Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Phytochemical prospection and larvicidal bioactivity of the janaguba (*Himatanthus drasticus*) Mart. Plumel (Apocynaceae) latex against *Aedes aegypti* L. (Diptera: Culicidae)

Prospecção fitoquímica e bioatividade larvicida do látex da janaguba (*Himatanthus drasticus*) Mart. Plumel (Apocynaceae) sobre *Aedes aegypti* L. (Diptera: Culicidae)

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Abstract

The aim of this study was to carry out phytochemical prospecting and evaluate the larvicidal activity of *Himatanthus drasticus* latex extracts against *Aedes aegypti*. The extracts were obtained by maceration from 5 g of latex powder concentrated separately in 100 mL of methanol, ethyl acetate, and hexane solvents. The concentrations of 100, 200, 300, 400, and 500 ppm of each extract were tested in triplicate with a solution of pyriproxyfen as the positive control and distilled water and dimethylsulfoxide as the negative control. The phytochemical prospection of the methanolic extract showed the presence of phenolic compounds, such as anthocyanins, anthocyanidins, catechins, chalcones, aurones, leucoanthocyanidins, and condensed tannins. The insecticidal bioactivity was most significant for the methanolic extract. The methanolic extract lethal concentrations (LC) of 50 and 90% were 190.76 and 464.74 ppm, respectively. After 48 hours of exposure, the extracts using methanol, ethyl acetate, and hexane at their highest concentrations (500 ppm) caused larval mortality of 100, 73.33, and 66.67%, respectively. These extracts also promoted changes in the external morphology of the larvae, such as damage to the anal papillae, darkening of the body, and reduction in the number of bristles. The methanolic extract showed greater expressivity for morphological changes. The latex of *H. drasticus* has larvicidal activity against third-stade larvae of *A. aegypti* and it is more significant when obtained through maceration in methanol. The methanolic extract of *H. drasticus* latex contains phenolic compounds with insecticidal activity against *A. aegypti larvae.*

Keywords: biolarvicide, arboviruses, dengue, phenolic compounds.

Resumo

O objetivo deste estudo foi realizar a prospecção fitoquímica e avaliar a atividade larvicida de extratos de látex de *Himatanthus drasticus* contra *A. aegypti*. Os extratos foram obtidos por maceração de 5 g de látex em pó concentrado separadamente em 100 mL dos solventes metanol, acetato de etila e hexano. As concentrações de 100, 200, 300, 400 e 500 ppm de cada extrato foram testadas em triplicata com uma solução de piriproxifeno como controle positivo e água destilada e dimetilsulfóxido como controle negativo. A prospecção fitoquímica do extrato metanólico mostrou a presença de compostos fenólicos, como antocianinas, antocianidinas, catequinas, chalconas, auronas, leucoantocianidinas e taninos condensados. A bioatividade inseticida foi mais significativa para o extrato metanólico. As concentrações letais (CL) do extrato metanólico de 50 e 90% foram 190,76 e 464,74 ppm, respectivamente. Após 48 horas de exposição, os extratos utilizando metanol, acetato de etila e hexano em suas maiores concentrações (500 ppm) causaram mortalidade larval de 100, 73,33 e 66,67%, respectivamente. Esses extratos também promoveram alterações na morfologia externa das larvas, como danos às papilas anais, escurecimento do corpo e redução do número de cerdas. O extrato metanólico apresentou maior expressividade para alterações morfológicas. O látex de *H. drasticus* possui atividade larvicida contra larvas de terceiro estádio de *A. aegypti* e é mais significativa quando obtido por maceração em metanol. O extrato metanólico do látex de *H. drasticus* contém compostos fenólicos com atividade inseticida contra larvas de *A. aegypti*.

Palavras-chave: biolarvicida, arboviroses, dengue, compostos fenólicos.

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Received: December 5, 2022 – Accepted: March 23, 2023

1. Introduction

Aedes aegypti L. is a mosquito of great importance in tropical countries. It is a transmitter of arboviruses such as urban yellow fever, chikungunya, zika, and dengue (Chantawee and Soonwera, 2018). Dengue has the greatest epidemiological impact, with an estimated 400 million infections per year worldwide, of which approximately 100 million clinically manifested cases (Excler et al., 2021).

In the Americas, unplanned urbanization, disorderly population growth, and intense human migration combined with a poor water supply and inadequate disposal of solid waste have compromised the actions for vector control. This set of factors combined with the tropical countries' climatic conditions led to a series of changes in the environment that favored the proliferation of *A. aegypti* (Gregianini et al., 2017; Silva et al., 2020).

