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# Larvicidal Potential of Pequi (*Caryocar Brasiliense* Cambess) in *Aedes Aegypti* (Diptera: Culicidae): An Ecological Alternative

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**Abstract** There is a need to find sustainable alternatives to control this arbovirus vector due the increasing resistance of *Aedes aegypti* to conventional chemical insecticides especially in tropical and subtropical countries. This study aims to evaluate the larvicidal potential of the *Caryocar brasiliense* fruit pulp as an eco-friendly alternative to the control of *Aedes aegypti*. Using mosquito third instar larvae, *in vitro* and *in vivo* toxicity tests were performed with the fruit pulp of *Caryocar brasiliense*. Larvicidal activity was observed at all concentrations tested, with no evidence of toxicity *in vitro* and *in vivo*, indicating safety for non-target organisms. The results suggest that the fatty esters present in the pulp of *Caryocar brasiliense* may be a promising alternative for the control of *Aedes aegypti*. These findings pave the way for further studies on the structure-activity relationship of natural molecules in combating mosquito-borne diseases.

***Keywords:*** *Caryocar brasiliense, fatty acids, larvicidal activity, bioactive molecules, eco-friendly*

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## 1. Introduction

*Aedes aegypti* is the main vector of several viruses that cause dengue, zika and chikungunya in developing countries, representing a major challenge for public health due to its adaptability in urban environments. The main strategy for controlling the mosquito population is the use of chemical insecticides. However, the resistance of mosquito populations to conventional insecticides represents a substantial challenge in control strategies [1] [2].

The use of plant-derived compounds has become an alternative for vector control, especially in developing countries where access to chemical insecticides is limited and biological vector control technologies are expensive. Botanical insecticides are possible ecological methods of insect control because they are derived from plants that have lower toxicity in non-target organisms, so they are considered an eco-friendly alternative [3]. They are derived from natural molecules that emerge as a promising strategy in the management of resistance to conventional insecticides, because they have biodegradable and non-toxic compounds, and unlike traditional chemical insecticides, they act in a more specific way, reducing the selection capacity of populations [4].

The biodiversity of the Brazilian Cerrado presents a variety of plant species with valuable natural chemical compounds of various nutritional, therapeutic and bioactive properties. Among the species, the pequi tree (*Caryocar brasiliense* Cambess.), a perennial native fruit tree, considered a symbol tree of the Cerrado biome, stands out. Its fruits have commercial and gastronomic potential because they are nutritious sources, with exotic flavor and aroma [5].

Studies are carried out with various oily plants and

fruits against vectors [6] [7] [8]. However, with the pulp of the ripe fruit of *Caryocar brasiliense* there are still no reports in the literature. It is known that many ripe fruits are rich in fatty acids, such as palmitic, oleic, linoleic, linolenic acids, stearic acids, among others [9], and these have been reported as potent bioactives. This is due to the conformity of their molecular compounds of long linear hydrocarbon chains attached to a carboxyl group (-COOH). These molecules have an amphipathic character, *i.e.*, a portion composed of a long nonpolar hydrocarbon chain (hydrophobic), with or without unsaturation, and a polar end (hydrophilic) composed by a carboxyl group, similar to the phospholipid structures of the lipid bilayer of the plasma membrane. The long unsaturated chains play an important role in bioactivity, since the presence of unsaturations increases the fat solubility of the molecule, which makes them permeable to the plasma membrane and consequently their intracellular bioactivity, being a considerable factor in larvicidal activity [10] [11].

Despite the availability of natural resources in the Brazilian flora, the control of *Ae. aegypti* is still concentrated on synthetic chemicals. The use of natural compounds presents itself as a sustainable and safe alternative for mosquito control in emerging countries and opting for these compounds not only reduces the dependence on synthetic chemicals that are harmful to the environment and human health, but also promotes sustainable development and public health. In this sense, the present study aims to evaluate the larvicidal potential of the *C. brasiliense* fruit pulp as an ecological alternative.

# 2. Material and Methods

## 2.1. Plant material and Preparation of Extracts

Ripe fruits of *C. brasiliense* were collected from the soil, in perfect morphological conditions of the bark in the months of December 2021 and January 2022, in the rural area of the municipality of Coxim (18º30'25"S; 54°45'36" W), in the Mato Grosso do Sul State, Brazil (Figure 1).

