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Chemical composition, oviposition deterrent and larvicidal activities of the wood extracts of *Tabebuia avellanedae* from the Cerrado of Brazil

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Tabebuia avellanedae **is an important timber source belonging to the family of Bignoniaceae. The latter is known for its richness in terms of variety of bioactive chemical constituents, and it has been used in folk medicine for treatment of various diseases. The aim of this work was to investigate the chemical composition, oviposition deterrent and larvicidal activities of the wood extracts of** ***T. avellanedae*** **from the Cerrado of Brazil. Extracts of acetone, ethyl acetate and ethanol from** ***T. avellanedae*** **were obtained using various extraction methods. Quantitative analysis of phytochemical screening confirmed the presence of phenols and tannins in the wood extracts, however, anthraquinones, coumarins and alkaloids were absent. The toxicity of** ***T. avellanedae*** **extracts against 3rd instar larvae of** ***Aedes aegypti*** **using maceration and Soxhlet extraction methods was analyzed. The acetone and ethyl acetate extracts obtained by Soxhlet extraction were more toxic against 3rd instar** ***A. aegypti*** **larvae, with $CL_{50}$of 100.1 and 151.0 µg/mL, respectively. The mortality values (LT_{50} and LT_{95}) were 38.66 and 66.74 min for ethyl acetate extract, respectively, and 53.47 and 119.96 min for acetone extract, respectively. In all cases, the assay showed that all extracts presented mortality of 100% to 3rd instar larvae after 12 h. The oviposition assay showed that gravid** ***A. aegypti*** **females laid their eggs preferentially in the control ovitraps. The ethanol extract at 333.3 µg/mL strongly deterred oviposition by 89.89% while the ethyl acetate and acetone extracts presented 89.04 and 68.10% deterrence, respectively. The bioactive compounds in** ***T. avellanedae*** **make it a potential source for the control of** ***A. aegypti*** **vectors, without promoting deforestation of trees.**

Key words: Mosquitoes, larvae, toxicity, Ipê-roxo, *Aedes aegypti*, phytochemical, phenolic compounds.

INTRODUCTION

The mosquito *Aedes* (Stegomyia*) aegypti* (Linneus, 1792) (Diptera: Culicidae) transmits dengue and is

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responsible for the transmission of other virus diseases, including yellow fever, chikungunya and Zika virus (Olagnier et al., 2016). Dengue, Zika and chikungunya viruses deserve special attention particularly because these viruses are currently causing negative impacts on public health and economic damage around the world (Mayer et al., 2017). To date, vaccines and medications for treatment of dengue are not available. Disease prevention solely depends on the elimination of mosquito by killing larvae with larvicides (Kumar et al., 2010).

Many insecticides may be used to control mosquitoes, however, many of them are not selective and can harm beneficial insects, increase the resistance of these insects and produce environmental contamination (Moreira et al., 2016). For the past several decades, the problem of vector resistance to insecticides has developed in many vector-borne disease endemic areas throughout the world. Therefore, new compounds to overcome this problem are being developed. Botanical insecticides are less likely to bio-accumulate, as they are biodegradable (Bosire et al., 2014).

Insecticidal plants and their compounds have been intensively screened in terms of their insecticidal properties (Rajeswary and Govindarajan, 2014; Tennyson et al., 2015; Thongwat et al., 2017). The use of phytochemicals is one such strategy that may be suitable for mosquito control (De Omena et al., 2007; Ribeiro et al., 2009; Garcez et al., 2009). The family Bignoniaceae, order Lamiales (Dahlgren, 1989), consists of about 120 genera and 800 species. Among these species, the chemistry of *Tabebuia* spp. has been extensively studied, following its use in popular medicine, and several biologically active constituents have been isolated, including furanonaphtoquinones, quinones, benzoic acid, cyclopentene dialdehyde, flavonoids, iridoids, phenolic glycosides and naphthoquinones (Alonso, 2004). Other chemical compounds of *Tabebuia* spp. have investigated for their biological properties. Cavalcanti et al. (2015) reported the antimicrobial activity of β-lapachone encapsulated into liposomes against methicillin-resistant *Staphylococcus aureus* and clinical strains of *Cryptococcus neoformans*.

Tabebuia avellanedae has several popular names including *pau d'arco*, *ipê*, *ipêroxo*, *lapacho*, *tahuari*, *taheebo*, trumpet tree, *tabebuia ipê* and *tajy*. It has been used for its anti-inflammatory and antioxidant activities. An anticancer effect of a series of furanonaphthoquinones based on the naphtho [2,3-b] furan-4,9-dione skeleton were detected in water extracts of the inner bark (Zhang et al., 2015).

The constituents (particularly quinones) of several *Tabebuia* spp. have been shown to have potent larvicidal activity against *Toxocara canis in vitro*, indicating the relevance of studies in this area (Santos-Mota et al., 2015). Lapachol, an important representative of the quinone group, is isolated from plants of the Bignoniaceae family.Recent research by Kim et al. (2013) demonstrated high larvicidal activity of methanolic extractsand fractions of the bark of *T. avellanedae* against the mosquito species *A. aegypti*, *Culex pipiens pallens* (Coquillett, 1898) (Diptera: Culicidae) and *Ochlerotatus togoi* (Theobald, 1907) (Diptera: Culicidae).

