; *Licaria puchury-major*, bioassay; safrole

1. Introduction

Essential oils have been reported as efficient natural pesticides, without human hazards [1,2]. The pesticide effect of the *Licaria puchury-major* (Mart.) Kosterm. (Lauraceae) essential oil was evaluated in this work against three most common pests in tropical countries, which are currently causing severe damages in human health and agriculture: *Aedes aegypti* Linn. larvae, *Tetranychus urticae* Koch. mites and Cerataphis lataniae *Boisd*. aphids. Mosquito-transmitted diseases continue to be

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The article was published by ACG Publications
www.acgpubs.org/RNP © Published online 10/10/2017 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.25.17.05.036

one of the leading causes of human death worldwide, and *Aedes aegypti* Linn., the yellow fever mosquito, has been widely distributed in tropical zones. Mosquito larvae control is a successful way of reducing mosquito densities [3]. Furthermore, the two-spotted spider mite, *Tetranychus urticae* Koch., is an important pest of agriculture, and many food crops or ornamental plants might be subject to their attack, resulting in its premature drying [4]. Finally, the palm aphid, *Cerataphis lataniae* Boisd., is a local pest of açaí (*Euterpe oleracea* Martus and *E. precatoria* Martus) palm's that may cause death of young plants or early fall of flowers and fruits [5]. In Amazon region, the control of this aphid has been nowadays based on its hand removal.

Studies reporting the larvicidal, acaricide and insecticidal bioactivities of the *L. puchury-major* essential oil have not been reported, to our knowledge, in scientific literature. Puxuri, puchury or pixuri [6] is native from Amazon and has been often used in the Brazilian northern folk medicine. Their aromatic seeds are resistant to decomposition due to its antimicrobial constituents and hardness shell [7]. A tea prepared with a powdered seed (known as "abafado") is used to treat stomach and intestinal diseases, as well as to treat insomnia and irritability [8]. Few studies reporting this species have been found in scientific literature, and they basically report its chemical composition [6-12]. The first chemical report of *L. puchury-major* [13] suggests a large amount of safrole. Previous work [9] has reported 36% and 11% of safrole and eugenol, respectively, in the essential oil from seeds. These same compounds were also found in the essential oils from leaves, branches and trunk-woods [11,12]. Previous work [10] has reported 58.4% of safrole as the major compound. Furthermore, this essential oil reduced motor activity and anesthetized mice [6]. To date, the more recent study concerning this species has reported the use of ethanol extract from seeds to inhibit cell proliferation in human leukemia cell line [14].

The evaluation of essential oils as alternative controlling agent may lead to the discovery of new natural products for pest control. For this reason, the present work aims to assess the potential of the *L. puchury-major* essential oil against the *A. aegypti* Linn., *T. urticae* Koch. and *C. lataniae* Boisd. The evaluation of acetylcholinesterase (AChE) inhibitory and antioxidant activities of some Lauraceae species have been extensively investigated [15,16]. However, few studies have described the antioxidant activity of essential oils from *Licaria* species, as well as the evaluation of acetylcholinesterase (AChE) inhibitory activity in order to correlate these results with the lethal dosage obtained in the performed bioassays. These results would be useful for the development of a natural agent for control based on this plant due to the abundance of these seeds in the Amazon region and to the high essential oil yield.

2. Materials and Methods

2.1. Materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-on (quercetin), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate ($K_2S_2O_8$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), ferrous sulphate ($FeSO_4$), acetylcholine idodete (AChI), acetylcholinesterase from *Electrophorus electricus* (electric eel), neostigmin bromide, sodium phosphate buffer and 5,5'-dithiobis(2-nitro-benzoic acid) (DTNB) were purchased from Sigma-Aldrich® and dimethyl sulfoxide (DMSO) were purchased from Tedia Hight Purity Solvents®.

2.2. Plant material and Essential Oil Extraction

Seeds of *L. puchury-major* were collected in Belém/PA – Brazil and dried in controlled humidity at 30 °C until reach constant weight. The botanical identification was carried out at the Federal University of Amazonas (UFAM). Then, 150 g of powdered seeds were subjected to a Clevenger-type apparatus for 3 h at 100 °C. The essential oil was treated using anhydrous sodium sulphate and stored at

-18 ºC. Essential oil yield was obtained by the ratio between the extracted oil volume to the plant material mass.