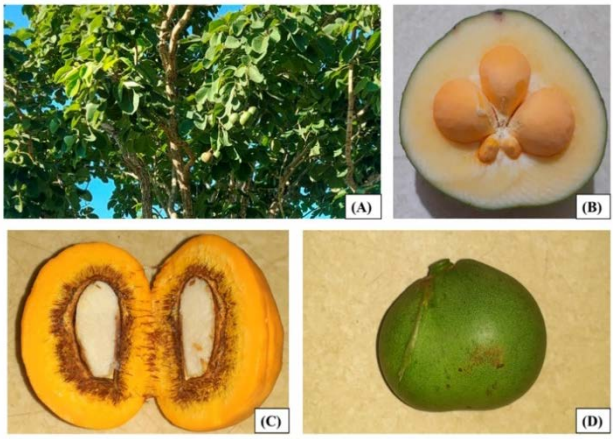


**Figure 1.** Image of *Caryocar Brasiliense*. (A) pequi tree (*Caryocar brasilense*); (B) Cut fruit with exocarp (skin), thick outer mesocarp, and five pyrenes. (C) longitudinal section of a pequi pyrene showing the inner mesocarp (yellow pulp) that covers the endocarp composed of a resistant layer of thorns where it houses the almond. (D) Globose fruit with exocarp or peel. Source: Authors.

The pulp was chopped, weighed and frozen. An amount of 975 g was lyophilized in a freeze dryer (Alpha 1-2 LDplus, Christ) for 76 hours, weighed once again and stored away from light, moisture and heat. After the freeze-drying process, the material was placed in an amber bottle with ethyl alcohol in a 2:1 portion, where it remained in a solvent maceration at exhaustion for 15 days at room temperature, and the solvent was changed every 5 days and subsequently filtered by vacuum. After this period, the extract was route-evaporated for total elimination of the solvent.

## 2.2. Fatty Acid Esterification Reaction

The fatty acid esterification reaction was performed according to the Ce-1F-96 Method from the American Oil Chemists' Society (AOCS).

The sample was added to a glass tube and the weight of each was noted. It was added 1.5 mL of NaOH 0.5M in methanol to the glass tube, stirred for 1 minute in vortex and left in a water bath at 100ºC for 5 minutes. After being cooled under running water, 2 mL of boron trifluoride 13% ($BF_3$) was added to a fume hood and agitated for 30 seconds in a vortex, left in a water bath at 100 ºC for 30 minutes and cooled again under running water. Next, 2 mL of isooctane was added, vortexed for 30 seconds, 5 mL of saturated NaCl solution was added, and vortexed for 30 seconds. Subsequently, 1 mL of the isooctane phase containing methyl ester (upper phase) was removed and placed in a vial to dry with commercial gaseous nitrogen and kept under freezing (-18°C) until injection into the gas chromatograph.

## 2.3. Gas Chromatography Coupled to Mass Spectrometry (GC/MS)

For the qualitative analysis of fatty acids, a Shimadzu gas chromatograph coupled to the mass spectrometer was used. The capillary column used was a DB5 (methyl silicon with 5% phenyl groups) with 60 m length, 0.25 mm internal diameter and 0.25 µm stationary phase and helium as carrier gas with a flow rate of 1 mL/min. The injection was in split mode (1:10). The injector was kept at 280°C and the interface at 300°C. The heating of the furnace followed the following sequence: initial temperature 210°C (0.0 min) - 228°C (0.0 min) -10°C/min-250°C (10 min). The system operated in SCAN mode, allowing the comparison of the spectra obtained with those from the equipment's spectra library (WILEY e NIST).

## 2.4. Analysis of Chemical Compounds of the *C. Brasiliense* Fruit Pulp

The ChemSpider database [12] was used to recover synonyms, formulas, and molecular weights of chemical compounds.

## 2.5. Larvicidal Bioassay

The eggs of *Ae. aegypti* belonging to the Rockefeller lineage, kindly donated by the Laboratory of Biology, Control and Surveillance of Insect Vectors LBCVIV, of the Oswaldo Cruz Institute - FIOCRUZ (RJ) Brazil,

through the TTM IOC Fiocruz 21-40, were used for the bioassay. The assays were performed according to the methodology adapted from the World Health Organization guidelines for determining lethal concentrations 50% (LC50) and 90% (LC90) to recover synonyms, formulas, and molecular weights of chemical compounds [13].

