SCIENTIFIC ARTICLE

Ovicidal, pupicidal, adulticidal, and repellent activity of *Helicteres velutina* K. Schum against *Aedes aegypti* L. (Diptera: Culicidae)

Atividade ovicida, pupicida, adulticida, e repelente de *Helicteres velutina* K. Schum contra *Aedes aegypti* L. (Diptera: Culicidae)

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Abstract

Aedes aegypti is a vector of emerging and neglected diseases, such as dengue, chikungunya, and Zika. *Helicteres velutina,* known as "pitó" in Brazil, is traditionally used as an insect repellent, and several studies have demonstrated its larvicidal activity. The aim of this study was to investigate this species and evaluate its potential ovicidal, pupicidal, adulticidal, and repellent activity. The viability of the eggs was evaluated using different concentrations of the test substances for 25 days. The hexane fraction killed 72.7% of the eggs, while dichloromethane killed 67.7%. The survival of the pupae and adults was verified after 72 h and 48 h, respectively. The LC_{50} for the hexane and dichloromethane fractions was 0.12 mg/mL and 8.85 mg/mL for pupae, 8.01 mg/mL and 0.74 mg/mL for adults (tarsal test), and 0.05 mg/mL and 0.23 mg/mL for adults (body test), respectively. Repellency was assessed for 240 min using neonatal Wistar rats on a Y-tube olfactometer. The hexane fraction attracted mosquitoes to the test chamber, while the dichloromethane fraction had a repellent action. The 7,4'-di-*O*-methyl-8-*O*-sulfate flavone provides greater repellency, and this finding is similar to the results of the *in silico* studies that have shown the potential of this substance against adult mosquitoes. This suggests that 7,4'-di-*O*-methyl-8-*O*-sulfate flavone may be one of the substances present in the extract from aerial parts of *H. velutina* that is responsible for the repellent activity mentioned in traditional medicine. These findings provide a better understanding of the insecticidal and repellent activity of the extract, fraction, and compounds isolated from *H. velutina* against *Ae. aegypti,* thereby revealing its potential in the development of a more effective botanical insecticide.

Keywords: *Helicteres velutina,* inseticidal activity, repellent activity, *Aedes aegypti,* neglected diseases.

Resumo

Aedes aegypti é o vetor de doenças emergentes e negligenciadas, como dengue, chikungunya e Zika. *Helicteres velutina*, conhecida como 'pitó' no Brasil, é tradicionalmente usada como um repelente de insetos, estudos anteriores comprovaram sua atividade larvicida. O objetivo desta pesquisa foi investigar esta espécie, avaliando seu potencial ovicida, pupicida, adulticida e repelente. A viabilidade dos ovos foi avaliada utilizando diferentes concentrações das substâncias teste durante 25 dias, a fração hexano causou a inviabilização de 72,7% dos ovos, enquanto a diclorometano matou 67,7%. A sobrevivência de pupas e adultos foi verificada após 72 e 48 horas, respectivamente. A CL_{50} da fração hexano e diclorometano foi de 0,12 e 8,85 mg/mL para pupas; 8,0 e 0,74 mg/mL para adultos (teste tarsal); 0,05 e 0,23 mg/mL para adultos (teste corporal), respectivamente. A repelência foi avaliada durante 240 min utilizando neonatos de ratos Wistar em um olfatômetro de tubo Y. No teste de atração-repelência, a fração hexano atraiu mosquitos para camara teste, enquanto a fração diclorometano causou repelência. A 7,4'-di-*O*-metil-8-*O*-sulfato flavona proporcionol maior repelência, corroborando com estudos *in silico* que mostram potencial dessa substância em mosquitos adultos. Sugerindo que 7,4'-di-*O*-metil-8-*O*-sulfato flavona pode ser uma das substâncias presente no extrato das partes aéreas de *H. velutina*, responsável pela atividade repelente citada na medicina tradicional.

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Esses achados proporcionam uma melhor compreensão da atividade inseticida e repelente do extrato, fração e compostos isolados de *H. velutina* frente a *Ae. aegypti* mostrando potencial no desenvolvimento de um inseticida botânico mais eficaz.

Palavras-chave: *Helicteres velutina,* atividade inseticida, atividade repelente, *Aedes aegypti,* doenças negligenciadas.

Introduction

Emerging infections, including dengue, Zika, and chikungunya, caused by arboviruses that are transmitted by *Aedes aegypti* L. are of great concern to the World Health Organization (Leta et al., 2018; Rojas-Pinzón et al., 2018). Globally, 2.5 billion people live in high-risk areas, especially in the tropical and subtropical regions of the world, where temperature and humidity promote the proliferation of such vectors (Kraemer et al., 2015; Reegan et al., 2014).

Mosquito control is an effective alternative for preventing the spread of these diseases, and management programs focus as much on the control of the immature vectors (egg, larvae, and pupae) as on adult mosquitoes (Chellappandian et al., 2018; Wang et al., 2018).

Synthetic insecticides and repellents continue to be the first line of defense owing to their fast action and easy application (Murugan et al., 2012). However, prolonged use of these compounds leads to ecological imbalance and consequent harmful effects on non-target organisms in addition to the development of resistance. Therefore, the search for natural alternatives that are safe, selective, economically viable, and biodegradable is important (Ravindran et al., 2012; Vivekanandhan et al., 2018).

Several studies have shown that essential oils, extracts, fractions, and substances isolated from plants affect mosquitoes (Castillo et al., 2017; Kovendan et al., 2013). The species *H. velutina* K. Schum, family Sterculiaceae, popularly known as "pitó" and It is endemic in Brazil, and the indigenous Pankararé tribe in Bahia uses it as a repellent. A few studies have shown that this species has repellent activity against *Aedes aegypti* larvae (Santos et al., 2012; Fernandes et al., 2018; Fernandes et al., 2019; Fernandes et al., 2020a; Fernandes et al., 2020b).

Therefore, the aim of this study was to investigate the ovicidal, pupicidal, adulticidal, and repellent activity of *H. velutina* K. Schum against *Ae. aegypti*.

Materials and methods

All procedures were approved by the Universidade Federal da Paraíba Animal Care and Use Committee (protocol 095/2016).