Studies by Jiménez-González et al. (2013) provided evidence supporting the use of *Tabebuia* species for treating infectious diseases. Nevertheless, little work has been done on activity of *T. avellanedae* wood residues against *A. aegypti* mosquito larvae. Thus, in the present study the larvicidal and oviposition deterrent activities of extracts of *T. avellanedae* (Bignoniaceae) wood residues against 3rd instar larvae of *A. aegypti* were investigated.

MATERIALS AND METHODS

Sample materials

Wood residues from *T. avellanedae* were derived from trees planted in Gurupi, Tocantins, Brazil (11°44'27.05"S latitude, 49°3'52.70"W longitude). Branches containing leaves and flowers from *T. avellanedae* were collected for taxonomic identification. Taxonomic identification of dried samples was confirmed by teacher of Botany, Dr. Rodney Vianna, where it was deposited in the Herbarium of the Nucleus of Environmental Studies of the Federal University of Tocantins (Campus of Porto Nacional), where the specimen voucher was deposited under the code HTO-10000707.

Extraction

Extracts were obtained using maceration and Soxhlet methods, as described below. The classical maceration method (Vieitez et al., 2018) was as follows: Samples of wood residue from *T. avellanedae* (20 g) were placed in a flask and 200 ml of 70% ethanol was added. The solution was then stirred for 24 h. In similar fashion, acetone and ethyl acetate were used as solvents. The Soxhlet method (Silva et al., 2014) was as follows: plant material (wood residue) was milled with Willey-type knives. After pulverization, the material (500 g) was subjected to polarity increasing extraction with three different solvents, first with the apolar solvents ethyl acetate PA (500 ml) and acetone PA (500 ml), and finally the solvent polar ethanol PA (500 ml), separately in a Soxhlet extractor (Marconi, modelMA-487/6/25, Brazil). It should also be noted that, in the present study, solvent selection was based on polarity differences in order to verify and compare its toxic effects on *A. aegypti* larvae. The extracts obtained by the two methods were filtered using 125 mm, n°. 3 filter paper (Whatman), and were concentrated under low pressure in a rotary evaporator (Marconi, model MA120) to remove the solvent. Standard stock solutions were prepared at 1% by dissolving the residues in dimethyl sulfoxide (DMSO). From these stock solutions, various concentrations were prepared and the solutions were used for larvicidal, residual activity, time response and mosquito ovipositor-deterrence bioassays.

Qualitative phytochemical analysis

The phytochemical screening of the acetone, ethanol and ethyl acetate extracts obtained by the Soxhlet method was carried out as described by Matos (2009) and Abate and Mengistu (2018). Extracts (1 mg/ml) were used to detect phytochemicals through qualitative tests, involving precipitation reactions or color development.

Alkaloids

The determination of alkaloids from extracts obtained from the residue of *T. avellanedae* wood was performed according to Abate and Mengistu (2018) with some modifications. For this reaction, 2 ml of alkaline extract were mixed with 1 ml of hydrochloric acid (HCl) and 4 drops of oil in a test tube. Two milliliters of extract, 1 ml of HCl and 4 drops of drag oil were added to second tube, and 2 ml of extract, 1 ml of HCl and 4 drops of Mayer reactive were added to a third tube. The formation of insoluble and flocculent precipitates confirmed the presence of alkaloids.

Anthraquinones

The determination of anthraquinones from extracts obtained from the residue of *T. avellanedae* wood was performed according to Abate and Mengistu (2018) with some modifications. For each extract solution, a 2.0 ml methanolic solution was placed in a test tube and chloroform (5.0 ml) was added and stirred. It was allowed to stand for 15 min. The chloroform phase was collected and divided into two test tubes. In the first tube, 1 ml of 5% aqueous NaOH solution was placed. The purple coloration in the aqueous phase indicated the presence of anthraquinones (Borntraeger Reaction). In the second tube, 1 ml of 5% magnesium acetate solution in methanol was added. Purple staining indicated the presence of free anthraquinones.

Coumarins

The determination of coumarins from extracts obtained from the residue of *T. avellanedae* wood was performed according to Abate and Mengistu (2018) with some modifications. For this reaction, 2 ml of methanolic solution was placed in a test tube with filter solution dissolved with 10% NaOH solution and brought to a 100°C water bath for a few minutes. The filter papers were removed and examined under UV light. Yellow fluorescence indicated the presence of coumarins.

Phenols and tannins

The determination of phenols and tannins from extracts obtained from the residue of *T. avellanedae* wood was performed according to Matos et al. (2009). They were placed in a test tube with 2 ml of alcohol extract and 3 drops of an alcohol solution of Fe_2Cl_3. Precipitates with blue hue indicated the presence of hydrolysable tannins, and green indicated the presence of condensed tannins.

Flavonoids

The determination of flavonoids from extracts obtained from the residue of *T. avellanedae* wood was performed according to Matos et al. (2009).The cyanidin or Shinoda test (concentrated HCl and magnesium) was performed: 2 ml of extract, a piece of metallic magnesium and 2 ml of HCl were added. The end of the reaction was determined by the cessation of effervescence. Red coloration indicated the presence of flavonoids in the extract.