Some plants use substances from their secondary metabolism in their defense against insects, most notably terpenes, alkaloids, and phenolic compounds. These groups are stored in plant structures, such as glandular trichomes, vacuoles, resin ducts, and laticifers (Taiz et al., 2017). Laticifers are cellular structures specialized in producing a cytoplasmic content called latex. This fluid is exuded by some groups of plants when they suffer some mechanical damage to their tissues (Kitajima et al., 2018). In general, latex has a milky appearance, and its physiological role is associated with plant defense against herbivores, including insects, as well as, against attack by microorganisms (Ramos et al., 2019).

Himatanthus drasticus (Mart.) Plumel (Apocynaceae) is a laticiferous species, of arboreal habit, present in several Brazilian regions (Almeida et al., 2019). *H. drasticus* is popularly known as janaguba in the state of Ceará, and it is frequently found in the Araripe plateau, located in the extreme south of the state. This species is highly sought after by the population due to the popular use of its latex in folk medicine for the treatment of gastritis, hemorrhoids, anemia, inflammation, and even cancer (Morais et al., 2020). Scientifically it is proven that the janaguba latex has antibacterial (Nascimento et al., 2018), gastroprotective (Colares et al., 2008; Leite et al., 2009), anti-inflammatory (Almeida et al., 2019), healing (Santos et al., 2017), and antitumor activity (Santos et al., 2018).

For decades, the control of *A. aegypti* in Brazil has been done through the application of synthetic insecticides (Oliveira et al., 2017). However, the continuous use and in increasingly higher doses have generated problems such as environmental pollution, the risk of toxicity to non-target organisms, such as humans, and the selection of vector strains resistant to these products (Fernandes et al., 2019). This fact has aroused the interest of researchers that seeks alternatives for the control of this vector, including the use of bioactive compounds of plant origin since these substances are not associated with the aforementioned problems (Perumalsamy et al., 2015).

Therefore, this study aimed to evaluate the larvicidal activity of *Himatanthus drasticus* latex extracts under laboratory conditions on third-stage larvae of the *Aedes aegypti* mosquito and to determine the presence of phenolic compounds in the extract that presented the best larvicidal effect.

2. Material and Methods

2.1. Collection of botanical material

The latex of *H. drasticus* was collected in the early morning (between 6 and 7 am) in the rural community of Sítio Catolé (07°27'07 "S and 39°28'51 "W), at an altitude of 942m, in a Cerrado area in the Araripe plateau, located in the municipality of Moreilândia, Pernambuco, Brazil (Figure 1). The collection was performed by a specialized extractivist, as directed by the Brazilian Chico Mendes Institute for Biodiversity Conservation (ICMBIO), following the methodology of Nascimento et al. (2018). The sample was obtained through longitudinal insertions in the plant bark and dripping into sterile Falcon tubes.

2.2. Preparation of extracts

After collection, the latex was taken to the Laboratory of Agricultural Entomology at the Federal University of Cariri-UFCA, in the Center for Agricultural Sciences and Biodiversity (CCAB) to prepare the extracts for the bioassay.

According to the methodology of Rajkuberan et al. (2018), the latex was dispersed in 100 x 95 cm Petri dishes and kept in an oven Model SSD SOLIDSTEEL® at 45°C for 120 hours for sterilization and drying. Then the dried latex was ground in a blender to obtain a powder. The extracts were obtained by maceration, and 5 g of powder was dissolved separately in 100 mL of each of the methanolic, ethyl acetate, and hexane solvents for a period of 72 hours. After this time, each extract was homogenized in a FANEM® Model 257 magnetic stirrer for 30 minutes.

The mixtures were filtered through cotton and funnel, then concentrated in a FISATOM® model rotary evaporator with reduced pressure, rotation at 40 rpm, and temperature at 40ºC to remove the solvents. After this process, the extracts were transferred to a beaker and left in a water bath for 24 hours to ensure the complete elimination of solvents. The crude extracts were then stored separately in amber flasks and kept under refrigeration for approximately 48 hours until use in the experiments.

2.3. Collection and counting Aedes aegypti eggs

The *A. aegypti* eggs were obtained from ovitraps installed in residences in the municipality of Moreilândia, Pernambuco, Brazil. The traps consisted of a black polyethylene plant pot with a capacity of 400 mL, containing 200 mL of water and a 10% aqueous extract of hay fermented for seven days. This extract was used to attract *A. aegypti* females according to the method described by Reiter et al. (1991). In addition to the vase and the aqueous solution, a Eucatex straw was used (Platex type) with dimensions of 3 x 11 cm with a porous texture useful for adherence of the mosquito eggs. This straw was attached to the vertical position of the vessel wall with a clip (Monteiro et al., 2014).