2.3. Oil Characterization

The essential oil relative density was estimated at 20 °C. Two washed, dried capillary tubes were filled with distilled water (m_1) or essential oil (m_2) and weighed. Another capillary tube remained unfilled (m) to obtain the weight difference of the full and empty capillary tubes. The density value was obtained according to the equation [$d = (m_2 - m_1) / (m_1 - m)$] and converted using the water density table [17].

Raw essential oil's refraction index was estimated at 20 °C using an ABBE refractometer model DR-A1.

Thermogravimetric analysis was carried out using sample mass of 11.1080 mg in a SDT Q600 (TA Instruments – USA). The experiment was carried out under N_2 atmosphere (100 mL.min^{-1}) with heating rate of 10 °C.min^{-1} from 30 to 300 °C.

Quantitative chromatographic analysis was carried out using a Shimadzu™ GC2010-FID instrument equipped with a FID detector and a DB-5 (30 m x 0.25 mm, film thickness 0.25 μm film thickness) fused silica capillary column. Injector and detector temperatures were 250 ºC and 290 °C, respectively; helium was used as carrier gas (1.0 mL.min^{-1}); temperature column was from 60 °C to 250 °C with a rate of 3 ºC.min^{-1}. Split ratio 1:10 and 1.0 μL of hexane solution. Qualitative chromatographic analysis was performed using a Shimadzu™ GCMS-QP2010 instrument and the same column used in the GC-FID analysis. The operating conditions were same as that of GC-FID, whose ionization energy and mass range were 70 eV and 32 to 420 Da.

The identification of isolated compounds was established from their GC retention indices using a C_6-C_{30} *n*-alkanes homologous series, whose retention indices were calculated [18]. Moreover, essential oil compounds also were confirmed using published data [19], as well as through the Wiley 7.0 Registry of Mass Spectral Data [20], NIST [21] and Pherobase [22].

2.4. DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging activity

The essential oil radical scavenging ability was evaluated using the DPPH$^{\bullet}$ radical [23] with slight modifications. 100 μM of DPPH$^{\bullet}$ methanolic solution was prepared. 200 μL of essential oils in five concentrations obtained by series dilution (31.2 to 1.9 μg/mL) was added to 1800 μL of DPPH$^{\bullet}$ methanolic solution. Quercetin was used as positive control (25.0 to 1.6 μg/mL). The mixture was maintained in the dark at room temperature for 30 min. The absorbance at 515 nm was measured using a UV-Visible Spectrophotometer (Thermo Scientific, Evolution 220). The inhibition percentage was obtained according to the equation: [100 – (absorbance/average absorbance of control) x 100]. The LD_{50} was defined as the amount of essential oil needed to inhibit the DPPH$^{\bullet}$ radical formation by 50%. The bioassay was carried out in triplicate.

The essential oil radical scavenging capacity was evaluated using the ABTS method [24] with some modifications. The ABTS radical cation (ABTS$^{\bullet+}$) solution was prepared using ABTS (7 mM) and $K_2S_2O_8$ (140 mM) at room temperature for 16 h. The absorbance of the resulting ABTS$^{\bullet+}$ solution was adjusted for 0.70 ± 0.05 at 734 nm via dilution using ethanol. 30 μL of the resulting sample at different concentrations (from 62.5 to 1.9 μg/mL) was diluted in 3000 μL of the ABTS$^{\bullet+}$ solution. After 6 min, the mixture absorbance was measured at 734 nm using a UV-Visible Spectrophotometer (Thermo Scientific, Evolution 220). Trolox was used for the calibration curve (100 a 2000 μM). The results were expressed as μM of Trolox Equivalent Antioxidant Capacity (TEAC) by grams of essential oil.