To hatch the eggs, 500 mL of water was boiled for 5 min. (to remove diluted oxygen), transferred while still boiling to a graduated flask with a blue cap, sealed with Parafilm M®, and cooled to room temperature. Subsequently, the flask was opened rapidly, the eggs arranged on strips of filter paper were deposited and sealed again until the end of hatching (approximately 50 minutes). The hatched larvae were transferred to a 20x30x6 cm plastic tray with a distilled water column of about 2 cm and ornamental fish feed (Alcon Basic®) was added in a portion of 1mg/larva/day, covered with a disposable cap and taken to an incubator (28±1°C, 70 ± 10% humidity and 12h/12h cycle).

Third instar larvae were used for bioassays four days after hatching. After preliminary tests to verify the existence of mortality (minimum of 1%) and to determine the concentrations to be tested, the samples were added in a 2 mL microtube, weighed on an analytical balance (Analytical®), added in 1 drop of DMSO, 25 mg of crushed feed, shaken, transferred to transparent plastic cups with a capacity of 150 mL and kept at room temperature. A volume of 40mL of distilled water was added to each glass. A number of 25 randomly chosen larvae were placed in the cups, added feed and kept in the incubator for 24 hours. Concentrations of 0.3 to 1.0 mg/mL were tested. Three replicates were set up for each concentration and a negative control was made with only distilled water and feed. Mortality was observed 24 hours after the experiment and larvae that did not respond to stimuli or when they did not maintain the oscillation of descent and ascent to the surface of the solution were considered dead. Samples were collected for morphological analysis in a 1000x binocular microscope with USB camera.

## 2.6. Sample Preparation of Ethanolic Extract from *C. Brasiliense*

The extract was diluted in dimethyl sulfoxide (DMSO) at a concentration of 0.1 g/mL. The extract was added to the cells by serial dilution in culture medium, using concentrations of 0.25, 2.5, 25 and 250 µg/mL in triplicate. The maximum concentration of DMSO used was 0.25%, as it did not alter cell viability.

## 2.7. Cell Lineages

To evaluate the cytotoxicity of the samples, murine fibroblasts (3T3, ATCC CRL-1658) and human umbilical cord endothelial cells (HUVEC, ATCC CRL-1730) were used. The lineages were cultivated in RPMI (Roswell Park Memorial Institute) and DMEM (Dulbecco's Modified Eagle Medium) culture medium, respectively, containing penicillin and streptomycin (1%) and fetal bovine serum (FBS) (10%), and maintained in a humid incubator at 37ºC and $CO_2$ 5% until the formation of the monolayer with approximately 80% confluency. The dispersion of the adhered cells at the bottom of the flask was performed with the addition of 0.5 mL of trypsin-EDTA (0.25% + 1 mM EDTA) in PBS buffer, pH 7.4, and the suspension was transferred to a 15 mL conical tube containing complete medium (medium, antibiotics and FBS 10%), with 1.5 ml of trypsin to neutralize them. After cell detachment, the medium was resuspended, collected, and centrifuged at 1000 rpm for 4 min. The pellet was resuspended in complete medium, and the suspension was used in the cell counting process.

## 2.8. Sulforramine B (SRB) Assay

The cytotoxicity of the samples was evaluated by means of the SRB assay, which was performed as described by Skehan and colleagues [14]. The detached cells from the maintenance flasks were transferred to a conical tube with complete medium containing 1.5 mL of trypsin and centrifuged for 4 min. at 1000 rpm. 2 mL of culture medium was added to the pellet and the aliquot was removed from this suspension to dilute in dye (trypan blue 1:4) that identifies the non-viable cells that were excluded from the count. In a manual counter (Neubauer chamber) the suspension of enough cells for the inoculation of 7,500 cells in each well of the 96-well test plate was obtained. After counting, in the T0 (Time Zero) plate, which served as a parameter, the cell suspension of each lineage and the culture medium were added in triplicate, inoculated and after 24 hours stained with SRB. The cell suspension was added to the test plate, and after the initial incubation of 24 hours, the test samples were added at concentrations of 0.25 - 2.5 - 25 and 250 µg/mL, in triplicate, and incubated for 48 hours. The test plate contained the blank of each concentration of the test sample, the negative control (cells plus 100µL of medium) and the positive control composed of doxorubicin (DXR) (0.025 - 0.25 - 2.5 and 25 µg/mL). After 48-hour incubation, SRB staining was performed by adding 100 µL of 20% trichloroacetic acid (TCA) to fix viable cells. After 30 min of rest at 4ºC and protected from light, the plates were washed in running water and, after drying, 50 µL of 0.1% SRB (Sigma, USA) diluted in 1% acetic acid were added. The plates were kept at rest for 30 min. and then washed 5 times with 1% acetic acid to remove excess free dye. A total of 100 µL of Trizma Base buffer (10 mM, pH 10.5) (Sigma, USA) was added to the plate for the solubilization of the dye adhered to the proteins of the fixed cells. The plate was read in a spectrophotometer at 540 nm.