Plant material and test substances

The aerial parts of *H. velutina* were collected from Serra Branca/Raso da Catarina (Jeremoabo City, Bahia, 09°53'15.5", 09°44'34.6" S and 38°49'36.1", 38°52'20.4" W). The material was identified by Professor Adilva de Souza Conceição, and a voucher specimen was stored in the Herbarium of the Universidade Estadual da Bahia (HUNEB, Paulo Afonso Collection).

The plant material was oven-dried at 40 °C, and the powder was macerated with 95% ethanol for 72 h. The extract solution was dried at 40 °C under reduced pressure, and the crude ethanolic extract (CEE) was subjected to liquid-liquid chromatography using hexane, dichloromethane, ethyl acetate, and n-butanol, which resulted in their respective fractions and a hydroalcoholic fraction (Fernandes et al., 2018).

The hexane and dichloromethane fractions as well as two flavonoids isolated from the dichloromethane fraction: (1) 7,4'-di-*O*-methyl-8-*O*-sulfated flavone (sulfated flavonoid) and (2) Tiliroside (glycosidic flavonoid) (Figure 1) (Fernandes et al., 2019; Fernandes et al., 2020b) showed promising larvicidal activity against *Ae. aegypti*.

These fractions were used to evaluate the potential ovicidal, pupicidal, and adulticidal activity against *Ae. aegypti*. The CEE, the fractions, and the flavonoids acting against the larvae (*in vivo*) were used to investigate the repellent activity (Fernandes et al., 2019; Santos et al., 2012). In addition, an *in silico* study have shown that these substances have the

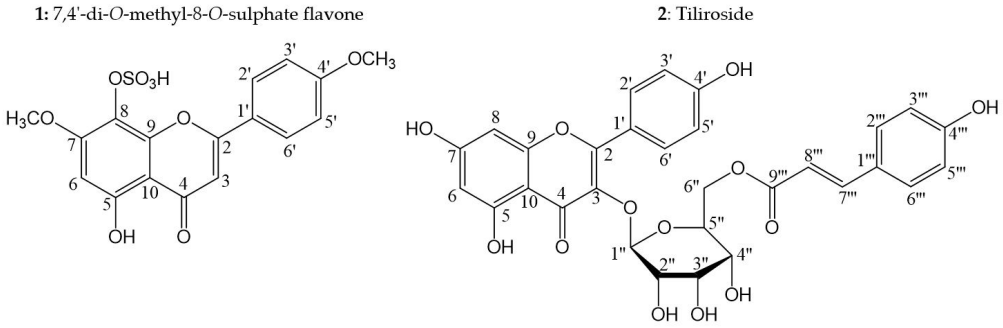

Figure 1. Compounds isolated from aerial parts of *Helicteres velutina* with potential activity against mosquitoes adult of *Aedes aegypti*.

ability to bind to proteins present in the adult mosquito (1YIY and 3UQI) (Fernandes et al., 2019), which has triggered the interest of researchers with regard to the investigation of their repellent activity.

The hexane and dichloromethane fractions were solubilized with distilled water and 1% DMSO (dimethyl sulfoxide). The CEE and isolated flavonoids were solubilized in distilled water.

This study has been registered in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen - A568B8A).

Mosquito maintenance

Aedes aegypti of the Rockefeller João Pessoa strain were obtained from the Laboratory of Biotechnology Applied to Parasites and Vectors in the Biotechnology Center at the Universidade Federal daParaíba. The insects were maintained in a biological oxygen demand (BOD) incubator, under controlled conditions of temperature 27 ± 2 °C, relative air humidity 75% ± 5%, and 12-hour light and dark photoperiod (Imam et al., 2014; Nunes et al., 2015; World Health Organization, 2013).

Ovicidal activity

The ovicidal activity was evaluated according to the methodology described by Reegan et al. (2015). Filter papers containing 30 freshly collected *Ae. aegypti* eggs were exposed to different concentrations of hexane and dichloromethane fractions (1.0–10.0. mg/mL). The eggs were then kept in a BOD incubator, and the hatchability was checked every five days until the 25th day of the experiment. The eggs exposed to water and 1% DMSO were used as the negative control (Silva et al., 2014), while a commercial insecticide (0.02% Imiprotin, 0.05% Permetrin, and 0.1% Esbiotrin) was used as the positive control. All assays were performed in triplicates (Nunes et al., 2015).

Pupicidal activity

Pupicidal activity was assessed according to the protocol provided by the WHO (World Health Organization, 2005). Twenty pupae of *Ae. aegypti,* with a maximum of 24 h of life, were placed in a plastic container containing 10 mL of the hexane and dichloromethane fractions at different concentrations (0.1–20.0 mg/mL). Pupae mortality or mosquito emergence was verified after 24, 48, and 72 h of exposure. The experiments were performed in a BOD incubator as described above. The negative and positive controls were maintained, and the number of replicates was assessed, as described previously for the ovicidal test.

Adulticidal activity

Two different protocols were used to evaluate the effect of the hexane and dichloromethane fractions on adult mosquitoes, as described by Nunes et al. (2019a).

In the tarsal test, the walls of the plastic containers were moistened with the test solutions and allowed to dry. Twenty *Ae. aegypti* mosquitoes (5–6 days of emergence) were placed in these containers,

and the mortality was checked after 24 and 48 h. This methodology reproduces the method of indirect application of insecticides on surfaces and allows the evaluation of their residual effect.

For the body test, a topical application of the test substances was carried out directly on the body of the mosquitoes. Twenty mosquitoes were anesthetized by exposure to cold (-4 °C for approximately 2 min) and transferred to a plastic container. Each mosquito received 10 μL of the test substances on the dorsal part of the body using an automatic pipette. Mortality was checked after 24 and 48 h. The negative and positive controls were maintained as described previously for the ovicidal test.

Attraction-inhibition assay

Repellency of the extract, fractions, and isolated flavonoids against adult female *Ae. aegypti* was evaluated using a Y-tube olfactometer, constructed according to the protocol provided by the WHO (World Health Organization, 2013). A chamber (12 × 8 × 8 cm) containing a neonate Wistar rat was placed in each arm of the Y-tube. In the test arm, 100 μL of the test substance (extract, fractions, and isolated substances) was pipetted directly onto the skin of the animal's dorsal region. Distilled water (100 mL) and 1% DMSO were used as the negative control, while 100 μL of the OFF® commercial repellent, active ingredient: 15% DEET (diethyltoluamide) was used as the positive control (Nunes et al., 2019a).

