# LARVICIDAL ACTIVITY AGAINST AEDES AEGYPTI AND ANTIOXIDANT, CYTOTOXIC AND MICROBIOLOGICAL EVALUATION OF THE CRUDE ETHANOLIC EXTRACT OF POGOSTEMON CABLIN BENTH

# ATIVIDADE LARVICIDA CONTRA AEDES AEGYPTI E AVALIAÇÃO ANTIOXIDANTE, CITOTÓXICA E MICROBIOLÓGICA DO EXTRATO ETANÓLICO BRUTO DE POGOSTEMON CABLIN BENTH

# ACTIVIDAD LARVICIDA CONTRA AEDES AEGYPTI Y EVALUACIÓN ANTIOXIDANTE, CITOTÓXICA Y MICROBIOLÓGICA DEL EXTRACTO ETANÓLICO CRUDO DE POGOSTEMON CABLIN BENTH

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## ABSTRACT

The present work aimed to evaluate the larvicidal, antioxidant, microbiological and cytotoxicity activity of the crude ethanolic extract of Pogostemon cablin (Blanco) Benth leaves. The chemical characterization was performed through staining and staining reactions for the detection of secondary metabolite classes. The larvicidal activity against Aedes aegypti was carried out according to the protocol of the World Health Organization. The antioxidant activity was evaluated by the sequestering ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH). As for the microbiological evaluation, the microplate dilution technique was used against three bacteria, according to the protocol of the Clinical and Laboratory Standards Institute. P. cablin presented as classes of secondary metabolites: steroids and triterpenoids, depsides and depsidones, which in synergy with the other substances potentiated the

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larvicidal action of the species with an LC50 of 63.91 µg.mL-1 in 24 h. There was no antioxidant activity at the tested concentrations, however, it showed inhibition of bacterial growth against E. coli with MIC of 31.25 µg.mL-1. The extract showed moderate toxic action with LC50 of 257.93 µg.mL-1. Therefore, the P. cablin species showed significant larvicidal potential, with bacteriostatic action, the absence of antioxidant action and moderate toxicity.

**Keywords:** Biocide. Patchouli. Lamiaceae. Vector Control. Oriza.

**RESUMO**

O presente trabalho teve como objetivo avaliar as atividades larvicida, antioxidante, microbiológica e de citotoxicidade do extrato etanólico bruto das folhas de Pogostemon cablin (Blanco) Benth. A caracterização química foi realizada por meio de reações de coloração e testes específicos para a detecção de classes de metabólitos secundários. A atividade larvicida contra Aedes aegypti foi conduzida de acordo com o protocolo da Organização Mundial da Saúde. A atividade antioxidante foi avaliada pela capacidade sequestradora do radical 2,2-difenil-1-picrilhidrazil (DPPH). Para a avaliação microbiológica, utilizou-se a técnica de diluição em microplacas contra três bactérias, conforme o protocolo do Clinical and Laboratory Standards Institute. P. cablin apresentou como classes de metabólitos secundários: esteroides e triterpenoides, depsídeos e depsidonas, os quais, em sinergia com outras substâncias, potencializaram a ação larvicida da espécie, com $CL_{50}$ de 63,91 µg·mL$^{-1}$ em 24 h. Não foi observada atividade antioxidante nas concentrações testadas; entretanto, verificou-se inibição do crescimento bacteriano contra E. coli, com CIM de 31,25 µg·mL$^{-1}$. O extrato apresentou ação tóxica moderada, com $CL_{50}$ de 257,93 µg·mL$^{-1}$. Assim, a espécie P. cablin demonstrou significativo potencial larvicida, ação bacteriostática, ausência de atividade antioxidante e toxicidade moderada.