2.5. Ferric Reducing Antioxidant Power (FRAP)

The FRAP test [25] was employed to evaluate the total antioxidant capacity of the essential oil. The FRAP reagent was freshly prepared adding acetate buffer (0.3 $mol.L^{-1}$, pH = 3.6), TPTZ solution (10 mM) and $FeCl_3.6H_2O$ (20 mM) in proportion 10:1:1. A sample aliquot (90 µL, 250 $\mu g.mL^{-1}$) or positive control (125 $\mu g.mL^{-1}$) was mixed with 270 µL of ultrapure water and added to 2700 µL of the FRAP reagent. After incubated for 30 min at 37 °C, the absorbance was measured at 595 nm using a UV-Visible Spectrophotometer (Thermo Scientific, Evolution 220). The results was determined by the corresponding regression equation of ferrous sulphate (250–2000 µM) and expressed in Fe(II) $(\mu M).g^{-1}$ of essential oil. The assay was performed in triplicate. Quercetin was used as the positive control.

2.6. Evaluation of Acetylcholinesterase (AChE) Inhibitory Activity

AChE inhibitory activity of the essential oil was performed based on the method described previously [26] with some modifications. Solutions prepared with 20 µL of sample (500.0 – 7.8 µg/mL), 80 µL of phosphate buffer (100 mM), 40 µL of dithiobisnitrobenzoic acid (DTNB, 2.5 mM), and 20 µL of AChE (1.0 U/mL) were added to each microplate well at 37°C for 10 min. Then, 40 µL of acetylcholine iodide (AtCHI, 10 mM) was added and incubated again in the same conditions. 60 µL of sodium dodecylsulphate (SDS, 1%) was added as reaction terminator. The mixture absorbance was measured at 405 nm. The percentage inhibition of AChE activity was calculated by [Inhibition (%) = $(A_2 - (A_1 - A_3) \times 100)/A_2$], where A_1 is the sample and enzyme absorbance, A_2 is the enzyme absorbance, and A_3 is the tested sample absorbance. The LD_{50} was obtained by curves of each inhibitor toward AChE. The bioassay was carried out in duplicate. Galantamine was used as standard from 25.0 to 1.6 µg/mL.

2.7. Statistical analysis

The Kolmogorov–Smirnov test [27] was performed to evaluate the data distribution. Multiple data comparison was carried out based on the Tukey test [28]. Pearson correlation coefficients were obtained with $p < 0.05$ and performed using the Minitab™ 13.0 software.

2.8. Bioassays of Oil Solution

Bioassays were conducted to test the *L. puchury-major* essential oil against *A. aegypti* Linn. larvae based on previous work [29] with some modifications. All bioassays were performed at 26 ± 2 °C and 80% RH in a photoperiod regimen of 12:12 h. Essential oil/dimethyl sulfoxide (DMSO) solutions were prepared at 500, 250, 100, 50 and 25 ppm. 500 larvae at 3rd instar were divided in 5 groups for each tested concentration. DMSO and temephos (0.12 µg/mL) were used as negative and positive control, respectively. This bioassay was carried out in quintuplicate and the larvicidal activity was estimated by the larvae mortality after 24 h, 48 h and 72 h of exposure. Data were analyzed in POLO PC® program [30] for calculations of the LD_{50} (Lethal Concentration that kills 50% of the exposed larvae), LD_{90} (Lethal Concentration that kills 90% of the exposed larvae), LCL (Lower Confidence Limit) and UCL (Upper Confidence Limit) with fiducially limits of 95%.

Evaluation of the acaricide and insecticidal potential of the essential oil was based on previous works [31,32]. Glass Petri plates (90 mm × 20 mm; 130 mL) were used for the evaluation of the essential oil's volatile phase effect. *T. urticae* Koch. and *C. lataniae* Boisd. were obtained, respectively, from stock colonies *of* papaya leaves (*Carica papaya L.*) and açaí palm (*Euterpe oleracea* Martus) without any pesticide exposure. Dorsal side leaves were placed on filter paper saturated with 1% sodium hypochlorite. Ten adults (mite or aphid) were transferred from stock using a soft paintbrush and allowed to settle before exposure. The tested oils were applied on filter paper disks at the inner surface of the Petri dish lid. Each concentration (10 µL) of essential oil/dimethyl sulfoxide (DMSO) solution (1.0, 0.50, 0.25, 0.10, 0.062 and 0.031%) was added on filter paper disk placed at the inner surface of the

Petri dish lid. Plates were sealed. These bioassays were carried out in triplicate. DMSO and thymol (3.0 $\mu g.mL^{-1}$) were used as negative and positive control, respectively. Mortality was evaluated after 24 h, 48 h and 72 h of exposition. Mites or aphids were considered dead if they did not move when prodded with a fine paintbrush. The mortality data were subjected to the PROBIT analysis [33] for calculations of the LD_{50}, LD_{90}, LCL and UCL with fiducially limits of 95%.