Twenty female mosquitoes, after 24 h of fasting, were placed in the Y-tube olfactometer and observed at an interval of 2 min to record the arm of choice (Nunes et al., 2019b). The duration of the repellent activity was evaluated for 4 h at intervals of 0, 30, 120, and 240 min after application (Rodriguez et al., 2015). The tests were performed in triplicates.

The following formula was used to assess the spatial activity index (SAI):

$$SAI = \left[\frac{(Nc - Nt)}{(Nc + Nt)}\right] \times \left(\frac{Nm}{N}\right) \quad (1)$$

where *Nc* is the number of mosquitoes in the control chamber, *Nt* is the number of mosquitoes in the treatment chamber, *Nm* is the total number of mosquitoes in the two chambers, and *N* is the total number of mosquitoes in the test unit (20 mosquitoes).

The SAI varies from -1 to 1, where zero indicates no response; -1 indicates that all mosquitoes moved into the treatment chamber, resulting in an attractant response; and 1 indicates that all the mosquitoes moved into the control chamber (away from the treatment source), resulting in a spatial repellent response (World Health Organization, 2013).

To calculate the percentage of landing inhibition, the number of mosquitoes that landed on each neonate during the intervals was recorded according to the formula given below:

$$\%landing\ inhibition = 100 \times \left[\left(\frac{Cl - Tl}{Cl}\right)\right] \quad (2)$$

where *Cl* is the number of mosquitoes landing in the control space and *Tl* is the number of mosquitoes landing in the treatment space.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). For the ovicidal, pupicidal, adulticidal, and landing inhibition assays, the significant differences between the groups were analyzed using ANOVA and post-Tukey test ($P < 0.05$). For the spatial activity assay, the significant differences between the groups were analyzed using ANOVA and Bonferroni post-hoc test ($P < 0.05$). The LC_{50} and LC_{90} were calculated using non-linear regression, considering a 95% significance level.

Results

Different concentrations of the *H. velutina* fractions were tested against *Ae. aegypti*. Both the hexane and dichloromethane fractions of *H. velutina* were able to inhibit 100% egg

hatching at a concentration of 5 mg/mL (Figure 2). Even at the lowest tested concentration (0.1 mg/mL), these substances were able to inhibit egg hatching (67.7% inhibition by hexane and 31.1% by dichloromethane) during the experimental period of 25 days. Despite the similarity of both fractions, the hexane fraction showed higher inhibition at concentrations of 0.1–1.0. mg/mL (Figure 2).

The pupicidal activity of the hexane fraction was significantly higher than that of the dichloromethane fraction. The hexane fraction killed 85% and 100% of pupae at concentrations of 0.15 mg/mL and 0.20 mg/mL, respectively, after 24 h of exposure, while the dichloromethane fraction exhibited lethal activity only at a concentration of 10.0 mg/mL after 72 h of treatment (78% mortality) (Figure 3). The LC_{50} and LC_{90} of the hexane fraction were 0.12 mg/mL and 0.14 mg/mL, respectively, while those for the dichloromethane fraction were 8.85 mg/mL and 26.93 mg/mL, respectively (Figure 4) (Table 1).

The adulticidal activity was evaluated, and we found that both fractions possessed adulticidal activity. The tarsal test shows the residual effect of the test substance after its evaporation from the container's wall. In this test, the adulticidal activity of the dichloromethane fraction was higher than the hexane fraction (LC_{50} of 0.74 mg/mL vs. 8.01 mg/mL and LC_{90} of 43.89

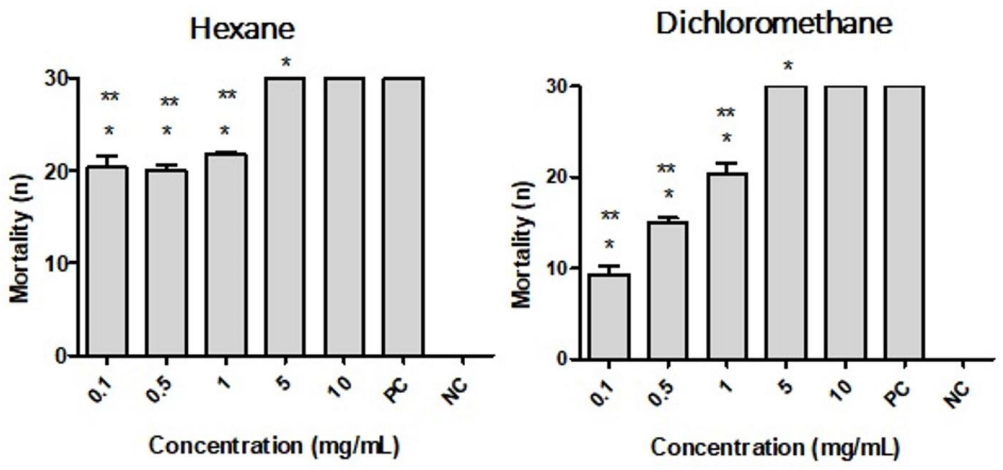

Figure 2. Ovicidal activity of different concentrations of fractions of *Helicteres velutina* on *Aedes aegypti* eggs after 25 days. PC = Positive Control, NC = Negative Control. (*) Results significantly different from negative control. (**) Results significantly different from positive control.

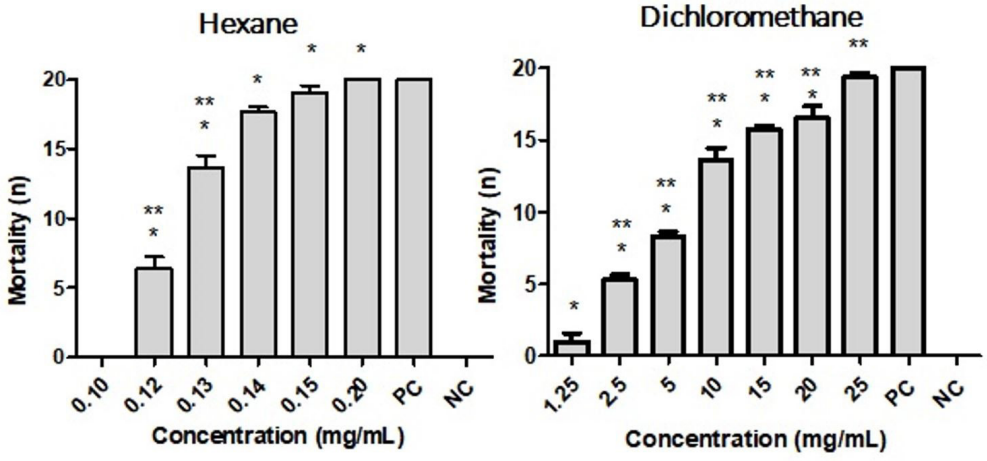

Figure 3. Pupicidal activity of different concentrations of fractions of *Helicteres velutina* on *Aedes aegypti* pupae after 72 hours. PC = Positive Control, NC = Negative Control (*) Results significantly different from negative control. (**) Results significantly different from positive control