**Palavras-chave:** Biocida. Patchouli. Lamiaceae. Controle de Vetores. Oriza.

**RESUMEN**

El presente trabajo tuvo como objetivo evaluar las actividades larvicida, antioxidante, microbiológica y de citotoxicidad del extracto etanólico crudo de las hojas de Pogostemon cablin (Blanco) Benth. La caracterización química se realizó mediante reacciones de coloración y pruebas específicas para la detección de clases de metabolitos secundarios. La actividad larvicida contra Aedes aegypti se llevó a cabo de acuerdo con el protocolo de la Organización Mundial de la Salud. La actividad antioxidante se evaluó mediante la capacidad secuestradora del radical 2,2-difenil-1-picrilhidrazilo (DPPH). Para la evaluación microbiológica se utilizó la técnica de dilución en microplacas frente a tres bacterias, conforme al protocolo del Clinical and Laboratory Standards Institute. P. cablin presentó como clases de metabolitos secundarios: esteroides y triterpenoides, depsidos y depsidonas, los cuales, en sinergia con otras sustancias, potenciaron la acción larvicida de la especie, con una $CL_{50}$ de 63,91 µg·mL$^{-1}$ a las 24 h. No se observó actividad antioxidante en las concentraciones evaluadas; sin embargo, se evidenció inhibición del crecimiento bacteriano frente a E. coli, con una CIM de 31,25 µg·mL$^{-1}$. El extracto mostró una acción tóxica moderada, con una $CL_{50}$ de 257,93 µg·mL$^{-1}$. Por lo tanto, la especie P. cablin presentó un importante potencial larvicida, acción bacteriostática, ausencia de actividad antioxidante y toxicidad moderada.

**Palabras clave:** Biocida. Patchouli. Lamiaceae. Control de Vectores. Oriza.

## 1 INTRODUCTION

Liquid and solid synthetic insecticides are used in vector control of *A. aegypti.* They are generally accepted as effective, but they are carcinogenic, hazardous to the environment and non-target organisms. The compound N, N-diethyl-3-methylbenzamide, also known as DEET, is the product with significant insect repellency efficiency [1]. However, due to neurotoxicity allied to environmental claims, the population began to worry about its widespread use. [2, 3].

In the last years, the secondary metabolites found in plants have aroused researchers' interest to be used as alternatives to chemical insecticides [4-6]. In fact, insecticides of botanical origin have several advantages such as rapid action and degradation, low toxicity to mammals, greater selectivity and low phytotoxicity [5,7].

The mosquito *A. aegypti* L. (Diptera: Culicidae) is the transmitter of dengue, yellow fever, Chikungunya, and Zika, which cause severe morbidity and mortality in humans [8-10]. The etiology of *A. aegypti* influences its wide dispersion, favored in urban environments, preferably in the domiciliary conditions offered by human's way of living. The presence of the breeding grounds in an environment of human conviviality favors the rapid proliferation of the species, due to two aspects: ideal breeding conditions and feeding sources [1].

The use of insecticides to control adult (adulticidal) and larval (larvicidal) mosquito populations can be done through focal and non-focal treatment by the aerospace spraying of ultra-low volume insecticides (UVB). Repellents can be applied to the individual's skin to repel mosquitoes and avoid stings [11].

However, there is increasing the resistance of mosquitoes to synthetic insecticides, as well as negative impacts on the environment. Thus, it is important to search for alternative methods to be used in the control of *A. aegypti*, which are efficient, low cost, biodegradable and more selective [12].

In this context, species of the Lamiaceae family present potential for obtaining essential oils and plant extracts, they have several biological functions used in the treatment of diseases in folk medicine, as well as reports of anti-influenza, insecticide, repellent, antibacterial and anti-intestinal parasite activities [13].

*P. cablin* is a species of the Lamiaceae family, popularly known as Oriza or Patchouli, traditionally used for medicinal purposes, especially for the treatment of nausea, headache and heart problems, as well as proven biological activities such as antioxidant, analgesic, anti-inflammatory, antiplatelet, antithrombotic, aphrodisiac, antidepressant, ant mutagenic, antiemetic, fibrinolytic and cytotoxic [14-17].