Quantitative determinations of phytochemicals

Total phenolic content and total tannin content

The quantification of the total phenols was performed by the Folin–Ciocalteu method, as described by Amorim et al. (2008) using gallic acid as a standard. Methanolic solutions (0.2 ml) of the extract (1 mg/ml, w/v) or standard (0.1-1.0 µg/ml w/v) aqueous solutions were mixed with the Folin–Ciocalteu reagent (0.5 ml of 10%, v/v), sodium carbonate (1 ml of 75%, w/v) and 8.3 ml of Milli-Q water, gently agitated and maintained for 30 min in the dark. The absorbance was measured at 760 nm in a UV–vis Spectrophotometer (Biospectro® SP-220) equipped with quartz cells of 1 cm path length, calibrated with Milli-Q water. Total phenols were determined by interpolation of the absorbance of the samples against a calibration curve (GAE mg/g) ($y = 1.3644x + 0.0212$; $R^2 = 0.9617$) generated with concentrations of gallic acid standard, expressed as mg gallic acid equivalents (GAE) per gram of extract. To quantify the tannin content, the extract solution (10 ml) was mixed with an equal volume of casein solution (0.1 g/ml). This mixture was stirred for one hour and was then centrifuged at 1358 × g for 10 min at 10°C. In the clear supernatants, the non-tannin phenolics were determined in the way similar to that of the total phenolics. Tannin content was calculated as the difference between total phenolic and non-tannin phenolic content in the extracts. The total tannin content was expressed as milligrams of gallic acid equivalents per gram of *T. avellanedae* extract (mg GAE/g).

Condensed tannin content (CTC)

The determination of the condensed tannin content of the *T. avellanedae* was performed according to the vanillin method of Burns (1971) with some adaptations. Briefly, 2 ml of the extract were mixed with 3 ml of methanol and 2.5 ml of hydrochloric acid (10% in methanol). After 10 min, 2.5 ml of vanillin (1% in methanol) were added. The mixture was heated to 60°C for 10 min, and absorbance was measured at 500 nm. Total condensed tannins were determined by interpolation of the absorbance of the samples against a calibration curve (y = standard, expressed as mg catechin equivalents (CE) per gram of extract (CE mg/g).

Insect culture

A. aegypti mosquitoes were raised in the Entomology Laboratory of the Federal University of Tocantins, Gurupi campus, according to the methodology of Aguiar et al. (2015). Adult mosquitoes were maintained in a 10% aqueous sucrose solution and the blood of live Wistar rats (*Rattus norvegicus albinus*). The larvae were raised in plastic containers (35 cm × 5 cm) and were fed a sterilized diet (80/20 mix of chick chow powder/yeast). All bioassays were conducted at 26 ± 1°C, 60.0 ± 5% RH, with 12 h light-dark photoperiod. All applicable international, national, and institutional guidelines for the care and use of animals were considered.

Larvicidal bioassay

Standard methods for assaying larvicidal activity, as recommended by the World Health Organization (2005), were followed in all experiments with acetone, ethyl acetate and ethanol extracts against the 3rd instar larvae of *A. aegypti*to determine the LC_{50} and LC_{95}. Briefly, a stock solution (1000 µg/ml) of each extract was prepared with 0.5% dimethyl sulfoxide (DMSO) and was then diluted with dechlorinated water to obtain the desired concentrations. The concentrations used were for chloroform and hexane extracts were 33.3, 166.7 and 333.3 µg/ml. For each extract, 0.1 g was weighed and solubilized in 500 µl DMSO and diluted to 29.5 ml distilled water in 200 ml disposable cups. The concentrations used were between 33.3 and 333.3 µg/ml extract for the toxicity bioassay. Assays were carried out in triplicate using 25 larvae for each replicate assay. Mortality was verified after 24 and 48 h exposure of the 3rd instar larvae to extracts. After obtaining the

Table 1. Results of the phytochemical assay of three different extracts obtained from the wood residue of *T. avellanedae* by the maceration and Soxhlet method.

Phytochemicals	Types of extracts		
	Ethyl acetate extract	Ethanol extract	Acetone extract
Phenols	+	+	+
Flavonoids	-	-	-
Flavonones	-	-	-
Alkaloids	-	-	-
Anthraquinones	-	-	-
Coumarins	-	-	-

(+) sign indicates the presence of specific phytochemicals whereas (−) sign indicates the absence.

results, selective bioassays were performed to determine the LC_{50} and LC_{95}. The concentrations used for determination of LC were as follows: ethyl acetate extract: 33.3, 66.7, 100, 166.7, 173.3, 233.3 and 300 µg/ml; Acetone extract: 33.3, 100, 166.7, 200 and 333.3 µg/ml; ethanolic extract: 100, 166.7, 186.7, 333.3, 400, 466 and 500 µg/ml. The LC_{50} and LC_{95} were obtained for each extract and were carried out according to the Probit method (Finney, 1971).

Residual activity of extracts

The measurement of residual activity of acetone, ethyl acetate and ethanol extracts obtained by Soxhlet extraction was performed using a solution corresponding to LC_{95}. Bioassays were made in triplicate with 30 ml solution containing 25 *A. aegypti* 3rd instar larvae in each replicate. To determine residual activity the number of dead larvae, all were removed from the solution after 24 h, and 25 new larvae were added in each recipient with each extract. This process was repeated for seven days. The same procedure was repeated for the control group, with only water and water + DMSO. The percentage mortality was corrected by Abbott's (1925) formula according to Leong et al. (2018):

$$\text{Corrected \%mortality} = [(T - C) / (100 - C)] \times 100$$

Where, T = % mortality in test concentration. C = % mortality in control.