3. Results and Discussion

3.1 Oil Characterization

The extraction time has influenced the essential oil yield. The optimum extraction time was 3 h. The essential oil yield was found around 5.5% (w/v). Previous work [34] has reported that Lauraceae family plants presents essential oil yield higher than 1%. The essential oil density was found to be 0.24 g/mL and the estimated refraction index was 1.50.

Simultaneous TG/dTG curves of the raw essential oil are shown in Figure 1.

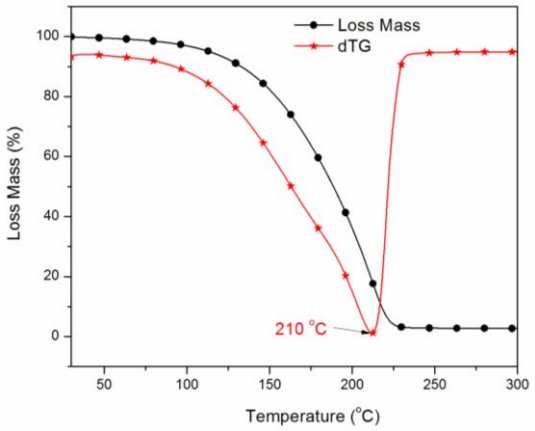

Figure 1. TG/dTG curves of the raw essential oil.

The essential oil remains stable up to ~100 °C, allowing the environmental exposure as a natural pesticide in tropical and subtropical countries due to its high thermal stability. In sequence, one stage related to loss mass/oil decomposition was observed from ~100 °C to 210 °C. Above 210 °C there are no further decomposition stages.

Chemical analyses of the *L. puchury-major* essential oil are given in Table 1. The identified compounds were grouped based on its functional groups. Twenty compounds were identified, representing 98.4% of the raw essential. Based on CG-FID and GC-MS characterizations, Safrole (38.8%) and Eucalyptol (21.7%) were recorded as the most abundant compounds, totalizing 60.5%.

As shown in Table 1, phenylpropanoids and oxygenated monoterpenes represent the main group (51.7 and 28.8%, respectively) of the raw essential oil. Non-oxygenated monoterpenes represent 17.5%, following by non-oxygenated sesquiterpenes (0.5%), with just one compound (*E*-Caryophyllene). Oxygenated sesquiterpenes have not been found.

Lauraceae species from Amazon region are described as having large amount of Safrole in their aerial parts [34,35]. Besides the insecticidal, microbial and bactericidal activities of Safrole or other plants presenting large amount of this compound [36,37], these species could become an alternative to obtain this phenylpropene. On the other hand, it has been observed that some phenylpropene derivatives present important pharmacological effects in the central nervous system [38].

Table 1. Essential oil composition from the seeds of *L. puchury-major*.

Compounds	*RI_{cal}	Percentage
α-thujene	924	0.18
α-pinene	932	2.01
sabinene	969	3.95
β-pinene	974	1.47
myrcene	988	0.83
α-terpinene	1014	0.17
o-cymene	1022	0.16
limonene	1024	8.27
eucalyptol (1,8-cineole)	1026	21.68
γ-terpinene	1054	0.33
Z-sabinene hydrate	1065	0.17
terpinolene	1086	0.09
δ-terpineol	1162	0.32
terpinen-4-ol	1174	0.77
α-terpineol	1186	5.82
safrole	1285	38.83
eugenol	1356	6.15
methyl eugenol	1403	6.56
E-caryophyllene	1417	0.47
elemicin	1555	0.16
Total (%)		98.4
Non-oxygenated Monoterpenes (%)		17.5
Oxygenated Monoterpenes (%)		28.8
Non-oxygenated Sesquiterpenes (%)		0.5
Oxygenated Sesquiterpenes (%)		---
Phenylpropanes (%)		51.7

*RI_{cal}: retention indices relative to C_7 -C_{30} *n*-alkanes on the HP-5 column

3.2 Antioxidant and AChE inhibitory activities

According to Table 2, the *L. puchury-major* essential oil has showed sequestration capacity of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, besides iron reducing capacity.