Considering the potentiality of species of the Lamiaceae family and the need for more studies aimed at solving public health problems, in particular, those caused by the *A. aegypti* vector, it is important to adopt alternative strategies with greater investments in appropriate methods. Thus, the present research had the objective of studying the larvicidal activity against *A. aegypti* of the crude ethanolic extract of *P. cablin* leaves. This also includes antioxidant, microbiological and cytotoxic activities, which will serve as a complementary study to evaluate the potentiality of the species for future formulation of a natural biocide.

## 2 MATERIAL AND METHODS

### 2.1 PLANT MATERIAL

The species *P. cablin* was collected in Fazendinha district (0 ° 01'08 "S and 51 ° 06'17" O) in Macapá-Amapá Municipality, Brazil. For botanical identification, the sample of the species was sent to the Herbarium of the Institute of Scientific and Technological Research of the State of Amapá (IEPA), and it was registered under number 019183.

### 2.2 VEGETABLE EXTRACT

The leaves of *P. cablin* were oven dried at 50 ° C for a period of 48 hours and manually ground (400g of the plant material). The plant material was placed in a suitable vessel and ethyl alcohol (96%) was added until complete submersion. Every 3 days, the ethanol extract was filtered and placed in a rotary vacuum evaporator (totaling three extractions), under the following conditions: temperature of 50ºC and pressure of 500 to 760mmHg [18].

### 2.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The qualitative phytochemical analysis of crude ethanolic extract was performed according to Barbosa et al. [19], in which methods of precipitation and staining reactions were applied to for the detection of cardiac glycosides, catechins, flavonoids, purines, anthraquinones, steroids and triterpenoids, depsides and depsidones, polysaccharides, phenols and tannins, proteins and amino acids, alkaloids, reducing sugars, azulenes, organic acids, and saponins.

### 2.4 QUANTITATIVE PHYTOCHEMICAL ANALYSIS

#### 2.4.1 Total phenolic content

The quantification of total phenolics was determined by the Folin-Ciocateu method, according to Amorim et al. [20], with modifications. An aqueous solution of gallic acid (5000 $\mu g.mL^{-1}$) was prepared for successive dilutions. Subsequently, the calibration curve was

carried out at concentrations of 10 to 500 µg.mL$^{-1}$ and 400 µL of Folin-Ciocateu (10%) aliquots were added to 1600 µL of Na2CO3 (75 g.L$^{-1}$). The mixture was incubated at 25 ° C for 2 hours and the absorbance was measured in a spectrophotometer with a wavelength of 760 nm. After reading the calibration curve from the samples, an aqueous solution of extract (1 mg.mL$^{-1}$) was prepared with 200 µL added in a 10 mL flask, 400 µL of Folin-Ciocateu (10%) and 1600 µL Na2CO3 (75 g.L$^{-1}$), in triplicate, to quantify the total phenolic content. The results were expressed as mg equivalent of gallic acid per gram of extract (mg EAG/g).

## 2.5 LARVICIDAL ACTIVITY

The larvae of *A. aegypti* used in the bioassay were from the colony kept in the laboratory of the Medical Entomology of the Institute of Scientific and Technological Research of the State of Amapá (IEPA), where the larvicidal test was carried out. Biological assays were conducted under controlled climatic conditions with a temperature of 25±2 °C, relative humidity of 75±5 %, and a photoperiod of 12 h.

The methodology used followed the standard protocol of the World Health Organization [21] with modifications. The extract of *P. cablin* (0.09 g) was dissolved in 85.5 ml of distilled water and 4.5 mL of Tween 80. For the negative control, 1% Tween 80 and distilled water were used. As for the positive control, Malathion larvicide was used in commercial concentration. The extract solution was separated in triplicates at concentrations of 20 to 100 µg.mL$^{-1}$ in Becker of 100 mL, and 25 larvae of the *A. aegypti* mosquito in the 3rd young stage (L3) were added. After 24 and 48 hours, the dead larvae were counted, they being considered as such all those that were unable to reach the surface.