The traps were installed in strategic locations, near water tanks, sewers, and tires. Every five days of installation, the straws and the water solution were replaced. The straws were taken to the Laboratory of Agricultural Entomology at UFCA. A stereoscopic magnifying glass was used to count the viable insect eggs in each straw.

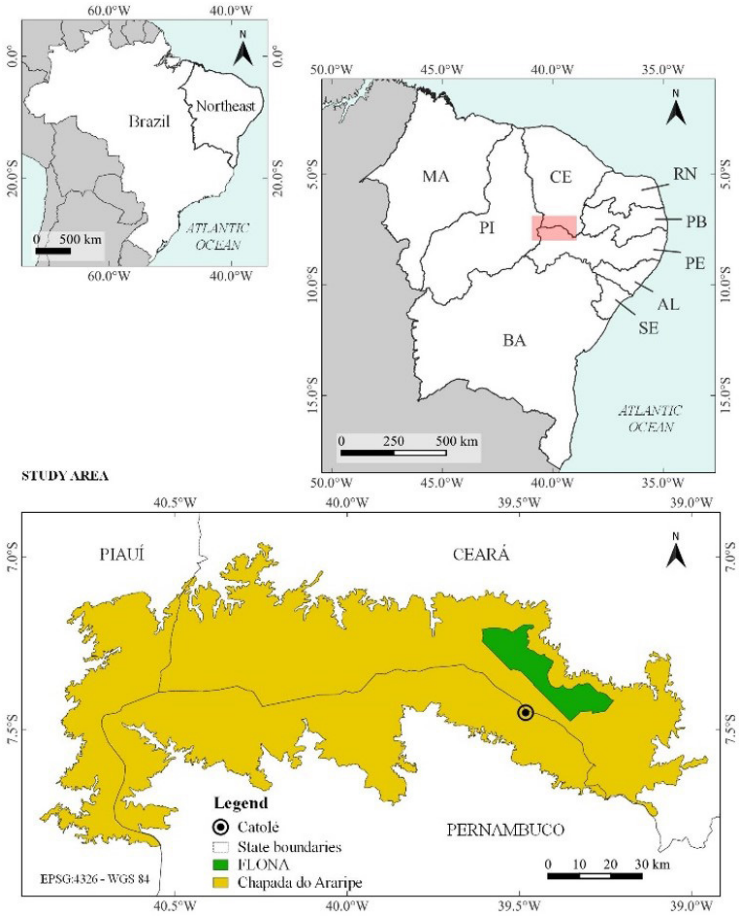

Figure 1. Location of the *H. drasticus* collection in the community of Sítio Catolé, Moreilândia, Pernambuco, Brazil. **Source**: Author (2022).

2.4. Obtaining the larvae of Aedes aegypti

The straws containing the eggs were placed in 22 x 19 cm white trays containing 3 L of water. Then the trays were taken to a BOD (Biochemical Oxygen Demand) (Eletrolab, EL202, São Paulo, Brazil) under controlled conditions of temperature of 25 ± 1 °C, relative humidity of 70 ± 10%, and photoperiod of 12 hours. After 24h, the straws were removed and the larvae were fed with fish food until they reached the third instar.

2.5. Larvicidal bioactivity

To evaluate the larvicidal bioactivity of each of the extracts, the concentrations used were prepared in Eppendorf microtubes with a capacity of 1,500 µL. The extracts presented low solubility in water, given this, a DMSO (Dimethylsulfoxide) solution was used. To prepare each stock solution, portions of 50, 40, 30, 20, and 10 mg of each extract were weighed on an analytical balance Shimadzu Model AX 200, then the extracts were added to 1,000 µL of 1% DMSO.

Before the experiment was performed, DMSO was calibrated at five concentrations (1 to 5%) to verify that DMSO would not influence larval mortality.

Polyethylene containers with a capacity of 50 mL were used for the experiment. Each container received 9,900 µL of distilled water, larval food, and 100 µL of the respective stock solutions at concentrations of 50,000, 40,000, 30,000, 20,000, and 10,000 ppm, obtaining concentrations of 500, 400, 300, 200, and 100 ppm, respectively. A group of third instar larvae was removed from the trays using a Pasteur pipette and placed in each of the containers.