Table 2. Antioxidant and enzymatic activities of the *L. puchury-major* essential oil.

Sample/Standard	$DPPH^{\bullet}$ IC_{50} ± SD	$ABTS^{\bullet+}$ TEs ± SD	FRAP Eq. Fe(II) ± SE	AChE GalanE
Essential Oil	27.83 ± 0.99	977.33 ± 25.17	548.20 ± 29.00	na
Quercetin	12.45 ± 0.10	-	1984.53 ± 89.07	-

IC_{50}: expressed in μg/mL; ABTS: expressed in μM TEs/g (Trolox Equivalents); FRAP: expressed in μM Eq Fe(II)/g; GalanE: Galantamine Equivalents; SD: Standard Deviation; na: not active.

The antioxidant activity in DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays reflects the hydrogen donating ability of a given compound. The *L. puchury-major* essential oil has shown LD_{50} = (27.8 ± 1.0) µg/mL, and 44.6% of activity rate when compared to Quercetin. The ABTS$^{\bullet+}$ radical scavenging activity was (977.3 ± 25.2) µM TEs/g of essential oil. The FRAP assay was performed to evaluate the reduction of Fe^{3+} to Fe^{2+} in the presence of antioxidants. The essential oil showed (548.2 ± 29.0) µM Fe(II)/g, and 44.3% of activity rate when compared to Quercetin in the tested concentrations. The calibration curves for the equivalence calculations for Trolox and ferrous sulfate are shown in Figure 2. Our results may be explained by the presence of the phenylpropanes, Safrole (38.8%) and Eugenol (6.1%), besides the oxygenated monoterpenes, 1,8-Cineole (21.7%), which present important antioxidant properties [39,40].

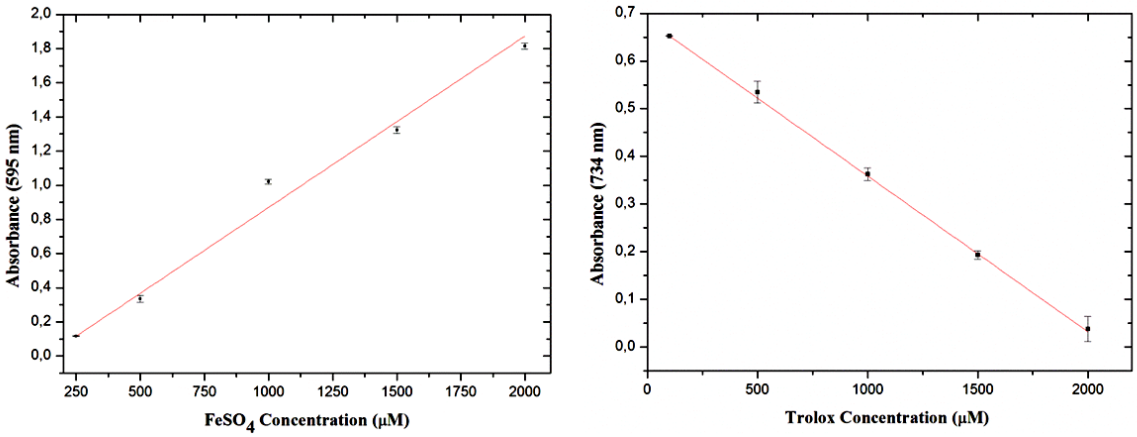

Figure 2. Calibration curves for the equivalence calculations for Trolox and ferrous sulfate.

No inhibition of acetylcholinesterase (AChE) was observed in the tested concentrations. Essential oils of Lauraceae family are rich in Safrole (54-59%), which has presented AChE inhibitory activity above 1000 µg/mL[41].