## 2.6 DETERMINATION OF ANTIOXIDANT ACTIVITY

The antioxidant activity was evaluated according to the methodology of Chen et al. [22] and Lopez-Lutz et al. [23] by the sequestering ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activity was calculated [24] as follows:

$$(\%AA) = 100-\{[(Abs_{sample} – Abs_{white})100]/Abs_{control}\}$$

%AA – percentage of antioxidant activity

$Abs_{sample}$ – Sample absorbance

$Abs_{white}$ – White absorbance

$Abs_{control}$ – Control absorbance

A methanolic solution of DPPH at the concentration of 40 µg.mL$^{-1}$ was prepared. The extract was diluted in methanol at different concentrations of 7.81 to 250 µg.mL$^{-1}$. Triplicates

with 0.3 mL volume of the extract solution per tube were performed with 2.7 mL of the DPPH solution. In parallel, the negative control was prepared with 2.7 mL of methanol and 0.3 mL of the methanolic solution. For the positive control, ascorbic acid was used in the same conditions of preparation of extract. After 30 minutes of incubation at room temperature and protected from light, the absorbance was measured in a spectrophotometer (Biospectro SP-22) at wavelength 517 nm, in a quartz cuvette.

## 2.7 ANTIMICROBIAL ACTIVITY

### 2.7.1 Bacterial strains and culture conditions

Two gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 25922 and *Escherichia coli* ATCC 8789) and gram-positive bacteria (*Staphylococcus aureus* ATCC 25922) were used in this bioassay.

A stock culture in BHI (Brain Heart Infusion) environment, with 20% glycerol-preserved at - 80 ° C was prepared for each microorganism. An aliquot of 50 µL of this culture was inoculated into 5 mL of sterile BHI broth environment and incubated for 24 hours at 37 ° C.

### 2.7.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The microplate dilution technique (96 wells) was used for determination of MIC and MBC, according to the protocol established by the Clinical and Laboratory Standards Institute [25], with adaptations.

Initially, the bacteria were reactivated with stock cultures and kept in BHI broth for 18 h at 37 °C. The inoculum in 0.9% saline solution was prepared for each microorganism, adjusted for the McFarland 0.5 scale, followed by dilution in BHI and tested at 2 x 106 CFU.mL$^{-1}$.

For MIC determination, the extract was diluted in Dimethyl sulfoxide (2% DMSO). The first well column of the plate was filled with 0.2 mL of the extract solution at the concentration of 2000 µg.mL$^{-1}$, the other wells were filled with 0.1 mL of 0.9% NaCl. Subsequently, base two serial dilutions were performed in the ratio of 1: 2 to 1: 128 until the dilution in a final volume of 0.1 mL. The cells (2 x 106 CFU.mL-1) with 0.1 mL adjusted according to the previous item were added to each well, resulting in a final volume of 0.2 mL. There were performed the control of the culture environment, the control of extract, and the negative control (DMSO 2%). For positive control, amoxicillin (50 µL.mL$^{-1}$) was used. The experiments were carried out in triplicates. The microplates were incubated in an oven at 37 °C for 24 hours. After this time, the plates were read in ELISA reader (DO 630 nm).

The MBC was determined based on the results obtained in the MIC test. Microplate wells were replicated in Müller-Hinton agar and incubated at 37 ° C for 24 h. MBC was established as the lowest extract concentration capable of completely inhibiting microbial growth in Petri dishes after 24-48 hours of growth.

The results were categorized in Microsoft Excel (Version 2010 for Windows) and then, analyzed in GraphPad Prism software (Version 6.0 for Windows, San Diego California USA). Significant differences between the groups were verified using the One-way ANOVA test with Bonferroni post-test, considering $p < 0.001$.