mg/mL vs. 36.14 mg/mL, respectively) (Figure 5) (Table 1). This difference was even greater at a concentration of 1.0 mg/mL, where the dichloromethane fraction killed 58.3% of the mosquitoes, while the hexane fraction killed 8.33% after 48 h (Figure 6). It is important to note that both fractions modified the insect behavior. Initially, they became lethargic, with abnormal flight, followed by death, and these changes became more evident as the concentration of the test substances increased.

In the body test, the hexane fraction presented better activity than the dichloromethane fraction (LC_{50} of 0.05 vs. 0.23 mg/mL and LC_{90} of 1.12 vs. 2.10 mg/mL) (Figure 7 and Figure 8) (Table 1). Similar to our observation in the tarsal test, the mosquitoes became lethargic, paralyzed, and then died.

To evaluate the repellent activity, we calculated the SAI and the percentage inhibition of landing. In the spatial repellency analysis, 7,4'-di-*O*-methyl-8-*O*-sulfate flavone (0.182 mg/mL) showed the best repellent activity at the beginning of the assay (time 0 min). For the hexane fraction (3.88 mg/mL), the best repellent activity occurred in 30 min. For the CEE (2.98 mg/mL),

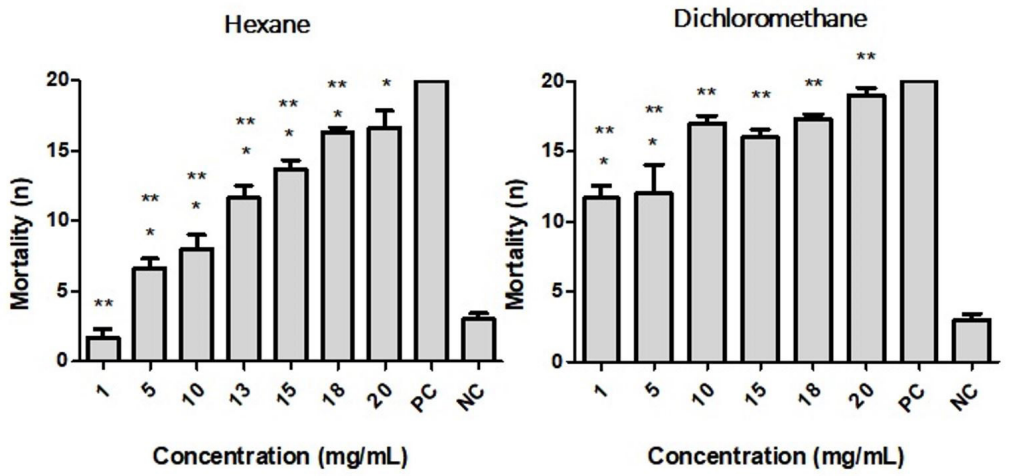

Figure 4. Dose-response curve of mortality (n) of *Aedes aegypti* pupae versus concentrations (mg/mL) of fractions of *Helicteres velutina* after 72 hours. The LC 50 and LC90 were calculated using non-linear regression, considering a 95% significance level. (*) Results significantly different from negative control. (**) Results significantly different from positive control.

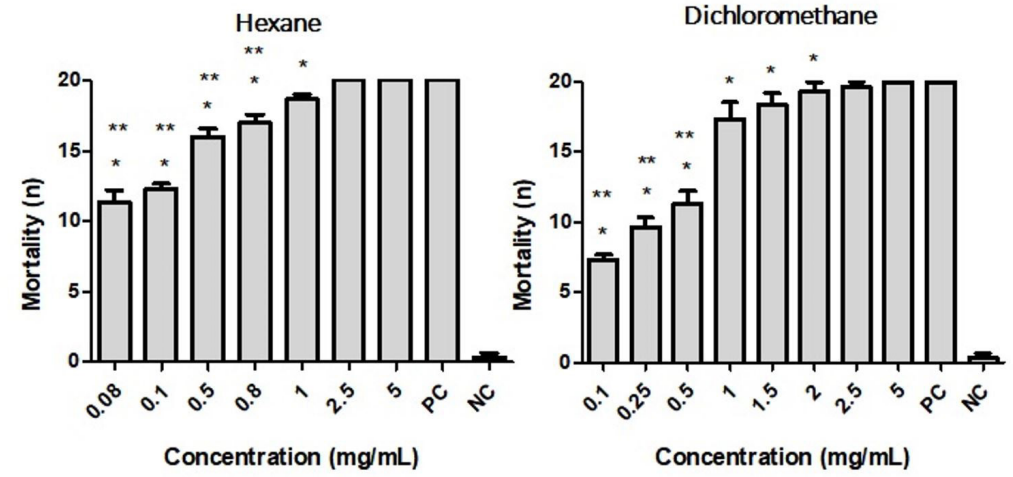

Figure 5. Adulticidal activity - tarsal contact of different concentrations of fractions of *Helicteres velutina* on *Aedes. aegypti* mosquito after 48 hours. PC = Positive Control, NC = Negative Control (*) Results significantly different from negative control. (**) Results significantly different from positive control.