The experiment was also conducted in a BOD and the same conditions were maintained for the larvae-hatching process. The experimental design adopted was a 3 x 5 factorial scheme, corresponding to the three types of *H. drasticus* latex extracts and the five concentrations, and each concentration was accompanied by triplicates, totaling 45 experimental units. The experiments were accompanied by a positive control composed of the insecticide SumiLarv® which has pyriproxyfen as the active ingredient, at the usual concentration recommended by the World Health Organization of 100 ppm (Brasil, 2014), and a negative control, containing distilled water + DMSO at a concentration of 1%. The reading of the tests was performed 24 and 48h after exposure, the larvae were considered dead as they did not react to the mechanical stimulus caused by the bristles of a thin brush.

The efficiency of the extracts for larval mortality was determined in percentage using Abbott (1925) formula (Equation 1).

$$E(\%) = \frac{Nc - Nt}{Nc} \times 100 \quad (1)$$

Where: E = Mortality efficiency; Nc = Number of live individuals in the control treatment; Nt = Number of live individuals in the treatments.

2.6. Morphological analysis of Aedes aegypti larvae

After the larvicidal bioactivity test, five larvae were randomly removed from the three extracts and the control groups to be mounted on glass slides and then observed in a Motic optical microscope model BA210, coupled to a 3Mp-Moticam camera. The photos were recorded using the Motic software version 3.0 (Huber and Reis 2011).

2.7. Phytochemical prospection of Himatanthus drasticus latex

The tests for phytochemical characterization were performed at the Natural Products Research Laboratory (LPPN) of the Regional University of Cariri (URCA). The latex of *H. drasticus* was submitted to a series of tests using specific reagents following the method described by Matos (2009), to elucidate the classes of phenolic compounds.

2.7.1. Tannin determination

For the tannin test, a solution containing 30 mg of *H. drasticus* methanolic extract was prepared in a container, where 3 mL of iron chloride ($FeCl_3$) was added. After stirring it was checked for color variation or precipitate formation. The formation of green precipitate indicated the presence of condensed tannins.

2.7.2. Determination of anthocyanidins and anthocyanins

To detect the presence of anthocyanidins and anthocyanins, in separate containers, two solutions containing 30 mg of the methanolic extract of *H. drasticus* were prepared. The first was acidulated with the addition of Hydrochloric acid (HCl) at pH 3 and the second was alkalinized to pH 8.5 with the addition of Sodium hydroxide (NaOH). In the pH 3 container, the red tint indicated the presence of anthocyanidins and in the pH 8.5 container, the purple tint indicated the presence of anthocyanins.

2.7.3. Determination of leucoanthocyanidins and catechins

To determine leucoanthocyanidins and catechins, a solution containing 30 mg of the methanolic extract of *H. drasticus* was prepared in a container. This solution was acidulated by adding hydrochloric acid (HCl) to pH 3. It was then heated carefully with the aid of an alcohol lamp for 2 to 3 minutes. The red color indicated the presence of leucoanthocyanidins and the brownish-yellow color indicated the presence of catechins.

2.8. Statistical Analysis

The average larval mortality ratio and its standard deviations were calculated for each experiment. The CL50 and 90 with 95% confidence intervals were determined for each extract. The data were submitted for analysis of variance (ANOVA). The means were compared by Tukey's test at a 95% significance level. The R CORE TEAM program, 2022, was used for data processing.

3. Results

3.1. Toxicity of Himatanthus drasticus latex extracts on Aedes aegypti larvae

The pyriproxyfen-based positive control killed 100% of the larvae at the manufacturer's recommended concentration (100 ppm/L), while the negative control (water + DMSO) caused no larval mortality.

For the 500 ppm concentration and 24 hours of exposure, the percentage of larval mortality caused by the methanolic extract of *H. drasticus* latex reached 56.66%, while the ethyl acetate and hexane extracts were less efficient, with 33.33% and 30.00% mortality, respectively (Table 1).

After 48 hours of larval exposure, the methanolic extract caused 100% mortality of the larvae at 500 ppm, while the

Table 1. Average percent mortality of *Aedes aegypti* larvae treated at different concentrations of *Himatanthus drasticus* extracts and controls after 24 h of exposure.

Concentration (p.p.m)	Mortality (Proportion mean ± standard deviation)		
	Methanolic	Ethyl acetate	Hexanic
Distilled water + DMSO	0 ± 0a	0 ± 0a	0 ± 0ab
100	10 ± 0ab	3.33 ± 0.58ab	0 ± 0ab
200	16.67 ± 0.58ab	10 ± 0ab	3.33 ± 0.58ab
300	23.33 ± 0.58bc	13.33 ± 0.58ab	13.33 ± 0.58ab
400	33.33 ± 0.58bc	20 ± 1ab	16.67 ± 0.58abc
500	56.66 ± 1.73c	33.33 ± 1.53bc	30 ± 1c
Piriproxyfen – 100	100.0 ± 0.0d	100.0 ± 0.0c	100.0 ± 0.0d

Different letters in the columns indicate statistically significant differences by Tukey's test.