### 2.8 CYTOTOXIC ACTIVITY

The evaluation of the cytotoxic activity was performed against the larvae of *Artemia salina* Leach [26, 27] with adaptations. A solution of 250 mL of synthetic sea salt at 35 $g.L^{-1}$ was prepared, 25 mg of exposed saline eggs were incubated in 24 h photoperiod to reach the methanuplion stage. The stock solution was prepared to contain 0.06 g of extract, 28.5 mL of the solution of synthetic sea salt and 1.5 mL of Tween 80. Seven groups of samples were divided in triplicate in the concentrations of 50 to 1000 $\mu g.mL^{-1}$, and 10 methanuplii were added in each test tube. In the end, the number of non-survivors for $LC_{50}$ determination was counted using the SPSS® software PROBIT analysis.

### 2.9 STATISTICAL ANALYSIS

The data analysis was performed through analysis of variance (ANOVA) and the Tukey test, in the BioEstat program, in order to identify significant differences between the averages. The differences that presented probability levels less than and equal to 5% ($p \leq 0.05$) were considered statistically significant. The results were expressed as mean ± standard deviation (SD). The $LC_{50}$ values were determined in the PROBIT regression, through the SPSS program (Statical Package for the Social Sciences).

## 3 RESULTS

### 3.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of the extract of the leaves of *P. cablin* showed the presence of steroids and triterpenoids, depsides and depsidones, according to table 1.

**Table 1**

*Identification of the secondary metabolites of the extract of P. cablin*

| Tests | Results |
|---|---|

| | |
|---|---|
| Cardiac glycosides | - |
| Catechins | - |
| Flavonoids | - |
| Sesquiterpenolactones and other lactones | - |
| Purines | - |
| Anthraquinones | - |
| Steroids and triterpenoids | + |
| Depsides e depsidones | + |
| Polysaccharides | - |
| Phenols and catheter tannins | - |
| Proteins and amino acids | - |
| Organic acids | - |
| Saponins | - |
| Azulenes | - |
| Alkaloids | - |
| Reducing sugars | - |

Signal (+) indicates presence of secondary metabolite, while signal (-) indicates absence

The identification of steroids and triterpenoids is due to the result of the appearance of the staining that ranges from blue evanescence to persistent green, which occurs due to the loss of the hydroxyl that activates the conjugated system of the steroid nucleus [28]. While depsides are esters of two or more units of hydroxybenzoic acids, and depsidones are biogenetically derived from depsides through an intramolecular oxidative coupling [29], the positive result of this class is indicated by the appearance of green, blue or gray coloration.

## 3.2 QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Table 2 shows the total phenolic content found in *P. cablin* leaves. The content of phenolic compounds was 4.02%, a relatively low result in which may be related to the absence of antioxidant activity.

**Table 2**

*Total phenolics of extract from leaves of P. cablin*

| Ethanolic Extract | Phenolic compounds (mg GAE/g) | Phenolic compounds (%) |
|---|---|---|
| EE | 40,27 µg/pipe | 4,02 |

3.3 LARVICIDAL ACTIVITY

The extract of *P. cablin* presented expressive larvicidal action, with $LC_{50}$ of 63.91 $\mu g.mL^{-1}$ in 24 h, and $LC_{50}$ of 64.58 $\mu g.mL^{-1}$ in 48 h, with $R^2$ of 0.914. These did not present statistical difference between the 24 h and 48 h periods with $p > 0.05$, as shown in table 3.