## 2.9. In Vivo Acute Oral Toxicity Assay

### 2.9.1. Experimental Animals

The acute oral toxicity test was performed following the Organisation for Economic Co-operation and Development (OECD) guideline 423 for acute oral toxicity [15]. Thirteen male BALB/C mice, weighing an average of 26 g, at six weeks of birth were obtained from the Central Vivarium of the Institute of Biosciences (INBIO) of the Federal University of Mato Grosso do Sul (UFMS-Brazil). The animals were kept in collective cages (size 40 x 35 x 17 cm), in the amount of three animals/cage, at a temperature of approximately 24°C,

with a light/dark cycle of 12 hours, receiving standard food (NUVITAL® CR1) and water *ad libitum*. The study was approved by the Animal Ethics Committee of the Federal University of Mato Grosso do Sul, Brazil (1306/2023).

#### 2.9.2. Experimental Design

*In vivo* tests were initiated at a concentration of 300 mg/kg, as information on lethal doses for the most active extract is unknown, as recommended by the aforementioned guideline, and later 2000 mg/kg, 50 mg/kg and 5 mg/kg. The experiments were carried out in four groups with three animals each. A control group (G5) that received olive oil and four test groups that received doses of 2000 mg/Kg (G1), 300 mg/Kg (G2), 50 mg/kg (G3) and 5 mg/kg (G4) of the extracts diluted in olive oil. In all groups, the animals received a single dose (200 µl) via gavage after 8 hours of fasting. After the administration of the different doses, a hippocratic screening of the animals was performed by means of individual observations of the animals in the periods of 30 min, 1, 2, 4, 6, 12 and 24 h, and daily for 17 days. The following parameters were evaluated: anesthesia, general activity, grasping strength, response to touch, tremors, locomotion, vocal thrill, irritability, contortion, ptosis, lacrimation, breathing, ataxia, water and food feeding.

#### 2.9.3. Histological Analysis

Immediately after euthanasia, kidneys and liver were collected from each animal for a previous macroscopic analysis of the organs and then weighed and fixed in 10% buffered formaldehyde for 24 hours. Then, the samples were dehydrated in alcohol and xylol, embedded in paraffin, and cut in a microtome with a thickness of 5 µm. The sections were stained with hematoxylin-eosin (HE). The analysis was based on morphological changes in the following parameters: steatosis (liver), hydropic degeneration, hyaline degeneration and necrosis (kidneys). For each parameter, scores were assigned to each area analyzed according to the intensity of the lesion found.

### 2.10. Statistical Analysis

The Lethal Concentration ($LC_{50}$) estimate was determined by Probit analysis with the aid of IBM SPSS Statistcs 27 software, with a significance level of $p < 0.05$. Larval mortality was expressed as mean ± standard error (SE) using the ANOVA test followed by Tukey's post-test using the SigmaPlot software (version 12.0), at a significance level of 5%. The calculation of $IG_{50}$ was performed according to [16].

## 3. Results

The fatty esters extracted were palmitoleic, hexadecanoic, stearic and methyl oleate acids, listed in Table 1.

The chromatographic profile by analysis (GC-MS) of the ethanolic extracts of the pulp of *C. brasiliense* is shown in Figure 2.

The mean larval mortality rates are shown in Table 2. The larvicidal effect depends on the concentration used. The mean mortality was directly proportional to the concentration of extract used to which the larvae were exposed. The larvae that were not exposed to any treatment, only fed with feed, were not observed lethality in 24 hours, showing that the effect of the extract on the larvae was significant at all concentrations.

Mortality presented in mean standard error of the mean. Bold p-values indicate a significant difference between the observed and expected values in the negative control ($p<0.05$). Asterisks differ significantly between < 0,005. Note: Negative Control (NC) group treated with H2O and ration. Source: Authors

Based on the results, it was possible to establish values for the lethal concentrations $LC_{50}$ and $LC_{90}$, analyzed by the Probit model, presented in Table 3. The ethanolic extract tested showed good efficacy and third instar larvae of *Ae. aegypti*, with $LC_{50}$ values of 0.64 and $LC_{90}$ 1.69 mg/mL in 24 hours.