Table 2. Average percent mortality of *Aedes aegypti* larvae treated at different concentrations of *Himatanthus drasticus* extracts and controls after 48 h of exposure.

Concentration (p.p.m)	Mortality (Proportion mean and standard deviation)		
	Methanolic	Ethyl Acetate	Hexanic
Distilled water + DMSO	0 ± 0a	0 ± 0a	0 ± 0a
100	23.33 ± 0.58b	16.67 ± 0.58a	13.33 ± 0.58a
200	46.67 ± 0.58c	26.67 ± 0.58b	23.33 ± 0.58abc
300	66.67 ± 0.58d	43.33 ± 1.15b	36.67 ± 0.58bc
400	83.33 ± 0.58e	56.67 ± 0.58bc	46.67 ± 1.53bc
500	100 ± 0f	73.33 ± 1.53cd	66.67 ± 0.58c
Piriproxyfen – 100	100.0 ± 0.0f	100.0 ± 0.0e	100.0 ± 0.0d

Different letters in the columns indicate statistically significant differences by Tukey's test.

ethyl acetate and hexane extracts caused less mortality, 73.33% and 66.67%, respectively (Table 2).

It was observed that regardless of the extract, the concentration of 500 ppm promoted the highest mortality. It was also noticed that the increase in concentration was proportional to the increase in dead larvae. The concentration of 500 ppm and the period of 48 hours of exposure were the most appropriate conditions to cause the highest mortality rate of *A. aegypti* larvae.

The analysis of variance revealed that there was a significant association between the different *H. drasticus* latex extracts and the exposure period, and between the concentration and exposure period (Table 3).

After 24 hours of exposure, it was found that the extract that showed the highest toxicity against *A. aegypti* larvae was the methanolic with CL_{50} = 743.96 and CL_{90} = 1,386,44. The ethyl acetate extract showed CL_{50} = 857.42 and CL_{90} = 1,751.48. While the hexanic extract obtained CL_{50} = 987.65 and CL_{90} = 1,549.61. Similarly, in the period of 48 hours of exposure, the extract that showed lower lethal concentration (CL) against *A. aegypti* was also the methanolic extract with CL_{50} = 190.76 ppm and CL_{90} = 464.74, and was considered the extract of greater toxicity when compared to the others. The ethyl acetate extract had a CL_{50} = 321.24 ppm and CL_{90} = 1,188.78 ppm. For larvae exposed to the hexanic extract, the CL_{50} was 390.65 and CL_{90} was 1,549.61 (Table 4).

Table 3. Analysis of variance of the effect between extracts, exposure period and concentration.

Variation Factor	Degrees of freedom	Mean Square	*P value*
Extract	2	30.53	<0.001
Exposure Period	1	208.54	<0.001
Concentration	4	58.63	<0.001
Extract x Period	2	4.58	<0.001
Extract x Concentration	8	0.64	0.458
Period x Concentration	4	7.46	<0.001
Extract x Period x Concentration	8	0.41	0.752
Waste	60	0.66	

3.2. Morphological changes in Aedes aegypti larvae submitted to Himatanthus drasticus extracts after 48 hours of exposure

In the control group, the larvae were active and vermiform in appearance. The head, thorax, and abdomen regions were well defined, with lateral bristles and anal papillae intact, the body was transparent, and the segments were visible (Figure 2 [a, b]).

The *H. drasticus* latex extracts caused external morphological changes in the larvae (Figure 2, [c-h]). On exposure to the methanolic extract, the larvae exhibited strong darkening in the cephalic capsule and respiratory siphon, and a strong reduction in the number of bristles along the body (Figure 2 [c, d]). While on exposure to the ethyl acetate extract, the larvae showed moderate darkening of the cephalic capsule and reduction in the number of bristles, in addition to the destruction of the anal papillae and narrowing of the posterior region (Figure 2 [e, f]). As for the hexanic extract, the larvae showed a slight darkening of the cephalic and posterior region, as well as a slight reduction in the number of bristles, furthermore, damage to the anal papillae was observed (Figure 2 [g, h]).

3.3. Phytochemical prospection of the methanolic extract of Himatanthus drasticus latex

The methanolic extract of *H. drasticus* latex was chosen for analysis because it showed higher activity on *A. aegypti* larvae. Thus, the phytochemical analysis of the methanolic extract of *H. drasticus* revealed the presence of some classes of secondary metabolites, such as anthocyanins, anthocyanidins, catechins, chalcones, aurones, leucoanthocyanidins, and condensed tannins.