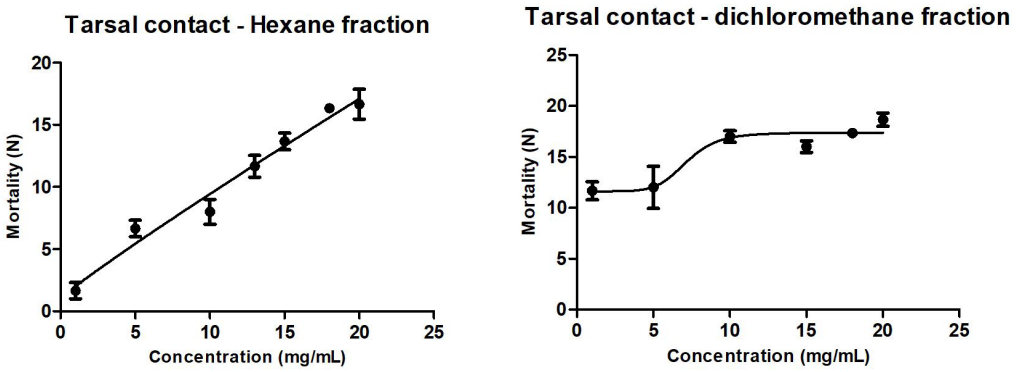

Figure 6. Dose-response curve of mortality (n) of *Aedes aegypti* adult (tarsal contact) versus concentrations (mg/mL) of fractions of *Helicteres velutina* after 48 hours. The LC 50 and LC90 were calculated using non-linear regression, considering a 95% significance level.

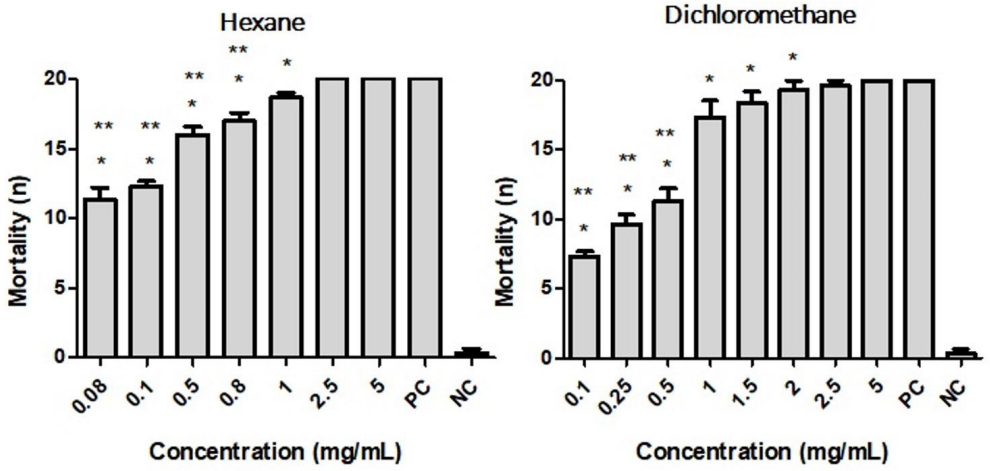

Figure 7. Adulticidal activity - body contact of different concentrations of fractions of *Helicteres velutina* on *Aedes aegypti* mosquito after 48 hours. PC = Positive Control, NC = Negative Control (*) Results significantly different from negative control. (**) Results significantly different from positive control.

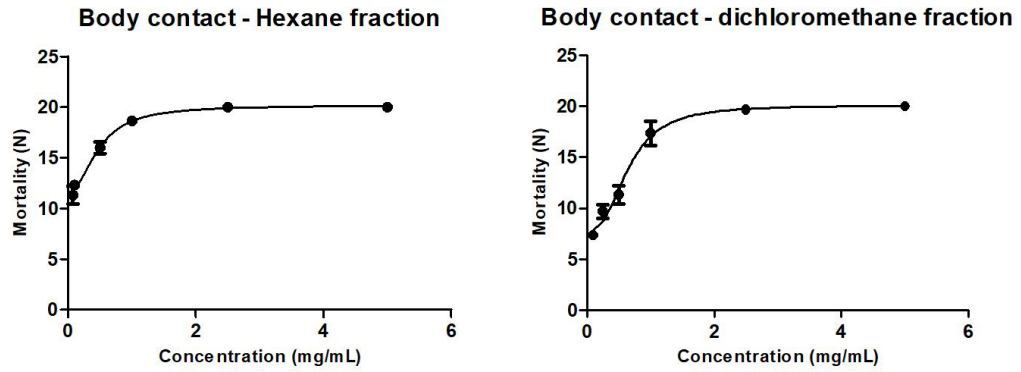

Figure 8. Dose-response curve of mortality (n) of *Aedes aegypti* adult (body contact) versus concentrations (mg/mL) of fractions of *Helicteres velutina* after 48 hours. The LC 50 and LC90 were calculated using non-linear regression, considering a 95% significance level.

dichloromethane fraction (5.80 mg/mL), and tiliroside (0.275 mg/mL), the best repellent activity occurred in 120 min (Figure 9). The dichloromethane fraction had higher spatial repellency of 0.27 after 240 min compared to that of the positive control. An increase the concentrations of the substances resulted in the highest SAI at 0 min for CEE (10.0 mg/mL), at 30 min for tiliroside (1.0 mg/mL), at 120 min for the hexane fraction (5.0 mg/mL), and at 240 min for both dichloromethane (10.0 mg/mL) and 7,4'-di-*O*-methyl-8-*O*-sulfate flavone (1.0 mg/mL) (Figure 9).

The repellency of the test substances was evaluated by analyzing the landings of the mosquitoes in neonates. At the initial concentrations, landing inhibition of the dichloromethane fraction fluctuated over time, with greater activity at 120 min. The hexane fraction showed a gradual decrease over time, while the extract exhibited higher inhibition at 0 and 120 min. The 7,4'-di-*O*-methyl-8-*O*-sulfate flavone showed maximum activity at 0 min, while tiliroside did not cause the inhibition of landing at any of the evaluated intervals (Figure 10). As the concentration of the test substance increased, it was observed that the extract reached 100% inhibition at 120 min, while the activity of the hexane fraction gradually decreased. The dichloromethane fraction exhibited the highest inhibition at 120 min, and the inhibition by 7,4'-di-*O*-methyl-8-*O*-sulfate flavone remained constant over 240 min (Figure 10).