Time response

For each of extract, larvae mortality was initially estimated by LC_{95} response curves. Mortality was recorded after 26-119 min of exposure to extracts of *T. avellanedae* and the lethal response time (LT) activity was reported as LT_{50} and LT_{95}, in minutes. To obtain the LT_{50} and LT_{95} of each extract, we used concentrations of acetone extract at 222.3 µg/ml, ethanol extract at 620 µg/ml and ethyl acetate extract at 319.3 µg/ml.

Mosquito oviposition deterrence

The effect of acetone, ethyl acetate and ethanol extracts of *T. avellanedae* on the oviposition response of female *A. aegypti* was carried out according to previously described methodology (Aguiar et al., 2015). A total of 25 *A. aegypti* females (3 days after blood feeding) were transferred to a cage containing four plastic vessels (10 cm diameter), one containing 30 ml of 0.5% (v/v) DMSO solution in distilled water (control) and the others containing 30 ml of extract solution at concentrations of 33.3, 166.7 or 333.3 µg/ml. These concentrations were determined from the toxicity test. The vessels were placed at diagonally opposite corners of the cage. A piece of filter paper was placed in each vessel as a support for oviposition. The females were maintained at 27 ± 0.5°C with 70 ± 10% relative humidity for 14 h in the dark. After this period, the eggs deposited in each vessel were manually counted with the aid of a stereomicroscope. The test was repeated three times. The eggs were counted 24 and 48 h after treatment. The oviposition-inhibition percentage was calculated as described by Mulla et al. (1974).

Statistical analysis

From the mortality data, each value of LC_{50} and LC_{95} and LT_{50} and LT_{95} was estimated according to Finney's probit method, using the program Polo Plus. The repellency and emergence data were corrected using the Abbott formula (Abbott, 1925). The mean values and standards deviations were calculated from replication data. One-way analysis of variance (ANOVA) was used to determine the significance of the treatments and means were determined by Tukey's test comparisons using SISVAR 4.6 (Ferreira, 2011). Significant differences were considered when $p< 0.05$.

RESULTS

Phytochemical screening of *T. avellanedae* wood residue extracts revealed the presence of tannins and phenols (Table 1). We found no other metabolites such as alkaloids, flavonoids, steroids, anthraquinones or coumarins. After phytochemical screening performed quantitative analysis of phenolic compounds in acetone, ethanol and ethyl acetate extracts.

Total phenolic content (TPC)

The quantification of total phenols in the extracts obtained from the wood residue of *T. avellanedae* showed that phenolic compounds were present in significant quantities. For the acetone, ethanol and ethyl acetate extracts by the Soxhlet method, the values ranged from 237.272 ± 6.770 to 57.461 ± 5.863 mg

Table 2. Analysis content of phenolic compounds and tannins present in wood *T. avellanedae* by the Soxhlet method.

Extracts	Phenolic compounds (mg GAE/g)	Phenolic compounds (%)	Total tannins	Total tannins (%)	Condensed tannins (mg CE/g)	Condensed tannins (%)
Ethyl acetate	57.461±5.863	5.75	16.075±5.44	1.61	8.116±0.916	0.81
Acetone	237.272±6.770	23.73	182.69±5.65	18.27	14.196±0.397	1.42
Ethanol	98.505±5.863	9.85	75.442±2.90	7.54	10.099±0.229	1.01

Values were expressed as mean ± S.D. (n = 3); GAE = gallic acid equivalent; CE = catechin equivalent.

Table 3. Results of concentration response ($LC_{50}$and LC_{95}) values of the acetone, ethanol and ethyl acetate extracts of *T. avellanedae* against3rd instar larvae of *A. aegypti*.

Extraction process	Extracts	Slope±SEM	LC_{50} (µg mL^{-1})	CI(LC_{50}) (µg mL^{-1})	LC_{95} (µg mL^{-1})	CI(LC_{95}) (µg mL^{-1})	X^2	P
Soxhlet	Acetone	4.804±0.86	100.1	80.0–118.3	222.3	183.3–312.0	1.43^{ns}	0.0581
	Ethanol	3.13±0.45	185.0	125.3–249.3	620.6	159.9–412.3	5.06^{ns}	0.2750
	Ethyl acetate	4.537±0.70	151.0	132.0–172.7	319.3	262.7–437.3	3.71^{ns}	0.0271
Maceration	Acetone	4.32±0.60	1.499	1.33 – 1.67	3.599	2.99 – 5.33	4.17^{ns}	0.0001
	Ethanol	5.93±1.03	4.633	4.33 – 5.33	8.766	6.99 – 13.33	3.53^{ns}	0.0001
	Ethyl acetate	-	-	-	-	-	-	

SEM: standard error of the mean; LC_{50}: lethal concentration with 50% mortality; LC_{95}: lethal concentration with 95% mortality; CI: Confidence interval the probability of 95%; (1) lower limit of the confidence interval at 95% probability (LCL); (2) upper limit of the confidence interval at 95% probability (UCL); X^2: chi square; Was not significant to the chi-square test ($p < 0.05$). -: Not detected.

GAE/g (Table 2). Thus, the acetone extracts had a higher percentage of total phenolic compounds with 23.73%, followed by the ethanolic extract with 9.85% and the ethyl acetate extract with 5.75%.

Condensed tannin content (CTC)

The acetone extract gave 14.196 mg CE/g ± 0.397, followed by the ethanolic extract with 10.099 mg CE/g ± 0.229 and the ethyl acetate extract with 8.116 mg CE/g ± 0.916. The acetone extracts showed maximal amounts of extractable tannin content as compared to other solvents (Table 2). Among the extracts, acetone presented a larger amount of condensed tannins with 1.42%, followed by ethanol 1.01% and ethyl acetate with 0.81%.