Table 4. Lethal concentrations (CLs) of methanolic, ethyl acetate and hexanic extracts of *Himatanhtus drasticus* latex on *Aedes aegypti* larvae after 24 and 48 h of exposure.

Extracts	Time (h)	CL_{50} (p.p.m) (95% CI)	CL_{90} (p.p.m) (95% CI)
Methanolic	24	743.96 (490.4 - 926.52)	1,386.44 (1,106.25 - 1,865.31)
	48	190.76 (154.13 - 224.66)	464.74 (377.23 - 651.94)
Ethyl Acetate	24	857.42 (586.05 - 1,263.32)	1,751.48 (1,510.42 - 2,532.39)
	48	321.24 (257.79 - 423.57)	1,188.78 (747.99 - 1483.66)
Hexanic	24	987.65 (541.89 - 1.063,32)	1,851.48 (987.19 - 2,732.39)
	48	390.65 (309.55 - 575.9)	1,549.61 (887.12 - 2,740.22)

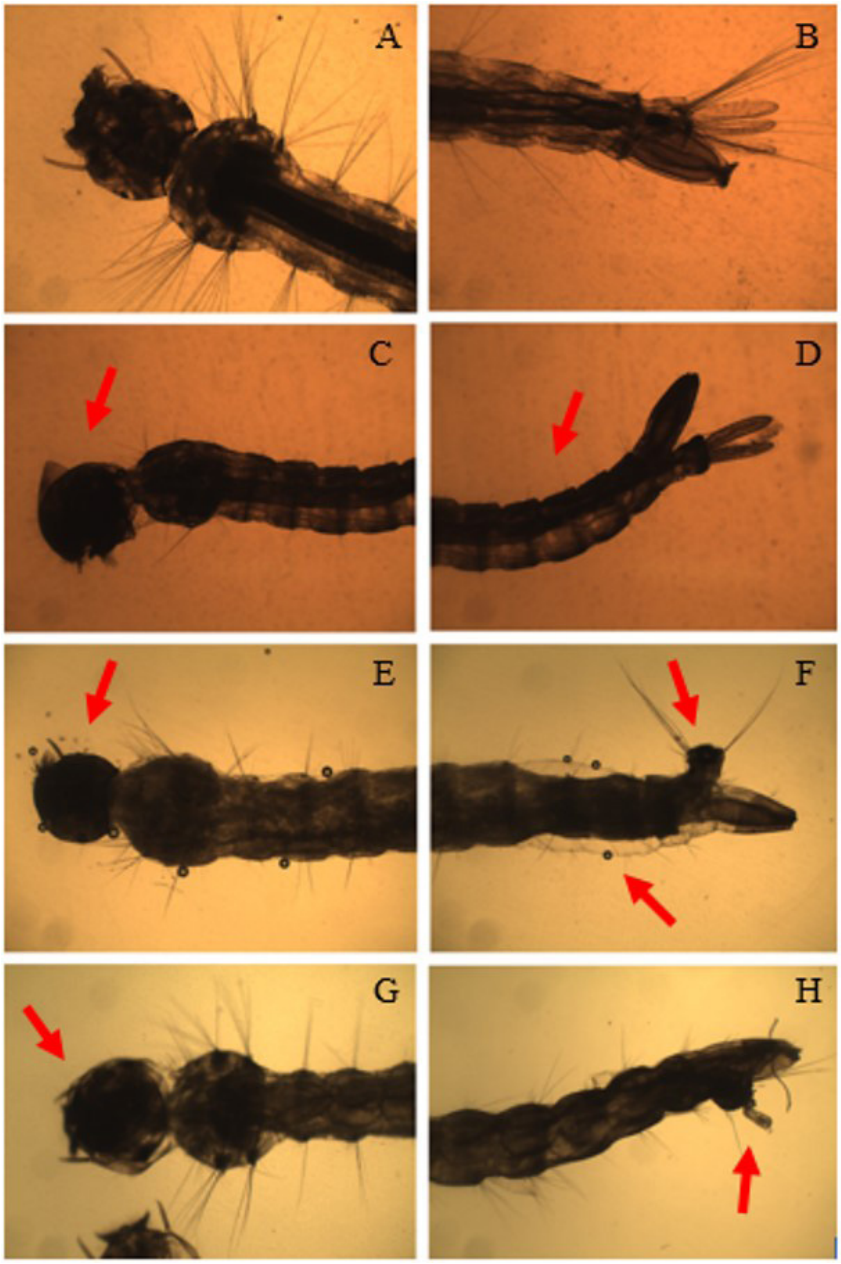

Figure 2. Morphological aspects of *A. aegypti* larvae exposed to 500 ppm concentration of *H. drasticus* latex extracts. Legend: A, B - Control + DMSO, C, D - methanolic extract, E, F - ethyl acetate extract, G, H - hexanic extract. **Source**: Author (2022).