Discussion

Studies have demonstrated the larvicidal activity of the extracts obtained from the roots, stem, and aerial parts of *H. velutina* against the larvae of *Ae. aegypti* (Fernandes et al., 2018; Santos et al., 2012), and a study has also highlighted the promising effects of the hexane and dichloromethane fractions (Fernandes et al., 2019). Triterpenes and pheophytins are the main classes of compounds in the hexane fraction, while the flavonoids constitute the major class

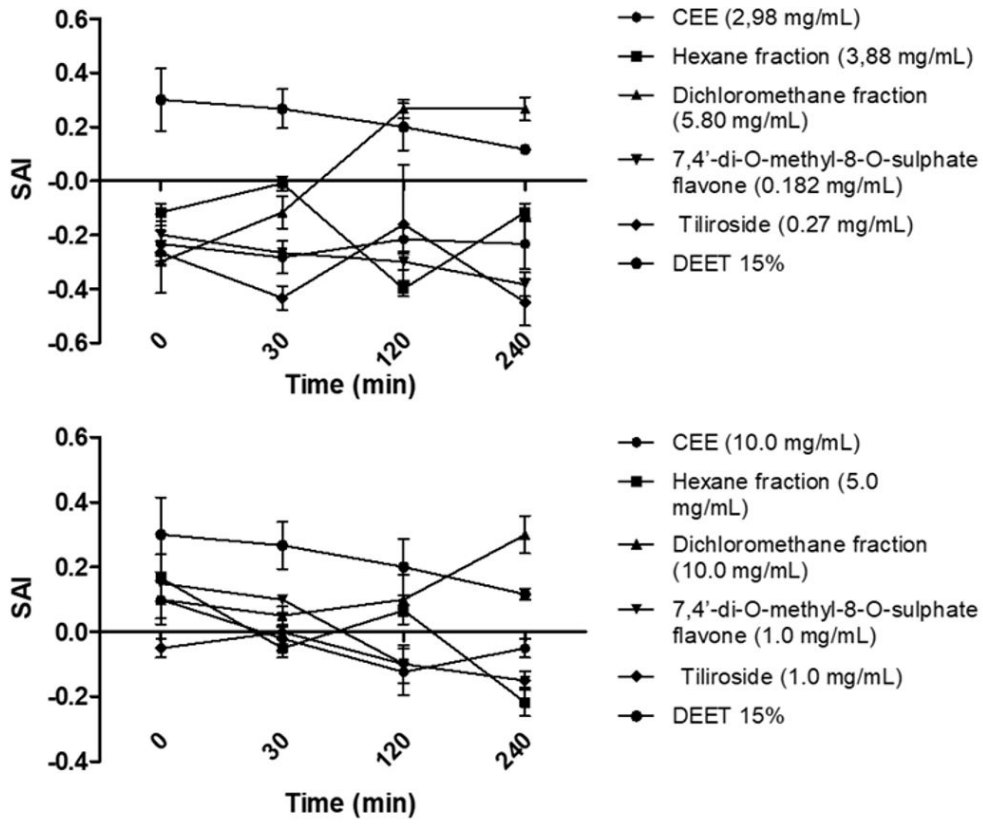

Figure 9. Spatial Activity Index (SAI) of the CEE, fractions and substances isolated from *Helicteres velutina* on females of *Aedes aegypti*.

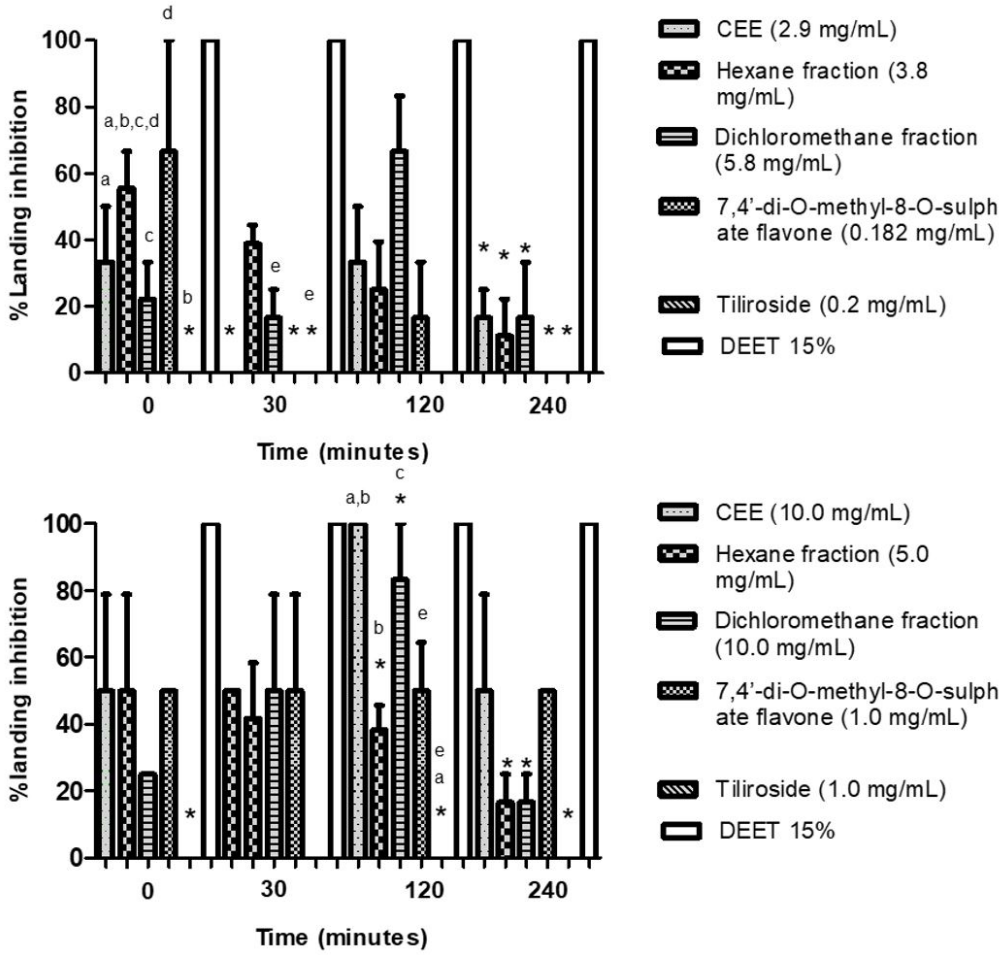

Figure 10. Landing inhibition percentage provided by the CEE, fractions and substances isolated from *Helicteres velutina* on females of *Aedes. aegypti*. Bars with * are significantly different in relation to the positive control (DEET 15%) and bars with the same letter are significantly different by Tukey test, p value < 0.05.

Table 1. Statistical details of the lethal concentration 50 and 90.