Larvicidal activity

The biological activities of acetone, ethanol and ethyl acetate against the 3rd instar larvae of *A. aegypti* were measured in extracts obtained by maceration and Soxhlet methods. In Table 3 we listed the values of LC_{50} and LC_{95} for all extracts that had larvicidal activity against 3rd instar larvae of *A. aegypti* after 24 h. The extracts obtained by the maceration method showed the following LC_{50} and LC_{95} (Table 3): acetone extract values were 1.499 µg/ml (1.33-1.67) and 3.599 µg/ml (2.99-5.33), respectively; ethanolic extract values were 4.333 µg/ml (4.33-5.33) and 8.766 µg/ml (6.99 –13.33), respectively. The extracts obtained by Soxhlet extraction showed the following LC_{50} and LC_{95} (Table 3): the acetone extract had an LC_{50} value of 100.1 µg/mL (80.0-118.3) and a LC_{95} of 222.3 µg/ml (183.3-312.0). The ethyl acetate extract had promising LC_{50} and LC_{95} values of 151.0 µg/ml (132-172.7) and 319.3 µg/ml (262.7-437.3), respectively, after 24 h against *A. aegypti*.

Time-mortality response

Lethal time (LT_{50} and LT_{95}) of *A. aegypti* exposed to acetone, ethanol and ethyl acetate extracts of *T. avellanedae* obtained by the Soxhlet method were obtained using the LC_{95} concentrations determined for 3rd instar larvae of *A. aegypti* (Table 4). The times required to achieve 50 and 95% mortality of the ethanolic extract were 26.22 and 54.51 min, respectively for of *A. aegypti* 3rd instar larvae; for ethyl acetate extract the values were 38.66 and 66.74 min, respectively; and for acetone extracts the values were 53.47 and 119.96 min, respectively (Table 4).

Residual activity of extracts

Measurement of residual activity of extracts of *T.*

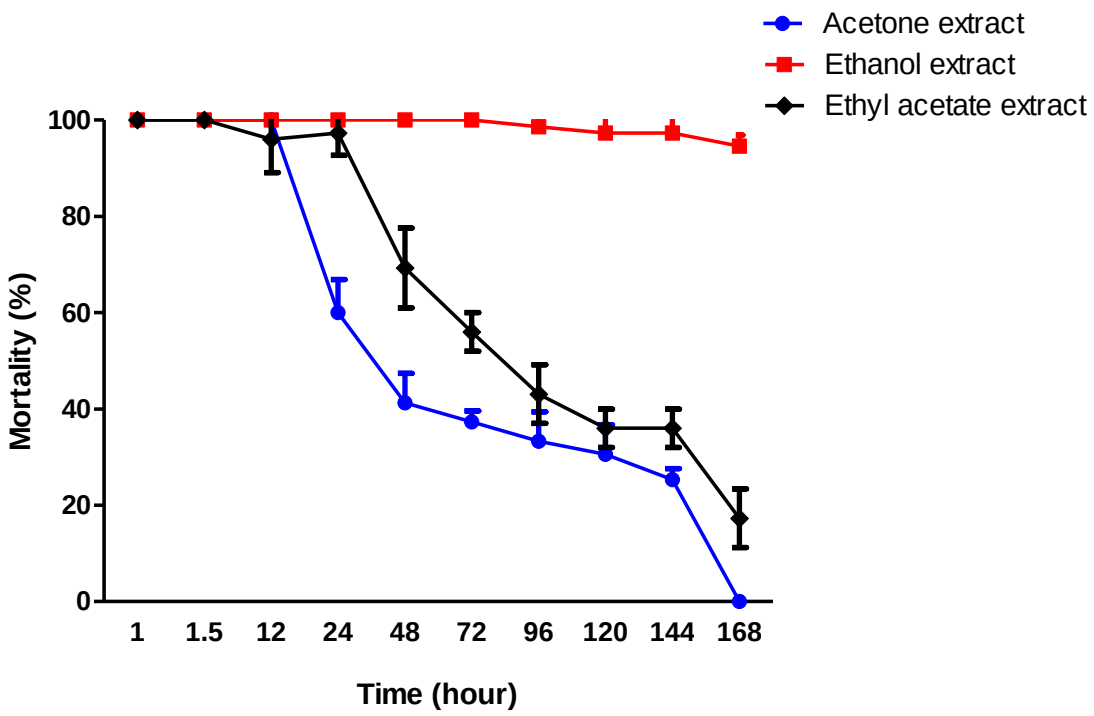

Figure 1. Residual activity of the acetone, ethanol and ethyl acetate extracts from *T. avellanedae* wood residueagainst 3rd instar larvae of *A. aegypti*. Values are mean of three replications ± SE. Treatments followed by the same capital letter in the column did not differ among themselves by the Tukey test (p <0.05).

Table 4. LT_{50} and LT_{95} values of extracts of *T. avellanedae* against 3rd instar larvae of *A. aegypti* using the LC_{95}.