**Table 3**

*Percentage mortality (%) of A. aegypti larvae at different concentrations of P. cablin extract in two periods*

| Concentrations ($\mu g.mL^{-1}$) | Larvicidal activity (%) | |
|---|---|---|
| | **24 h** | **48 h** |
| **20** | 0.0 | 0.0 |
| **40** | 4.0 | 4.0 |
| **60** | 52.0 | 54.0 |
| **80** | 78.66 | 78.66 |
| **100** | 80.0 | 81.33 |
| **Control (+)** | 100 | 100 |
| **Control ( - )** | 0.0 | 0.0 |

3.4 ANTIOXIDANT ACTIVITY

In the evaluation of the antioxidant activity, the results showed the absence of this activity, since in the highest concentration (250 $\mu g.mL^{-1}$) the DPPH consumption was 24.52%, which was lower than the expected 50% of consumption, which caused a high $IC_{50}$ of 900.98 $\mu g.mL^{-1}$, according to table 4.

**Table 4**

Mean and standard deviation of the percentage of antioxidant activity of extracts of *P. cablin* in different concentrations.

| **Concentrations ($\mu g.mL^{-1}$)** | **% AA** |
|---|---|
| 7,81 | 14.29± 0.25 |
| 15,62 | 15.45± 0.73 |
| 31,25 | 17.80±0.83 |
| 62,5 | 18.24 ±0.62 |
| 125 | 20.5 ± 0.29 |
| 250 | 24.52 ± 0.42 |

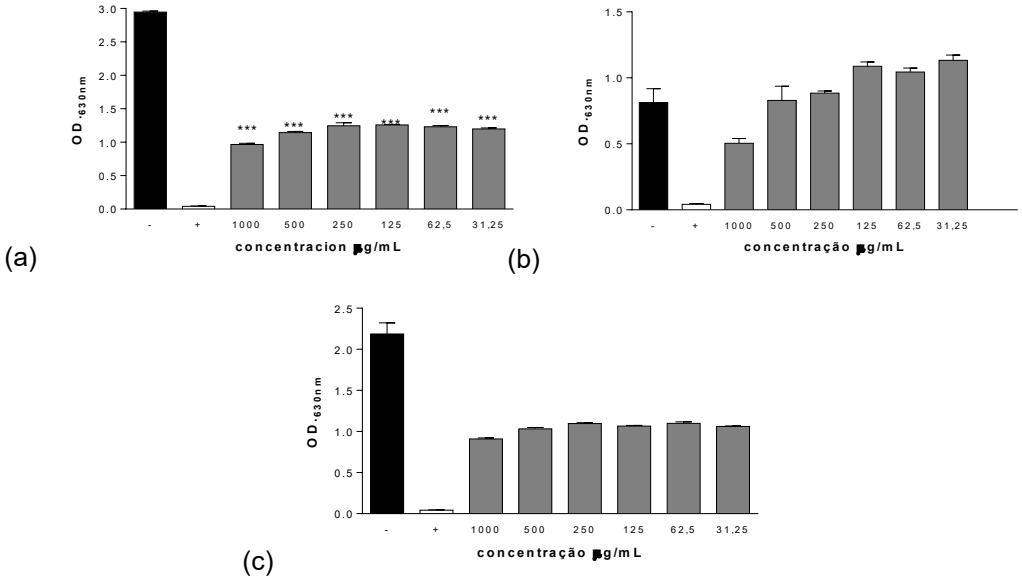
3.5 ANTIMICROBIAL ACTIVITY

The antimicrobial activity showed that *P. cablin* extract had a better bacteriostatic potential against *E. coli* bacteria, with a MIC of 31.25 $mg.mL^{-1}$ than for *S. aureus* and *P.*

*aeruginosa* bacteria. Regarding MBC, extract did not demonstrate bactericidal activity against the bacteria under test, according to Figure 1.

**Figure 1**

*Sensitivity Test (MIC) and (MBC) OE of P. cablin against the E. coli, S. aureus and P. aeruginosa*




MIC and MBC of the EO of *P. cablin* against (a) *E. coli,* (b) *S.aureus* and (c) *P. aeruginosa*Source: Own author. Substance test (■), BHI with 2% DMSO (□) and Amoxiline (▩). * P <0.001 statistically significant in relation to the negative control, # p <0.001 statistically significant in relation to the positive control.