Pupicidal Test				
Substance	**LC_{50} (mg/mL)**	**LC_{90} (mg/mL)**	**Hill slope**	**R^2**
Hexane fraction	0.12 (0.122-0.126)	0.14 (0.137- 0.146	2.26	0.98
Dichloromethane fraction	8.85 (6.33-12.37)	26.93 (20.52-35.33)	1.60	0.54
Adulticidal tarsal test				
	LC_{50} (mg/mL)	**LC_{90} (mg/mL)**	**Hill slope**	**R^2**
Hexane fraction	8.01 (6.32-10.16)	36.14 (24.73-52.81)	1.62	0.82
Dichloromethane fraction	0.74(0.26-2.13)	43.89 (12.50-154.1)	-6.97	0.70
Adulticidal body test				
	LC_{50} (mg/mL)	**LC_{90} (mg/mL)**	**Hill slope**	**R^2**
Hexane fraction	0.05(0.03-0.08)	1.12(0.48-2.60)	0.72	0.83
Dichloromethane fraction	0.23 (0.18-0.31)	2.10 (1.15-3.85)	-2.59	0.95

in the dichloromethane fraction. A few of these substances (tiliroside and 7,4'-di-*O*-methyl-8-*O*-sulfate flavone) are known to possess activity against *Ae. aegypti* larvae (Fernandes et al., 2019; Fernandes et al., 2020b), and these results have triggered interest with respect to studying other stages of the vector life cycle.

The development of resistance in insects to common insecticides is a major problem. However, plant extracts contain several metabolites with insecticidal properties that may be considered an alternative to these commonly used insecticides (Govindarajan, 2011; Govindarajan & Sivakumar, 2014; Kumar et al., 2012; Mukandiwa et al., 2016). Several factors influence the insecticidal activity of the extract and its fractions from plants, and a few of these factors are associated with the plant itself, such as the age of the plant as well as the extraction solvent (Kumar et al., 2018; Torres et al., 2015).

Our findings showed that the hexane and dichloromethane fractions (both 0.5 mg/mL) were able to inhibit *Ae. aegypti* hatching by 66.6% and 50.0% after 25 days, respectively. Govindarajan (2011) found that chloroform and hexane extracts from leaves of *Cardiospermum halicacabum* Linn. (Sapindaceae) exhibited activity at concentrations of 0.5 mg/mL and 0.6 mg/mL, respectively. These findings show that these extracts possess good activities in eggs of *Ae. aegypti* at low concentrations.

Munusamy et al. (2016) showed the ovicidal potential of the species belonging to the Asparagaceae family. The authors revealed that the methanolic extract of *Rubia cordifolia* roots (0.5 mg/mL) inhibits *Ae. aegypti* hatching by 70%. Moreover, the hexane extract of *Scilla peruviana* roots inhibits *Ae. aegypti* hatching by 43.2% at the same concentration. Reegan et al. (2015) studied the species belonging to the Rutaceae family and found that the hexane extracts of *Limonia acidíssima* (0.5 mg/mL) and *Aegle marmelos* (0.5 mg/mL) inhibit *Ae. aegypti* hatching by 60.0% and 48.8%, respectively.

A comparative analysis between the ovicidal activity of the hexane and dichloromethane fractions of the CEE of aerial parts of *H. velutina* and those found in the literature, previously mentioned, showed great similarity. These results emphasize that the ovicidal activity depends not only on the extracted solvent but also on the chemical profile of the species (Govindarajan & Sivakumar, 2014; Kovedan et al., 2013).

The hexane and dichloromethane fractions showed significantly different pupicidal activity (LC_{50} of 0.12 mg/mL vs. 8.85 mg/mL, respectively). A study showed that the dichloromethane extract from *Dalbergia oliveri* (Fabaceae) has a higher pupicidal activity (LC_{50} 1.00 mg/mL) than the hexane extract from the same species (LC_{50} 1.21 mg/mL) (Pluempanupat et al., 2013). Fractions from the hexane extract of *Limonia acidíssima* (Rutaceae) leaves showed promising pupicidal activity, with LC_{50} between 0.004 mg/mL and 0.032 mg/mL (Reegan et al., 2014).

In addition, the extracts obtained from other solvents such as the methanolic extract from *Solanum xanthocarpum* (Solanaceae) (LC_{50} of 0.27 mg/mL) (Kumar et al., 2012) and the ethanolic extract from *Citrus sinensis* (Rutaceae) (LC_{50} of 0.49 mg/mL) (Murugan et al., 2012) have reported pupicidal activities against the vector.

In the adulticidal tarsal test, the hexane fraction possesses higher LC_{50} compared to that of the dichloromethane fraction of *H. velutina* (LC_{50} of 8.01 mg/mL vs. 0.74 mg/mL). Govindarajan & Sivakumar (2012) analyzed extracts of leaf of *Eclipta alba* and *Andrographis paniculata* and revealed that the chloroform extract possesses greater adulticidal activity (LC_{50} of 0.20 mg/mL) than the hexane extract (LC_{50} of 0.25 mg/mL) after 24 h of exposure.

Amerasan et al. (2012) found similar results for the chloroform and hexane extracts from *Cassia tora* (Fabaceae) leaves, with an LC_{50} of 0.30 mg/mL and 0.32 mg/mL, respectively. The chloroform and hexane extracts from the leaves and seeds of *Albizia lebbeck* (Mimosoideae) showed an LC_{50} of 0.09 mg/mL and 0.11 mg/mL, respectively (Govindarajan & Rajeswary, 2015).

Ajaegbu et al. (2016) reported better adulticidal activity of the dichloromethane fraction from the leaf extracts of *Spondias mombin* compared to that of the hexane fraction (LC_{50} of 2.17 mg/mL vs. LC_{50} 4.42 mg/mL, respectively). Govindarajan & Sivakumar (2014) showed that the chloroform extract of *Erythrina indica* (Fabaceae) at a concentration of 0.25 mg/mL killed 97% of the adults, which was slightly higher than that of its hexane extract, which killed 94% of the adults at a concentration of 0.30 mg/mL. These results suggest a similarity between the adulticidal activities and the concentrations of the hexane and dichloromethane fractions.

In the adulticidal body test, the hexane fraction was more active than the dichloromethane fraction, with an LC_{50} of 0.05 mg/mL and 0.23 mg/mL, respectively. Our results are comparable with those of previous reports by Yu et al. (2015), where the chloroform extract of the seaweed *Bryopsis pennata* (Bryopsidaceae) was found to be more active (LC_{50} of 73.49 mg/cm^2) than its hexane extract (LC_{50} of 233.55 mg/cm^2).