**Table 1. Composition of fatty esters derived from fatty acids found in the ethanolic extract of *Caryocar brasiliense* pulp**

| CN | Compound | MF | RT (minutes) | Molecular weight (g/mol) |
|---|---|---|---|---|
| 1 | Palmitoleic acid | $C_{16}H_{30}O_2$ | 13,65 | 254,4 |
| 2 | Hexadecenoic acid | $C_{17}H_{34}O_2$ | 14,22 | 254.4 |
| 3 | Methyl oleate | $C_{19}H_{36}O_2$ | 19,39 | 296,5 |
| 4 | Stearic acid | $C_{18}H_{36}O_2$ | 20,94 | 284,4 |

CN = compound number; MF = molecular formula; RT =Retention time. Source: Authors

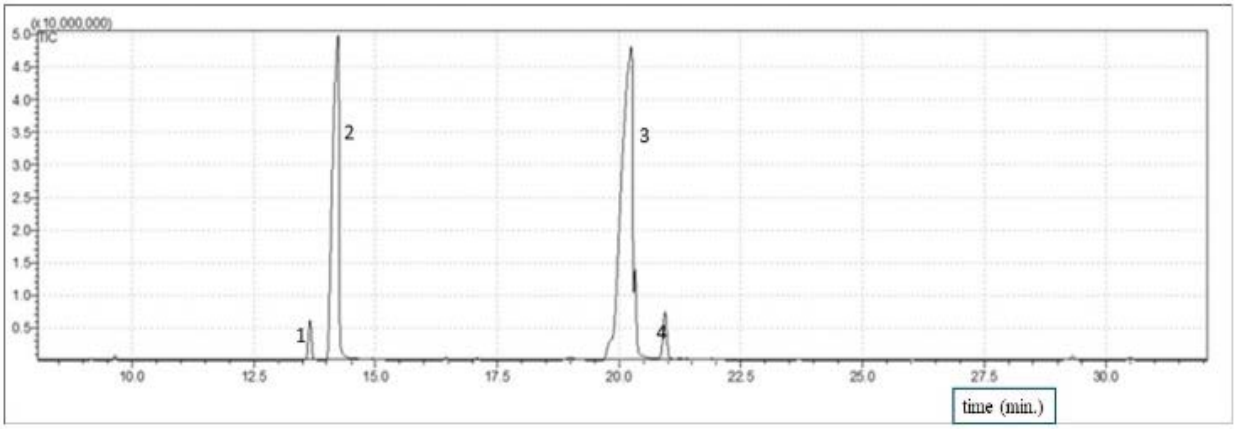


**Figure 2.** GC-MS chromatogram of the ethanolic extract of the pulp of the ripe fruit of *Caryocar brasiliense*

**Table 2. Larval mortality observed from the ethanolic extract of *Caryocar brasiliense* pulp in *Aedes aegypti* in relation to that expected in the negative control**

| Concentrations | Mortality | NC (p-value) |
|---|---|---|
| 0.3 | 2.33±0.33 | 0.020 |
| 0.4 | 5.33±0.33 | 0.004* |
| 0.5 | 12.67±0.33 | 0.001* |
| 0.6 | 13.33±0.67 | 0.002* |
| 0.7 | 13.67±0.33 | 0.001* |
| 0.8 | 15.00±0.58 | 0.001* |
| 0.9 | 16.00±0.58 | 0.001* |
| 1.0 | 16.67±0.88 | 0.003* |

**Table 3. Lethal concentrations ($LC_{50}$ e $LC_{90}$) for *Aedes aegypti* at various concentrations of ethanolic extract from the pulp of *Caryocar brasiliense* after 24 hours of exposure**

| Extract | $LC_{50}$ | | $LC_{90}$ | |
|---|---|---|---|---|
| | Concentration mg/mL | CI 95% | Concentration mg/mL | CI 95% |
| Pulp | 0.64 | (0.59 – 0.70) | 1.69 | (1.39 – 2.27) |

$LC_{50}$- lethal concentration estimated to kill 50% of larvae. CI - Confidence Interval at the significance level of 0.05. $LC_{90}$- lethal concentration estimated to kill 90% of the larvae. Source: Authors

It is important to note that even though they remained alive after 24 hours of treatment, the larvae showed a change in behavior, being less active when exposed to light, unlike the larvae of the negative control that remained active and agile after 24 hours.