Extracts	Slope±SEM	LT_{50} (min)	CI (LT_{50})	LT_{95} (min)	CI (LT_{95})	X^2	P
Acetone	8.38±1.88	53.47	45.87 – 59.02	119.96	95.54 – 209.92	0.25^{ns}	0.0050
Ethanol	9.26±1.96	26.22	23.98 – 29.13	54.51	42.98 – 95.37	0.20^{ns}	0.3953
Ethyl acetate	12.41±2.79	38.66	3.59 – 41.37	66.74	57.44 – 96.0	0.14^{ns}	0.1230

SEM: standard error of the mean; LT50: lethal time of lethal concentration with 50% mortality; LT95: lethal time of lethal concentration with 90% mortality; CI: Confidence interval the probability of 95%; (1) lower limit of the confidence interval at 95% probability; (2) upper limit of the confidence interval at 95% probability; X^2: chi square; Was not significant to the chi-square test (p<0.05).

avellanedae obtained by the Soxhlet method showed that mortality increased with increasing exposure time up to 12 h forall extracts tested (Figure 1). The ethanol extract at LC_{95} of 412.3 µg/ml induced rapid mortality for 3rd instar larvae with residual activity of 100% mortality for a period of 96 h of exposure. The ethyl acetate extract (LC_{95} = 319 µg/ml) residual activity promoted 100% mortality for a period of 24 h. The ethanol solution induced 98.6 ± 2.3% larval mortality rate in the first 120 h. After 168 h, the ethanolic extract was still killing at 82.6% (Figure 1). During the seven-day period, the ethyl acetate extract showed a mortality rate of 64.8 ± 37.1% (Figure 1).

Oviposition deterrent

The oviposition deterrence activity of extracts of *T. avellanedae* obtained by the Soxhlet method against *A. aegypti* is presented in Figure 2. The ethyl acetate extract (EAE) (at 33.3, 166.7 and 333.3 µg/ml) strongly deterred oviposition by *A. aegypti*, with a significantly lower proportion of eggs being laid on ovitraps containing extracts in comparison with controls ($p < 0.01$) (Figure 2). The percentage of effectiveness demonstrated by acetone, ethyl acetate and ethanolic extracts against oviposition were 68.10, 89.04 and 89.89% at 333.3 µg/ml, respectively. The acetone extract (AE) and ethanol extract (EE) were not statistically significant at all concentrations tested for oviposition deterrent activity in comparison with controls.

DISCUSSION

Maceration and Soxhlet extraction were used for wood

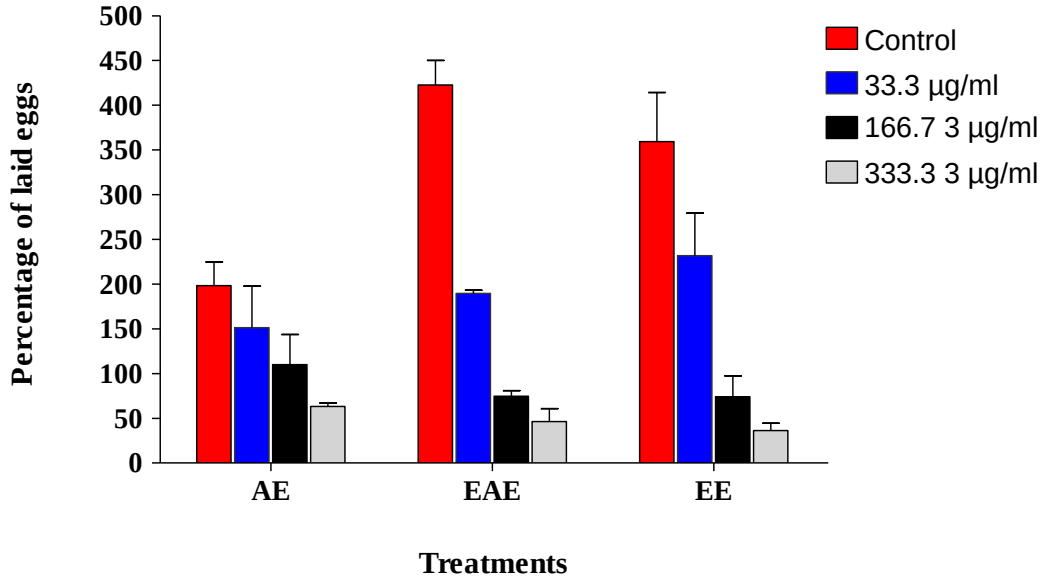

Figure 2. Effect of various concentrations of acetone, ethyl acetate and ethanolic extracts from *T. avellanedae* wood residue on oviposition by adult *A. aegypti* females.

residues of *T. avellanedae*. Solvent extraction is generally used for the preparation of plant material extracts because of its wide applicability, efficiency and ease of use. Most common organic solvents used for the extraction of phenolic compounds include methanol, ethanol, acetone, and ethyl acetate (Ross et al., 2009). Conventional solid liquid extraction techniques such as maceration are mostly used for obtaining bioactive compound extracts from plant material (Belova et al., 2009). However, conventional solvent extraction processes have certain limitations such as lower efficiency, low extraction yield, use of large quantity of solvents and mass transfer resistance (Jadhav et al., 2009).

Conventional Soxhlet extraction has some attractive advantages. The sample is repeatedly brought into contact with fresh portions of extractant, facilitating displacement of the transfer equilibrium. In addition, the system remains at a relatively high temperature by effect of the heat applied to the distillation flask reaching the extraction cavity to some extent. Finally, no filtration is required after leaching and sample throughput can be increased by performing several simultaneous extractions in parallel, facilitated by the low cost of the basic equipment (Castro and Priego-Capote, 2010).