3.3 Bioassays

Organophosphorate temephos are the most commonly employed larvicide for *Aedes* and *Anopheles* control. However, its toxicity has been increasingly reported besides the insecticide resistance of arthropods [42,43]. Most plant-derived compounds have shown great potential as natural pesticide, presenting low toxicity to non-target organisms, as well as to the environment. For this reason, the susceptibility of many species of mosquito larvae to plant products has been extensively studied [44,45].

Table 3. LD_{50} and LD_{90} values of the *L. puchury-major* essential oil against *A. aegypti* Linn.

Exposure	LD_{50} ± SD	(LCL–UCL)	LD_{90} ± SD	(LCL–UCL)	Regression Equation
24 h	98.9 ± 0.6	67.84 – 141.41	228.8 ± 0.6	141.41 – 156.23	Y= (-0.23 + 0.05) + 0.48X
48 h	79.1 ± 0.4	52.76 – 108.23	200.7 ± 0.4	140.31 – 431.64	Y= (-0.23 + 0.05) + 0.47X
72 h	59.8 ± 0.3	42.57 – 78.06	167.6 ± 0.3	122.10 – 293.43	Y= (-0.18 + 0.05) + 0.35X

LD_{50}: Lethal Dosage that kills 50% of the exposed larvae, expressed in µg.mL^{-1}; *LD_{90}*: Lethal Dosage that kills 90% of the exposed larvae, expressed in µg.mL^{-1}; *Y*: mortality rate (significant at *P<0.05* level); *X*: concentration (significant at *P<0.05* level); *SD*: Standard Deviation; *LCL*: Lower Confidence Limit; *UCL*: Upper Confidence Limit. Negative control: DMSO; Positive control: Temephos (0.12 µg.mL^{-1}).

Interest in the control of *A. aegypti* larvae lies in the fact that they represent serious public health problems in many developing countries. Plant extracts of the Lauraceae family have been reported as effective natural pesticides against 3th and 4th instars larvae stages [46]. Larvicidal activity of *L. puchury-major* essential oil was not reported earlier. Our work shows that the essential oil from its

seeds may be an efficient alternative to *A. aegypti* larvae control. After exposure to the essential oils, the *knock-down* effect was observed [35]. A significant larvicidal potential for 24 h of exposure was observed. The LD_{50} and LD_{90} values are shown in Table 3.

The LD_{50} and LD_{90} were found to be 98.9 and 228.8 µg/mL, respectively, for 24 h of exposure. For 48 h of exposure, the LD_{50} and LD_{90} were 79.1 and 200.7 µg/mL, respectively. Finally, for 72 h of exposure, concentrations of 59.8 and 167.6 were observed, respectively, for LD_{50} and LD_{90}. The LD_{90} provides information about the concentration that causes the maximum reduction of mosquito generation. Unfortunately, this data has not always been reported in scientific literature. Our results have shown that this essential oil may be applied as a natural larvicidal against the *A. aegypti*.

Acaricide and insecticidal activities of *L. puchury-major* essential oil were not reported earlier. Essential oils commonly contain compounds that present particular roles [47]. Such plant products have shown efficient insect toxicity in vapour phase, being reported as more toxic to the microorganisms than the contact form [32]. The total mortality of *T. urticae* Koch. and *C. lataniae* Boisd. was observed at 500 ppm and 250 ppm for 24 h of exposure. The LD_{50} and LD_{90} were, respectively, 30.8 and 87.1 µg/mL.

Although literature presents a range of similar studies showing the acaricide activity of essential oils, there is a deficit concerning the *T. urticae* Koch. Among the few published works [48], the sublethal effects of the *Cinnamomum zeylanicum* essential oil has shown LD_{50} = 23.4 µL/L. Similarly, the toxicity of two insecticides against moveable stages of immature *T. urticae* Koch. has been reported [49] after 24 h of exposure, with LD_{90} of 90.3 and 103.9 µg/mL for, respectively, vermitec® and spinetoram.

The insecticidal bioassay against the *C. lataniae* Boisd. has showed efficient activity. Studies reporting the use of essential oils against this aphid have not been found in scientific literature. After 24 h of exposure, 91% of mortality was observed. The LD_{50} and LD_{90} were, respectively, 13.5 and 72.8 µg/mL, as shown in Table 4.