The larvae that did not receive treatment were active, with an elongated and vermiform appearance, grayish coloration, head with a pair of antennae, well-defined thorax and abdomen, visible segments, pairs of lateral bristles present, respiratory siphon and anal lobe intact (Figure 3: A). The larvae treated with the extract showed alterations in the external morphological structures, mainly in the coloration with dorsal and ventral visibility (B-C), in the loss the digestive tract continuity (D-E) and alteration in the anal lobe (C-D).

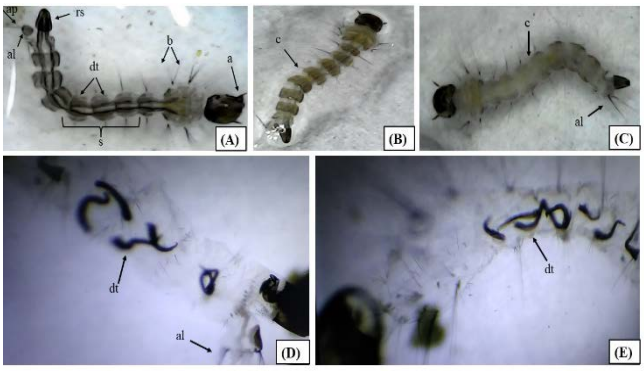


**Figure 3.** Morphology of *Aedes aegypti* larvae after 24 hours of treatment. (A) – Negative control; (B, C) - 200x magnification; (D, E) - 400x magnification. rs = Respiratory siphon; al = Anal lobe; b = Bristles; ap = Anal papillae; dt = Digestive tube; s = Segments; a = Antennas; c = Chitin. Source: Authors

In 3T3 (ATCC CRL-1658) and HUVEC (ATCC CRL-1730) lineages, the extract did not show cytotoxicity, with a $GI_{50}$ > 250µg/mL when compared to doxorubicin, Table 4. The $GI_{50}$ values obtained for the ethanolic extract of *C. brasiliense* fruit pulp were about 1,000 times higher in the fibroblast cell line when compared to doxorubicin and about 10,000 times higher in the endothelial cell line when compared to the same chemotherapy drug. Thus, we can infer that at the highest concentration tested in this assay (250 µg/mL), there was no cell growth inhibition. The results of the acute toxicological evaluations showed no signs of systemic toxicity in the groups treated with ethanolic extract of pequi pulp.

**Table 4. Cytotoxic activity ($IG_{50}$ µg/ml) of the ethanolic extract of *Caryocar Brasiliense* fruit pulp in 3T3 (ATCC CRL-1658) and HUVEC (ATCC CRL-1730) cells**

| Normal Cells | $IG_{50}$ of the ethanolic extract of *Caryocar brasiliense* | $IG_{50}$ Doxorubicin |
|---|---|---|
| 3T3 (ATCC CRL-1658) | >250 µg/ml | 0.25 |
| HUVEC (ATCC CRL-1730) | >250 µg/ml | 0.023 |

During the seventeen days of experimentation, no abnormal or major behavioral changes were observed. Normal values were observed in the hippocratic screening, and none of the animals died at the different concentrations tested. It is known that the hippocratic screening is part of a useful previous screening in the evaluation and pharmacotoxicological functioning of the substances evaluated, thus being able to consider that such alterations did not lead to relevant signs of toxicity. The morphological analysis of the liver and kidney analyzed the presence or absence of the following histopathological parameters: steatosis (fatty degeneration), necrosis, leukocyte infiltration, hyaline degeneration, hydropic degeneration and fibrosis. No histopathological alterations were found and the organs showed normal histological patterns, Figure 4 and 5.