The phytochemical screening of *T. avellanedae* compounds present in our acetone, ethyl acetate and ethanol extracts showed the presence of phytochemicals as well as total phenols and tannin compounds. In another species of the plant (*Tabebuia serratifolia*), Duarte et al. (2015) reported the presence of reducing sugars, organic acids, alkaloids, anthraquinones, depsides and depsidones, catechins, purines, foamy saponins and phenols and tannins in the ethanolic extract of the leaves. Reports in the literature demonstrated that plants of the Bignoniaceae family have a variety of classes of chemical constituents including quinolines, lignans, flavonoids, monoterpenes (mainly iridoids), triterpenes, cinnamic and benzoic acids (Von Poser et al., 2000).

In the present study, the values of total phenolic compounds were 237.27, 98.50 and 57.46 mg GAE/g in acetone, ethanol and ethyl acetate extracts, respectively (Table 3). Similar results were found by Salar and Seasotiya (2011), who investigated the total phenolic content of various extracts of *Butea monosperma* and *Hugoniamystax* and found that total phenolics ranged from 45-141 mg GAE/g in stem bark 262.2 ± 0.96 mg GAE/g in roots, respectively.

In this regard, the amount of phenols found in the wood residues of *T. avellanedae* in this study was higher. According to Romagnoli et al. (2013) this total amount of quinones and phenols was higher. The differences in the amounts of phenolic content may be associated with the agro-climatic conditions that were solely responsible for the occurrence of variable amounts of bioactive components in natural resources (Dhull et al., 2016).

Zarin et al. (2016) reported that of the total phenolic content of crude extracts from *Leucaena leucocephala* was 3.21 mg GAE/g extract, while purified condensed tannins were obtained at 2.06 mg GAE/g extract. Dhull et al. (2016) identified the presence of condensed tannin content in leaf extracts. Three solvents (ethanol, acetone and chloroform) were used to extract condensed tannin content from *Origanum majorana*. The authors found that the extract prepared with acetone gave the maximal

amount of extractable tannin content as compared to other solvents (6.02 mg CE/g). By contrast, in the present study, the maximum amount of extractable CTC was 14.196 mg CE/g (1.42%) in the acetone extract.

The results may differ depending on the type of plant, plant part, extraction temperature, extraction time and extraction phase used for recovery of bioactive compounds as well as the type of solvent used for extraction (Azwanida, 2015). This assertion can be confirmed through our studies with the various extracts (acetone, ethyl acetate and ethanol). Earlier studies reported that saponins, flavonoids and tannins were active insecticidal compounds. Generally, flavonoids and tannins are toxic phenolics that disrupt cellular structure (Julia et al., 2018).

The results we obtained for *T. avellanedae* revealed a chemical composition similar to or different from other studies. Romagnoli et al. (2013) reported an amount of lapachol in the wood extract of *T. serratifolia* of 27% (33.58 mg/g). Some authors reported the presence of lapachol, lapachol, α- and β-lapachone, in addition to furanonaphthoquinones isolated from several species of the genera *Tabebuia*, *Tecoma* and *Handroanthus* (Burnett and Thomson, 1968; Oliveira et al., 1990; Hussain et al., 2007). According to Oliveira et al. (1990), the species *Tabebuia incana*, family Bignoniaceae, there was another chemical constituent, 2-ethyl-5-hydroxy-naphtho [2,3-b] furan-4,9-quinone.

In another species from the same family, *Tabebuia ochracea*, lapachol (0.001%) and seven furanonaphthoquinones including 2- (1'-hydroxyethyl) naphtho [2,3-b] furan-4,9- dione were found in trunks. In the study with *T. serratifolia* using chromatographic fractionation of the ethanolic extracts of the wood, several substances were isolated, includingdehydro-α-lapachone, β-sitosterol, β-sitosterol glycoside, 4-hydroxy-3-methoxy-benzoic acid and lapachol. Our results for lapachol were higher than those of the hexanic and chloroform extracts (33.42 and 31.6%, respectively) in the cited studies.

Sensitivity of mosquitoes to various insecticides are highly variable. Jeon et al. (2011) carried out an insecticidal effect study using the topical active constituent of the *T. avellanedae* bark against *Nilaparvata lugens* and the small brown grasshopper, *Laodelphax striatellus*. In that study, the authors concluded that the constituents 2-hydroxy-3- (3-methyl-2-butenyl) -1,4-naphthoquinone and its derivatives had potential as new agents and that insecticidal activities of *T. avellanedae* methanolic extract against *N. lugens* and *L. striatellus* showed a significant dose-response relationship for toxicity in both insect species.

The extracts obtained by the maceration method showed that the LC_{50} and $LC_{95}$of acetone extract values were 1.49 and 3.59 µg/ml, respectively and for ethanolic extracts the values were 4.33 and 8.76 µg/ml, respectively. All the extracts obtained in the cold gave lower values forLC_{50} and LC_{95} than for extracts obtained by the Soxhlet method. According to Komalamisra et al. (2005), larvicidal activity can classified as follows: an extract is considered efficient when LC_{50} is less than 750 ppm; activity is moderate when LC_{50} is between 50 and 100 ppm and it is high when LC_{50} is less than 50 ppm. Thus, according to our results, the acetone extract obtained from wood residue is considered to have moderate activity, and those obtained from ethanol, methanol and ethyl acetate extracts were efficient. The acetone and ethanol extracts obtained by maceration can be classified as having high larvicidal activity.