The application of the substances tested directly on the body of the insect showed better results when compared to those of the tarsal contact test, requiring lower concentrations of the tested substances. This could be explained by the evaporation of the tested substance that occurs in the tarsal test. When the test substance is applied on the mosquito, certain volatile components evaporate and may no longer be present at the same concentration. These findings corroborate those of Oliveira et al. (2018); however, it is important to mention that studies related to the topical application of plant extracts on insects are quite rare.

Our results revealed that the hexane fraction obtained from the CEE of aerial parts of *H. velutina,* exhibited significantly higher activity against eggs, pupae, and adult mosquitoes, suggesting that components such as triterpenes, steroids, fatty acids, and pheophytins isolated from this fraction could possess activity against these phases of the vector's life cycle. In contrast, the dichloromethane fraction showed higher activity in the tarsal contact test, which may be associated with the species' repellent activity, since topical application of the repellent inhibits the landing of insects (Murugan et al., 2012).

To evaluate this repellent activity, the extract, fractions, and two flavonoids isolated from the dichloromethane fraction: tiliroside (glycosidic flavonoid), and 7,4'-di-*O*-methyl-8-*O*-sulfate flavone (sulfated flavonoid) were tested against *Ae. aegypti*. The two flavonoids are known to possess larvicidal activity and have shown potential to exhibit activity against the adult mosquito *in silico* (Fernandes et al., 2019).

While testing the repellent activity, we found that the hexane fraction attracted mosquitoes to the test chamber and that the CEE was less active than the dichloromethane fraction at all the analyzed concentrations and intervals, leading to the proposition that the compounds existing in the dichloromethane fraction would be responsible for the repellent action.

The dichloromethane fraction from *H. velutina* showed a better repellent effect than the positive control and exhibited the best repellency profile between the two concentrations. The tiliroside did not cause the inhibition of landing at any of the evaluated intervals; however, the 7,4'-di-*O*-methyl-8-*O*-sulfate flavone provided greater repellency, which is in accord with the results of the *in silico* studies that show the potential of this substance against adult mosquitoes (Fernandes et al., 2019). This suggests that 7,4'-di-*O*-methyl-8-*O*-sulfate flavone may be one of the substances present in the extract that is responsible for the repellent activity mentioned in traditional medicine.

In several cases, the stimulatory activity of the extracts cannot be attributed to one compound alone and involves the synergistic interactions among various compounds (Fernandes et al., 2018).

These findings corroborate the results of the tarsal test, which reveals higher adulticidal activity of the dichloromethane fraction, thereby suggesting that the substances present in this fraction, such as flavonoids, may be associated with this activity. Of the two tested flavonoids, it was possible to verify that the sulfated flavonoid presents greater repelling power than the glycoside flavonoid. The glycosidic unit in tiliroside may act as an attraction for the insect, considering that in the wild, these mosquitoes feed on plant sap and sugary solutions (Nunes et al., 2019b), while the sulfate group (OSO_3H) may act as an inhibiting agent (Fernandes et al., 2019). This study is the first to show the activity of sulfated flavonoids against adult *Ae. aegypti*.

Simmonds (2001) showed that flavonoids have been implicated as being important in host recognition and acceptance by adult insects and can modulate the feeding and ovipositioning behavior of insects. Glycosides flavonoids that stimulate feeding include isoquercetin, and the ones involved in ovipositioning include vicenin 2, narirutin, hesperidin, and rutin.

To date, there have been very few structure–activity studies on flavonoids against *Ae. aegypti*. Such studies can assist researchers in establishing the categories of flavonoids that have a greater influence on insect behavior and whether changes in the composition of substitutions on a flavonoid molecule can alter the responses of an insect to that particular compound (Simmonds, 2001).

A repellency study against female *Ae. aegypti* was performed with the CEE of *Vitex negundo*, and it was found that a concentration of 0.02 ppm provided complete protection in the period of 110–271 min. These results show that the peak activity of natural repellents increases over time (Kumar et al., 2011). Another study by Keziah et al. (2015) showed similar results, where the authors evaluated the repellent activity of *Ocimum gratissimum* from 0–180 min and recorded maximum protection up to 120 min for the crude methanolic extract and up to 150 min for the ethyl acetate phase.

The extract and fraction of *Clausena anisata* showed repellent activity. The average repellent activity of the extract (150.0 mg/mL) and hexane fraction (7.5 mg/mL) was 83% and 54%, respectively. DEET (15%) provided 90% protection against female landings over a period of 3 h (Mukandiwa et al., 2016). A study conducted with a 20% solution of *Ocimum sanctum*, *Mentha piperita*, and *Plectranthus amboinicus* essential oils showed results similar to those of the DEET positive control, with no mosquito landing until 6 h. Repellency of the oil from *Eucalipto globulus* lasted only for 90 min (Lalthazuali & Mathew, 2017).

Conclusions

This study showed that the popular use of *H. velutina* against *Ae. aegypti* has a scientific basis. Our results suggest that the compounds present in the crude ethanolic extract of the aerial parts of this species can be used against all the stages of the vector's life cycle, from eggs to adults. These fractions are promising and can be used in integrated control against *Ae. aegypti*.

The main findings of this study were as follows: the hexane and dichloromethane fractions of the crude ethanolic extract of the aerial parts of *H. velutina* K. Schum possess insecticidal and repellent activity against *Ae. aegypti*, with the potential to be used as a natural, effective, selective, and biodegradable alternative to combat the vector, thereby preventing the diseases caused by the arboviruses transmitted by these mosquitoes.

This is the first study to demonstrate the activity of sulfated flavonoids against adult *Ae. aegypti*, with considerable repellent activity *in vivo*.

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Ethics statement

Corpo dos Ethics statement.

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Conflict of interests

No conflict of interests declared concerning the publication.

Authors' contributions

DAF: Performed phytochemical study; DAF, LHGO, HLR and WGSS carried out the biological assay. FCN and MFVS supervised the work.

Availability of complementary results

All samples used in this study are in the possession of the authors.

The study was carried out at Laboratório de Biotecnologia Aplicada a Parasitas e Vetores - LAPAVET, Universidade Federal da Paraíba - UFPB, João Pessoa, PB, Brasil.

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