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Research Article

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Larvicide and antioxidant activity of the ethanol crude extract from the stem bark of *Pseudoxandra cuspidata* (Annoaceae)

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ABSTRACT

In Brazil, there is an epidemiological crisis scenario in relation to arboviruses like dengue, Chikungunya, and Zica, where all have in common vector, Aedes aegypti. The species Pseudoxandra cuspidate Maas (1983), is from Annonaceae family, popularly known as Lamuci, Envira, Amarela, Envira Preta, Envira Lamuci, and is referred to by traditional medicine with anti-inflammatory, antifungal and antimalarial; It is endemic in the north, but little studied in relation to its biological potential and its chemical constituents. The Pseudoxandra cuspidate following the chemotaxonomic profile of the Annonaceae family is rich in alkaloids. Based on these assumptions, this study has to analyze the potential larvicide with Aedes aegypti and antioxidant activity of the crude ethanol extract of P. cuspidata. In relation to the antioxidant activity of the crude ethanolic extract, IC50% = 326.85 µg/ml and in relation to larvicidal activity against Aedes aegypti intermediate stage of LC50% for 24 hours was 437.79 ppm for 48 hours and concentration was 270.85 ppm. In both activities, the result was significant, starting new possibilities of using the ethanol crude extract Pseudoxandra cuspidata.

Keywords: Medicine Plants, Natural Product, Lamuci, Antioxidants, Antimicrobial Activity.

INTRODUCTION

In Brazil, there is an epidemiological crisis scenario in relation to arboviruses such as Dengue, Zica, and Chikungunya, which all have in common vector, the *Aedes aegypti*, so natural products that contribute to the elimination or control early stages, intermediate or repellency have great value for the health of the population [1].

One of the ways to encourage the protection is sustainable use, making it possible the economic development of the region and use of forest resources in the Brazilian Amazon [2]. The family Annonaceae has about 2,500 genera and 135 species worldwide. In Brazil, there are 386 species distributed in 29 genera. The Amazon region has three-quarters of the Annonaceae of Brazil, with 27 genera and 280 species [3-6].

Gender Pseudoxandra sp. has 14 species in Brazil, most in Amazon forest. There are only two species in the Atlantic forest, *P. spiritus-sancti* Maas, and *P. bahiensis* Maas. The Pseudoxandra's group are trees characterized by leaves with prominent primary rib on the upper face, short and articulate pedicel with two to several bracts below the joint and none above, globular buttons, imbricated, rounded and concave petals, carpels pointed to a marginal egg and globose with a flat seed with equatorial groove [4,7].

The medicinal plants that have special metabolites also called natural active principles or secondary metabolites are typically classified from their biosynthetic route.

The main families of secondary metabolites are phenolic compounds, terpene compounds, steroids, and alkaloids. The alkaloids are defined as compounds having nitrogenous organic bases, they have a nitrogen in its formation, originating linked to amino acids (similar to proteins) and pharmacologically active [11,12]. The *Pseudoxandra cuspidata* following chemotaxonomic profile family Annonaceae is rich in alkaloids, in particular, aporphinoids [13].

Due to climatic conditions found in the state of Amapá (Brazil), which favor the development and proliferation of *A. aegypti* (vector of diseases known as arboviruses), it is the better look for alternatives for vector control, as the search for new insecticides agents from concentrated extracts.

Based on these assumptions, this study has to analyze the potential larvicide with *Aedes aegypti* and antioxidant activity of the crude ethanol extract of *Pseudoxandra cuspidata*.

EXPERIMENTAL SECTION

Plant Material

Study featured how qualitative and quantitative, based on knowledge empirical, experimental and exploratory. The plant species was collected in a particular field in Pedra Branca, a municipality which is located 188 km away from the state capital (Macapá - Amapá - Brazil). Vegetable leaves were removed (for botanical analysis) and the bark of the stem (for the preparation of extracts). The voucher specimen of the species was cataloged by a specialist in the field, in the Herbarium of the Federal University of Amapá, registration number: 458.

Sample preparation and obtaining the EBE

The husks were dried at 45 ° C for four days, then they were milled in a knife mill, TE-625 model. The dry milled material was used to prepare the crude ethanol extract (EBE) following the methodology proposed by Carvalho (2011) [14], macerated for 7 days, the solvent used was ethanol at 96 ° GL, the ratio was 1: 3 with respect to the raw material, the extraction solution was homogenized every 24 hours. It was filtered, and the solvent evaporated on rotaevaporator.