A recent study [50] have reported the LD_{50} against *A. craccivora* using plants collected from mid hills of western Himalayas in the range of 55–60 ppm. These results are comparable to chemical insecticides, which have demonstrated LD_{50} in the range of 25–51 ppm. Similarly, Lima et al., 2009 [35] have reported the lethal dosage of *Piper hispidinervum* essential oil and its effect on the comportment and/or mortality of the *Spodoptera frugiperda*. Safrole (82.0%) was reported as the major compound and the LD_{50} was 18.2 mg/mL for 24 h of exposure.

Then, our results concerning the *L. puchury-major* essential oil presented efficient insecticidal activity against the two-spotted spider mite and the palm aphid, making the puxuri a great candidate for a new natural agent for these pest control.

Table 4. LD_{50} and LD_{90} values of the *L. puchury-major* essential oil against *T. urticae* Koch. and *C. latanitae* Boisd.

Species	LD_{50} ± SD	(LCL–UCL)	LD_{90} ± SD	(LCL–UCL)	Regression Equation
T. urticae Koch.	30.8 ± 0.3	18.1 – 40.3	87.1 ± 0.3	66.9 – 145.0	$Y = (4.22 + 0.05) + 2.84X$
C. lataniae Boisd.	13.5 ± 0.8	1.4 – 25.7	72.8 ± 0.8	47.4 – 139.6	$Y = (1.98 + 0.05) + 1.75X$

LD_{50}: Lethal Dosage that kills 50% of the exposed larvae, expressed in µg.mL^{-1}; *LD_{90}*: Lethal Dosage that kills 90% of the exposed larvae expressed in µg.mL^{-1}; *Y*: mortality rate (significant at *P<0.05* level); *X*: concentration (significant at *P<0.05* level); *SD*: Standard Deviation; *LCL*: Lower Confidence Limit; *UCL*: Upper Confidence Limit. Negative control: DMSO; Positive control: Thymol (3.0 µg.mL^{-1}).

Many essential oils have been shown bioactivities against insects or invertebrates. However, few studies provide their mode of action in individual species. It is unknown whether the activities of the essential oil mixtures are due to the individual compounds or to synergistic interactions between their constituents. Bioassays performed with some essential oils resulted in characteristic symptoms of neurotoxic mode of action. The *knockdown* effect observed in the present work is similar to those produced by organophosphates and carbamates. Some phenylpropanoids (such as Safrole, identified as the major compound in this work) can block octopamine [51], which plays an important role in invertebrate systems [52]. The acute and sub-lethal behavioral effects of essential oil compounds in

invertebrates are consistent with an octopaminergic effect. The *knockdown* effect, toxicity and repellency of Safrole and other phenylpropanoids were reported in previous work [53]. Complementing these results, Eucalyptol (identified as the second major compound in this work) is also reported to has depressant effects on the central nervous system [54]. For this reason, these findings indicate that the route of action for the essential oil might be suggested via octopaminergic effect.

Furthermore, the *L. puchury-major* essential oil analysis showed two major compounds (Safrole and Eucalyptol) with confirmed bioactivities. For this reason, our results suggest this essential oil presented an efficient potential for use in the control of the yellow fever mosquito, the two-spottes spider mite and the palm aphid. In conclusion, this study clearly illustrated the efficacy of the investigated seeds, which encourages the development of a potential alternative as new natural pesticide. However, further studies will be conducted to evaluate the cost-efficacy of this essential oil on wide range of pests. Furthermore, the abundance of puxuri seeds in the Amazon region, as well as the large essential oil yield under hydrodistillation extraction makes this species a great candidate as an effective natural pesticide.

Acknowledgments

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Processo n° 403496/2013-6 and 401508/2016-1), Financiamento de Estudos de Projetos e Programas (FINEP, 0245/08 and 0391/09), Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM, 022/13-FIXAM/AM) financial supports, Centro de Biotecnologia da Amazônia (CBA, Manaus/AM), Instituto Nacional de Pesquisa da Amazônia (INPA), as well as the Central Analítica/UFAM.

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