## 3.6 CYTOTOXIC ACTIVITY

In the evaluation of the cytotoxic activity, the extract of *P. cablin* presented moderate toxic action, with $LC_{50}$ of 257.93 and $R^2$ 0.981, p <0.00. Table 5 shows the mean mortality readings performed in the 24 hour extract exposure against *A. salina* larvae.

**Table 5**

*Percentage of mortality of A. salina larvae of P. cablin extract in different concentrations*

| Concentrations (µg.mL-1) | Mortality (%) |
|---|---|
| 50 | 10.0 |
| 100 | 23.3 |
| 250 | 56.6 |
| 500 | 63.3 |
| 750 | 76.6 |
| 1000 | 86.6 |

## 4 DISCUSSION

The study for the development of biocidal herbs against *A. aegypti* is recent, beginning in the 1980s, in order to isolate and characterize such bioactive substances. Many plant-based products have active compounds that act synergistically or in isolation, and they have characteristics that can be effective in controlling and monitoring mosquito populations [30].

The plants have mechanisms against insect action and are able to synthesize, from different metabolic pathways, defense compounds as secondary metabolites and proteins that act as insecticidal toxins [18].

In this context, the phytochemical tests performed with *P. cablin's* extract, the classes of substances such as steroids and triterpenoids, depsids and depsidones showed positive results.

The identification of the steroids and triterpenoids is due to the result of the appearance of the coloration that goes from the blue evanescence to the persistent green, which occurs due to the loss of the hydroxyl that activates the conjugated system of the steroid nucleus in the reaction [31]. Steroids are derivatives of acetate, in which they act to reduce cholesterol absorption, reduce risks of cardiovascular diseases and inhibit the growth of malignant tumors [28]. The triterpenoids are a condensed compound derived from terpenoids and their biosynthetic source of isoprene. One of its main biological activities is the antispasmodic function, in which it has the function of relaxing the intestinal smooth muscle, reducing cramps [32].

In the staining reaction of depsides and depsidones, there was a positive result from the appearance of the greenish coloration. This class of metabolites consists of phenolic compounds of multiple properties such as antioxidant, antiviral, antibiotic, antitumor, allergen, inhibition of plant growth, anti-tuberculosis and enzyme inhibitory activity [33].

In the determination of the total phenolics, the extract of *P. cablin* presented 4.02%, a significantly low result, suggesting the absence of antioxidant activity, since the phenolic compounds present in the plants are related to the most abundant antioxidants. Therefore, the greater the number of phenolic compounds, the vegetable will have expressive antioxidant activity that contributes to the processes of inhibition of the risk of cardiovascular diseases and may act on oxidative stress, related to several chronic-degenerative pathologies, such as diabetes, cancer and inflammatory processes [34]. Currently, there is a shortage of studies indicating the content of phenolic compounds in the extract of *P. cablin.*

Studies have proven the activity of plant extracts in the control of different species of mosquito [35-37], including *A. aegypti* [1,4, 8-10, 12,13,30]. These plants synthesize several types of compounds that have recognized entomotoxic potential and arouse the interest of

several researchers in the search for alternative strategies for the chemical control of *A. aegypti* [18].

In this context, *P. cablin's* extarct presented a significant larvicidal potential with $LC_{50}$ of 63.91 μg.mL$^{-1}$ in 24h, since samples with $LC_{50}$ below 100 μg.mL-1 are considered to be good larvicidal agents [38]. The significant larvicidal activity may be related to the class of terpenes identified in the preliminary chemical test of this species, considering that biocidal studies of the *P. cablin* [39-42] species considered the sesquiterpenes (among them patchouli alcohol) as main responsible for their larvicidal potentiality.

It is important to emphasize that to relate larvicidal activity to some chemical compound is still a complex task, since the biological effect may reflect the action of the major component or is the result of the synergistic action of the constituents.