4. Discussion

The results showed that the extracts of *H. drasticus* latex prepared with different solvents affected differently the larvae of *A. aegypti,* the methanolic extract was the most significant. The toxic effect of *H. dastricus* on *A. aegypti* found in this study corroborates the work performed by Azevedo et al. (2019) where it was found that the ethanolic extract of the barks and leaves of this species were able to cause mortality in 94.4 and 83.3% of the third instar larvae of *A. aegypti*, respectively.

The same authors observed that the greatest effect on *A. aegypti* larvae exposed to the extract of *H. dastricus* bark is possibly related to the presence of flavonoids and tannins, as reported by Luz et al. (2014) when performing the phytochemical screening of the hydroalcoholic extract of the bark of this species and revealed the presence of these compounds. In the present study, the presence of the two aforementioned classes of metabolites was also verified in the methanolic extract of the latex. These data suggest that the chemical compounds of the bark and latex of this species present some similarity in their composition, which could explain the similarity between the results found by these authors and those of the present study.

No studies evaluating the insecticidal activity of other species of the genus *Himatanthus* on larvae or other stages of the biological cycle of *A. aegypti* were found in the literature, however, Silva et al. (2017) investigated the insecticidal activity of the ethanolic extract of the leaves of *H. articulatus (*Vahl) Woodson, on the cabbage bean aphid (*Brevicoryne brassicae* L. (Hemiptera: Aphididae), and observed mortality of 97.6% of the nymphs of this insect. Morais et al. (2021) evaluated the insecticidal action of a hydroalcoholic fraction of *H. dastricus* latex on the infestation of cowpea bean by *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) and found that this compound caused delays in the larval development of this insect, causing up to 100% mortality of larvae. These data point to the toxic potential of species of this genus on insects of various feeding habits.

Although this is the first report of the larvicidal activity of *H. drasticus* latex extract on *A. aegypti*, other authors working with latex extracts of other plant species have evaluated their toxic effects on this vector and demonstrated that plant latexes from different species have lethal properties against the mosquito.

Somani et al. (2017), tested the methanolic extract of *Euphorbia caducifolia* Haines latex on *A. aegypti* larvae and found CL_{50} values of 282 ppm and CL_{90} of 743 ppm, thus corroborating the data found in the present study. Similar results were also found by Rajkuberan et al. (2018) who verified the larvicidal activity of the methanolic extract of *Carica papaya* L. latex on *A. aegypti* and obtained CL_{50} of 187.81 and CL_{90} of 810. 83 ppm. Considering the values of CL_{50} and CL_{90} of the methanolic extract. These studies showed values very close to those found in this study, demonstrating that the larvicidal potential of the methanolic extract of *H. drasticus* latex on *A. aegypti* are in accordance with the findings in the literature.

The reduction in the number of larval bristles was also reported by Sutiningsih et al. (2018) the larvicidal effect of bucein, isolated from seeds of *Brucea javanica* (L.) Merr. Bristles have a sensory function and assist in buoyancy in the aquatic environment. Therefore, the decrease in the amount of these structures may have interfered with the survival of the larvae, since at this stage the larvae need to stay on the water surface to breathe through the respiratory siphon and spiracles (Consoli and Oliveira, 1994).

In *A. aegypti* larvae, the posterior and anal segment of the abdomen has four lobulated gills for osmotic regulation, better known as anal papillae. The effect of the extracts on the larvae may occur via ingestion of chemical compounds through these structures, causing asphyxiation. On the other hand, this product can cause cellular disorganization of the gills, contributing to a disorder in the osmotic regulation of the mosquito, which can lead to an imbalance in the absorption of ions from the water (Andrade et al., 2021).

Damage to the anal papillae of *A. aegypti* caused by plant extracts has been reported in previous studies. Kumar et al. (2010) observed alterations of the anal papillae in larvae exposed to ethanolic extract of three black pepper species. Warikoo and Kumar (2013) observed that extracts of *Argemone mexicana* L. also caused changes in the anal papillae of *A. aegypti* larvae.

Chaithong et al. (2006) point out that damage to the anal papillae leads to their dysfunctionality, which can result in a disruption of osmosis and ionic regulations. In addition, the extract disrupts the internal structure of the spiracular apparatus and causes the destruction of the hydrophobic surface of the stigmal plate causing water to enter the trachea, and impairing the respiratory system of the larvae (Neves Filho et al., 2009).