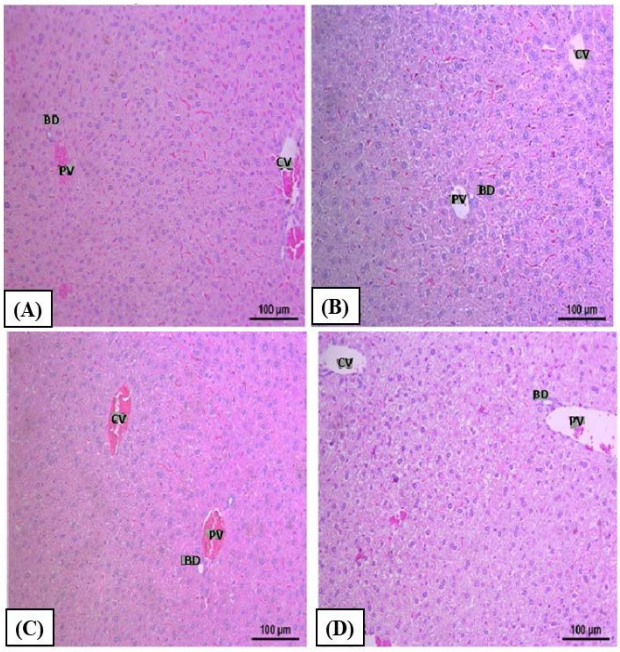


**Figure 4.** Photomicrographs of vital organs showing H&E staining of liver and kidneys. (A) (300 mg/kg). (B) (2000 mg/kg). (C) (5 mg/kg). (D) (50 mg/kg). Transverse section showing preserved bile duct (BD), Portal Vein (PV), central lobular vein (CV). 100x Increase. Source: Authors

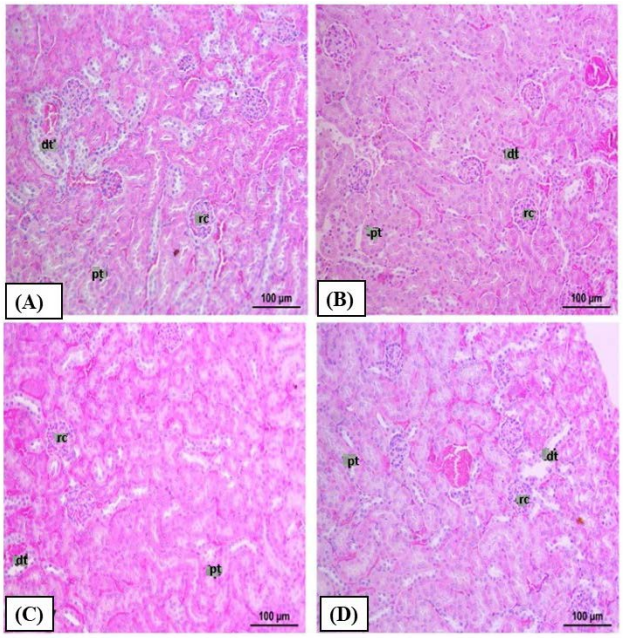


**Figure 5.** Photomicrographs of vital organs showing H&E staining of liver and kidneys. (A) (300 mg/kg). (B) (2000 mg/kg). (C) (5 mg/kg). (D) (50 mg/kg). Transverse section showing preserved renal twilight (rc), Proximal contorted tubule (pt); Distal contorted tubule (dt). 100x Increase. Source: Authors

## 4. Discussion

The *Ae. aegypti* is a mosquito vector responsible for several arboviruses transmission, such as dengue, zika, chikungunya and yellow fever and, due to this, the spread of these arboviruses has become a global public health concern, especially in emerging countries located in tropical and subtropical regions [17]. The growing population of this species in Brazil and in the world and the difficult control due to the high adaptability and resistance of the eggs to climatic factors reflects the urgent need to find sustainable and effective alternatives for mosquito control with the use of natural sources containing bioactive molecules found in oils, such as fatty acid esters [18]. The fatty esters identified in this study are basically three fatty acid molecules attached to a glycerol, resulting from the esterification reaction where the hydroxyl group (OH) from glycerin reacts with the carboxyl group (COOH) of the fatty acid, resulting in the formation of an ester bond and the release of a water molecule, also known as triacylglycerols or triglycerides [19].

Previous studies have shown the potential of these compounds in the mortality of Culicidae larvae. Esters extracted from *Solanum lycocarpumna* fruits showed larvicidal activity against *Culex quinquefasciatus* with $LC_{50}$ values between 0.70 and 27.54mg/L [6]; as well as reports of interference in the physiology of the insect, such as morphological changes, especially in the intestinal tract [20]. In addition, its natural origin offers advantages because it has low toxicity to non-target organisms. Glycerol, for example, present in fatty acid molecules has low acute and sub chronic toxicity, genotoxicity, carcinogenicity, and effects on reproductive development. As an endogenous mammalian metabolite, glycerol is rapidly absorbed, metabolized, and excreted [21].