As for the antioxidant analysis, Nascimento et al. [43] emphasize that the antioxidant test sample that has high potential in sequestering free radicals has a low IC 50 value. Thus, from a small amount of sample, there is a decrease in the initial concentration of the DPPH radical by 50%, to inhibit the radical oxidation by 50%. In conclusion, the results observed in this study did not demonstrate antioxidant activity, since the $IC_{50}$ of the correlation between antioxidant activity (%) and the extract concentration was 900.98 μg.mL$^{-1}$ when compared to the standard of ascorbic acid (vitamin C) with $IC_{50}$ of 16.71 μg.mL$^{-1}$. These results are linked to the phytochemical profile of the species, in which Maqsood et al. [44] state that ketone or phenolic substances present in plants influence the antioxidant activity.

In this scenario of research related to the search of natural bioactive compounds, the use of new substances with antimicrobial activity has aroused the interest of the scientific community, because some bacteria have resistance to synthetic antibiotics [45]. Thus, drugs that are manufactured from natural compounds appear as a promising alternative for the effective treatment of infectious diseases.

The plant extracts have compounds with antimicrobial potential, they act with a mechanism of action on the bacteria interconnected to the disturbance of the cytoplasmic membrane, cytoplasmic coagulation, change in electron flow, disruption of proton power, alteration of active transport and reduction of intracellular ATP pool [46,47].

In this study, in the evaluation of the antimicrobial activity, the extract of *P. cablin* prevented the bacterial growth only against E. coli bacteria, with MIC 31.25 μg.mL$^{-1}$, and it showed no bactericidal activity. Liu et al. [48] found the anti-microbial activity of *P. cablin* extract against Rhizopus nigricans, demonstrating its efficacy against infectious microorganisms. However, there are limited reports on the potentiality of the microbial activity of *P. cablin* extract, instigating further studies to clarify such biological activity.

The evaluation of the toxicity of a plant species is an important bioassay to verify if it can be used as herbal medicine. In this context, the preliminary toxicological bioassay with *A. salina* allows evaluating if the effects that a compound produces in these microcrustaceans are applicable to humans. It is necessary to make only mathematical corrections to verify the appropriate dose per unit of the body surface since the toxic effects caused in laboratory animals are approximately similar to those caused in humans [49].

The extract of *P. cablin* presented moderate toxicity, according to the classification of Lopez-Lutz et. al [23], in which high toxicity is considered $LC_{50}$ values less than 100 $\mu g.mL^{-1}$, moderate toxicity between 100 and 500 $\mu g.mL^{-1}$, weak toxicity between 500 and 1000 $\mu g.mL^{-1}$, and $LC_{50}$ above 1000 $\mu g.mL^{-1}$ are considered to be non-toxic.

The level of toxicity of a plant species depends on the chemical compounds that constitute it. In the species *P. cablin* it is estimated that the toxicity is related to the class of terpenes identified in its composition [50]. However, the plant extract of *P. cablin* may be more toxic than its isolated compounds, since the synergy between the substances potentiated the significant toxic action.

## 5 CONCLUSION

The preliminary chemical composition of *P. cablin* extract indicated the presence of the following classes of secondary metabolites: steroids and triterpenoids, depsides and depsidones.

*P. cablin's* extract demonstrated significant larvicidal potential and low toxicity in the $LC_{50}$ found in this study, which can be used to control mosquito larvae without causing a cumulative effect in humans and the environment.

As for the antioxidant evaluation, there was no evidence of antioxidant activity by the DPPH radical capture method when compared to the vitamin C standard.

The antimicrobial activity showed that the extract of *P. cablin* presented a bacteriostatic action in the concentration of 31.25 $mg.mL^{-1}$ only against E. coli bacteria.

The data show the relevance of the bioassays as a screening of the biological potential of the *P. cablin* species, as well as the importance of these products as a source of biocidal compounds. Also noteworthy is the lack of studies related to the biocidal activities of *P. cablin's* extract.

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