Indeed, stirring in the maceration method accelerated the extraction process, minimizing the contact time with the extracting solvent and preserving the bioactivity of the constituents. In addition, extraction at room temperature and the removal of the solvent at low pressure yielded the maximum amounts of compounds (Khoddami et al., 2013). In this context, various plant extracts of *Coleus aromaticus* leaf extract, lichen *Ramalina usnea* (L.) (Ramalinaceae) were tested as potential mosquito larvicides (Baranitharan et al., 2017; Moreira et al., 2016). The larvicidal efficacies of the tested extracts were dose-dependent. The biological activity of plant extracts is generally known to be due to the presence of various bioactive phytomolecules present in the plant, including alkaloids, terpenoids and phenolics (Vindhya et al., 2014; Francine et al., 2016).

This can be compared to our study in that the larvicidal efficacies of our tested extracts were also dose-dependent. The results from our toxicity assays demonstrated that the extracts of *T. avellanedae* had strong toxicity for *A. aegypti* larvae and depended on exposure time and concentrations used. Nevertheless, the differences in toxicity expressed by the various plant species and extracts of the same plant may be due to quantitative and qualitative variations in the chemical composition of the ethanolic extracts of *T. avellanedae* that induced full mortality (98.6%) of *A. aegypti* larvae within 96 h of exposure with a CL_{95} of 706 µg/ml.

We evaluated the larvicidal effects of *T. avellanedae* against *A. aegypti* at various concentrations for each extract obtained. The ethyl acetate extract exhibited high larvicidal activity (69.32%) at LC_{95} (319.3 µg/ml) for 48 h post-exposure. The use of quinolines in the treatment of bacterial pathogens, as well as in the defense against pathogens (allopathic and antimicrobial activity), have been shown to play important roles in several living organisms as cofactors of proteins in electron transport (Burnett and Thomson, 1968; McKallip et al., 2010) reviewed the efficacy of quinones that could be attributed to the strongly electrophilic character of the naphthoquinones that promoted their reactivity with thiol groups on proteins. Studies such as that of Pradeep et al. (2015) suggested that isoquinoline alkaloids were natural potential mosquito larvicides. Hussain et al. (20017) reported that lapachol and its enamine showed larvicidal and insecticidal activity against *Artemia salina* and *A.*

aegypti. In this way, we can suggest that the larvicidal activity measured in our extracts was related to lapachol.

The rapid and significant mortality ($p<0.05$) suggested potent insecticidal activity of wood extracts (acetone, ethyl acetate and ethanol) against larvae of *A. aegypti.* Similar time-response results were obtained from essential oils and extracts from other plant species. The essential oil of *S. guyanensis* at LC_{95} concentrations against *A. aegypti* and *Culex quinquefasciatus* required less than 33 min to kill 95% of insects (Aguiar et al., 2015; Soonwera and Phasomkusolsi, 2017). The response time was much lower than that of aqueous extracts of the seed kernel of *Azadirachta indica* A. Juss (Neem) tested on *A. aegypti* larvae that delivered 100% (LC_{100}) mortality at all concentrations used in the present study at 144 h (LT_{100}) (Ndione et al., 2007).

Species of the genus *Tabebuia* have been used empirically as anti-inflammatory agents, anti-cancer agents and antimicrobials in rural areas of the Colombia, Bolivia, Brazil and other countries in Latin America. Additionally, the constituents of several *Tabebuia* spp. have been shown to be potent insecticides (Jeon et al., 2012).

Extracts of *T. avellanedae* proved to be oviposition-deterrents against *A. aegypti*. The ethanol extract reduced oviposition of *A. aegypti* significantly ($p<0.01$). Previously, some investigators reported an oviposition deterrent effect of plant extracts against vector mosquitoes. Coria et al. (2008) reported a 100% oviposition deterrent effect obtained with *Melia azedarach* L. leaf ethanol extract at 1 g/L concentration against *A. aegypti*.

Autran et al. (2009) reported anoviposition deterrent effect of essential oils obtained from leaves, inflorescences, and stems of *Piper marginatum*. Leaves and stems of *P. marginatum* exhibited an oviposition deterrent effect at 50 and 100 ppm in that significantly lower numbers of eggs (<50%) were laid in glass vessels containing test solutions compared with those containing control solution.

The choice of oviposition sites by gravid female mosquitoes is guided by several factors. Initially, visual and olfactory cues are employed to find potential sites, after which the suitability of the location is verified by chemical and physical factors according to appropriate receptors distributed along the body of the mosquito. Clearly, when oviposition deterrents are detected, few, if any eggs are laid at the site (Day, 2016).

The oviposition repellent effect is a relevant property to be considered in the selection of a plant extracts for the control of vector insects. In general, the higher the repellency, the lower the infestation, resulting in reduction or suppression of oviposition, a reduced number of insects emerging and a decrease in cases of dengue.

Further studies on the larvicidal mode of action of *T. avellanedae* wood residue, their effects on non-target organisms and the environment, and formulations for improving the insecticidal potency and stability are needed to support their practical use as naturally occurring mosquito larval control agents. Nevertheless, wood residues of *T. avellanedae* can be an alternative source of mosquito control agents because they are a rich source of bioactive chemicals.

Conclusion

The present study clearly demonstrated that acetone, ethyl acetate and ethanol extracts from *T. avellanedae* had substantial larvicidal and oviposition repellency properties against *A. aegypti*. The presence of bioactive compounds in *T. avellanedae* makes it a potential source for the control of *A. aegypti* vectors, without promoting deforestation of trees.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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