Darkening of the larvae body was also observed by Oliveira et al. (2013) when exposed to the essential oil of *Piper aduncum* L. leaves. *Anopheles stephensi,* and *Culex quinquefasciatus,* submitted to the aqueous extract of *Annona squomosa* L. seeds. Possibly, this darkening occurred due to the action of the extract on the endocrine system of the larvae, which affects the secretion of ecdysone. The absence of this hormone prevents ecdysis from occurring, while cuticles overlap, giving a blackened appearance to the larval body (Abed et al., 2007).

There are few studies that portray the narrowing of the larval abdômen, however, Barreto et al. (2006) reported in their studies through morphological and histological evaluations in *A. aegypti* larvae submitted to ethanolic extract of *Sapindus saponaria* Lin. The narrowing of the body was also observed by Abed et al. (2007) when *A. aegypti* larvae were submitted to the oil-resin of *Copaifera reticulata* (Ducke). This autors cite that this narrowing is caused by peristaltic movements performed by the larvae to extrude the aggressive agent from its digestive tract.

With the exception of catechins and condensed tannins, the phytochemicals identified are subclasses of flavonoids and are widely found in the plant kingdom. Flavonoids are present in all plants, from mosses to angiosperms, acting in ultraviolet protection, flower coloration, species interaction, and defense against pathogens and herbivores (Martens and Mithöfer, 2005).

Flavonoids were also found by Santos et al. (2017) in photochemical screening studies and evaluation of the healing activity of ethyl acetate extract of *H. drasticus* latex

on mice. In addition, Santos et al. (2018) evaluated the antitumor activity of ethyl acetate extract of *H. drasticus* latex on mice and in photochemical screening, these authors also verified the presence of flavonoids in the tested extract. Both authors found significant effects on the biological activities evaluated, which supports that flavonoids present in the latex of the plant species in question are potent phytochemicals.

Condensed tannins and catechins were also found by Nascimento et al. (2018) by assessing the antibacterial activity of the ethyl acetate extract of *H. drasticus* latex, found significant effects in inhibiting multidrug-resistant strains of *Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumoniae.*

Tannins have the ability to inhibit the growth of fungi, bacteria, and insects (Nascimento et al., 2018). They are well known for their insecticidal activity, act against the attack of invertebrate and vertebrate herbivores, have astringent taste and are difficult to digest. Their detrimental effects on the insect diet are related to their interactions with food proteins, forming complexes responsible for compromising growth and low digestibility of the ingested food (Cavalcante et al., 2006).

Several authors have reported the insecticidal effect of tannins on mosquitoes. Silva et al. (2004) evaluated the effect of tannins isolated from the bark of the plant *Magonia pubescens* St. Hil on larvae of *A. aegypti,* found that these compounds showed significant larvicidal activity, with CL_{50} and CL_{90} of 3.1 and 36.6 ppm, respectively. Valotto et al. (2011) used extract of the stem cortex of the plant *M. pubescens* and evidenced that a fraction rich in tannins caused death of *A. aegypti larvae* through the destruction of midgut cells.

The larvicidal activity of catechin has already been isolated from *Leucas aspera* (Willd.) Link. and tested on larvae of *A. aegypti*, *A. stephensi* Listen and *C. quinquefasciatus* (Say). In these tests, damage to the anal papillae and midgut epithelial tissue was observed in addition to larval death (Elumalai et al., 2016). In the study by Silva et al. (2014) in which the larvicidal activity of the ethanolic extract of the stem of *Croton linearifolius* Mull. Arg. on *A. aegypti* larvae, 50% mortality of the larvae was observed within 24 hours of exposure. In the phytochemical prospection of the extracts of this species, these authors showed, among other compounds, the presence of catechin, pointing to the toxic effect of this compound on *A. aegypti* larvae.

Limitations of this study include the restricted observation of the effect of *H. drasticus* extracts on only one larval stage of *A. aegytpi*, as well as the evaluation of the mode of action only on the external morphology of the larvae. Furthermore, the phytochemical analysis was restricted to the level of phenolic compound classes.

5. Conclusions

H. drasticus latex was found to have larvicidal activity on third-stage larvae of *A. aegypti,* especially when obtained through maceration in methanol.

The three extracts promote changes in the external morphology of *A. aegypti* larvae, with more expressivity for the methanolic extract. In the latter, phenolic compounds with insecticidal activity on *A. aegytpi larvae* were identified.

Acknowledgements

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for their financial support.

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