The possible mechanisms of action of fatty acid esters on larval mortality may involve various biochemical and physiological processes that may vary depending on the specific structure of the ester and the mosquito species. One of the mechanisms of action is the systemic toxicity that occurs after ingestion or contact with fatty acid esters, where the substances are absorbed, causing the death of the insect [20]. For example, the leaf extract of *Schinus terebinthifolius* showed toxicity in *Ae. aegypti* larvae for causing injury to midgut [22].

Fatty esters can interfere with metabolic processes essential for insect development, such as protein synthesis, cellular respiration, or energy production [23]. They are also reported to act as endocrine disruptors, altering the functioning of the hormonal system of mosquito larvae resulting in death [24].

The relationship between the molecular structure of esters and their larvicidal activity can be complex and varied, since several factors involved in the structure of these substances can influence the action against Culicidae larvae. Some aspects of the molecular structure of esters are related to the size of the carbon chain, as the length of the chain can affect its solubility, cell permeability capacity, and affinity for biological targets [25,26]. In general, esters with longer chains tend to have higher larvicidal activity [10]. The presence of specific functional groups, such as hydroxyl, carbonyl or ether groups in the structure of esters, which can affect their physicochemical properties and interactions with biological targets, *i.e.*, functional groups can facilitate the penetration of ester molecules into cell membranes, which can increase the toxicity of mosquito larvae [27].

The degree of unsaturation of the esters may be involved with larvicidal activity. Double bonds in carbon chains (unsaturations) may exhibit different larvicidal activity from saturated ones. The presence of unsaturations can increase larvicidal activity through mechanisms related to interference in cell membrane integrity [28,29].

The spatial configuration of ester molecules can influence their ability to interact with specific biological targets, such as proteins or enzymes essential for the survival of mosquito larvae. In this sense, isomers with different configurations can exhibit distinct larvicidal activities [24].

Other compounds can even complement larvicidal activity, but fatty esters have biological activity ranging from attraction [30] and repellency [31] to insecticidal activity against *Ae. Aegypti* [10,32]. Fatty esters can act synergistically on mortality and, therefore, be considered a source of new natural bioinsecticides for the control of mosquito larvae [20].

In this study, the results obtained from the external morphological analysis show that the exposure to the ethanolic extract of the pulp of *C. brasiliense* directly influenced the modification of the structure of the larvae, especially in the continuity of the digestive tract, a structure composed of the foregut, midgut and hindgut, covered by a layer of columnar cells, important for nutrient absorption and survival and the loss of the internal structure of the digestive system are some of the alterations that consequently culminated in the death of the larvae. In addition, no histological alteration was observed, which means that the extract used does not pose

any risk, coinciding with the cytotoxicity values in normal cell lines with values higher than 250 µg/mL, indicating that it does not present cytotoxicity at the highest dose tested. Investigation of renal and hepatic functions in acute oral toxicity studies plays a crucial role in assessing the potential adverse effects of chemicals on the body. The liver and kidneys are vital organs responsible for metabolizing and eliminating toxins from the body, making them frequent targets of toxic-induced damage. As plant extracts have several phytochemical substances, it is possible that some develop liver and kidney damage [33], which was not observed in this study.

The understanding of the structure-activity relationship is essential for the rational development of new compounds with larvicidal potential and thus optimizing the efficacy and safety of these substances in the control of disease-transmitting mosquitoes.

## 5. Conclusion

The exuberant biodiversity of the Brazilian flora offers a variety of plant species rich in molecules with bioactive potentials that are still little explored. In this study, the ethanolic extract of the *C. brasiliense* fruit pulp proved to be efficient against of *Ae. Aegypti* larvae. The presence of saturated and unsaturated fatty esters is being considered in the literature as a promising alternative in the control of *Ae. aegypti*, and this is probably due to the structural conformity of its hydrophobic molecules that act synergistically with other compounds in cell membrane structures, which gives it bioactivity. Regarding the evaluation of the safety and feasibility of the compounds in cytotoxicity and acute oral toxicity tests, the ethanolic extract of the *C. brasiliense* fruit pulp proved to be safe, thus being considered an ecological alternative. The results of this study form the foundation for further studies aimed at understanding the structure-activity relationship of natural molecules in larvicidal activity against *Ae. aegypti*, and mosquito-borne disease control.

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