Evaluation of antioxidant activity of EBE against the kidnapping of free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH)

It was performed according to the methodology proposed by Souza et al. (2007) [15] Lopez-Lutz et al. (2008) [16] and Pitaro et al., (2012) [17] and as validation of the method, a calibration curve was prepared to evaluate the DPPH modifications during the change of concentration. It was prepared a DPPH solution of 40 µg/mL and some solutions with the EBE concentrations of 5; 2.5; 1; 0.75; 0.5 mg/mL and 0.25 mg/mL in methanol separately. After 30 minutes the decline of radical concentration was monitored by spectrophotometry visible at $\lambda = 517$ nm. Absorbance measurements were made in a spectrophotometer Biospectro SP-22. The experiment was performed in triplicate and the mean absorption was analyzed for each concentration and the positive control. The percentage of antioxidant activity was calculated according to Sousa et al. (2007) [15].

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

AA (%) = Percentage of antioxidant activity
Abs sample = absorbance of the sample
Abs white = white Absorbance
Abs control = control Absorbance

Larvicidal activity of EBE front of larvae in the L3 stage of *Aedes aegypti*

The larvae of *Aedes aegypti* used in bioassays are from the insectarium's Laboratory Arthropoda from the Federal University of Amapá, all generation F10 (generation Macapá - AP), the 3rd young stage. The biological tests were conducted in a room (3m x 4m) with controlled climatic conditions: temperature 25 ± 2 ° C, relative humidity of 75 ± 5%, 12-hour photoperiod.

The methodology followed the standard protocol of the World Health Organization [18], with adaptations, such as the modification of the test container. After analyzing preliminary test series concentrations were selected: 500, 400, 300, 200 and 100 ppm of EBE of the stem bark of *P. Cuspidata*.

A solution was prepared with 465 mg of SBS, and pre-solubilized in Tween 80 and dissolved in 93 ml of water to obtain the concentration of 5000 ppm. From this stock solution, serial dilutions were prepared to obtain concentrations of 500, 400, 300, 200 and 100 ppm. For each replicate of a treatment, it was used 10 larvae of *Aedes aegypti*, pipetted into a beaker containing 100 ml distilled water. During the experiment, the average temperature of the water was 25 °C. After 24 and 48 hours dead larvae were counted, all those being considered dead that did not move after stirring for a period of at least 1 minute. The Obtained data of mortality (%) x concentration (ppm) were analyzed by SPSS in Probit graph to determine the lethal concentration which causes 50% mortality of the population (LC50). The variation of the mean in biological assays was evaluated by the variance (ANOVA) with 95% confidence intervals.

RESULTS AND DISCUSSION

Antioxidant activity of EBE from *P. cuspidata*

For the validation of the analytical method was prepared an analytical curve for analyzing variations of DPPH and calibration of equipment, targeting specificity, precision and accuracy of the results obtained, as can be seen in Figure 1.

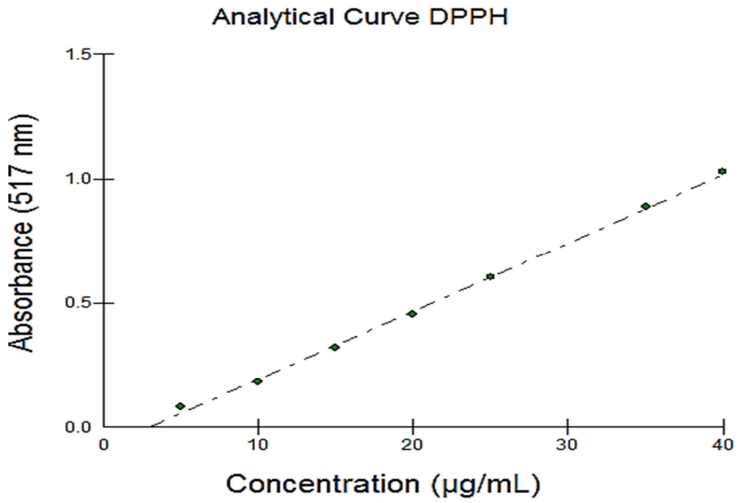

Figure 1 - Analytical Curve DPPH

The DPPH has free radical scavenging activity based on electron transfer; it is used during the test as a control to determine antioxidant activity [19]. In the table is presenting the percentage of antioxidant activity for the EBE *Pseudoxandra cuspidata*.

Table 1 - Mean and standard deviation of the percentage of antioxidant activity of crude ethanol extract *Pseudoxandra cuspidate*

Concentration (mg/mL)	Antioxidant activity (%)
5	95.321 ± 1.22 [a]
2.5	94.541 ± 0.31 [be]
1	94.444 ± 0.42 [bf]
0.75	94.086 ± 0.22 [bfg]
0.5	93.859 ± 0.45 [cefgh]
0.25	93.421 ± 0.31 [defh]

Vertically, values (% AA) followed by the same letter do not differ significantly for ANOVA (p <0.05)

Table 2 - Mean and standard deviation of the percentage of antioxidant activity of lower solutions concentration of Ethanoic crude extract of *Pseudoxandra cuspidata*

Concentration (µg/mL)	Antioxidant activity (%)
500	73.278 ± 4.87 [a]
250	41.471 ± 1.2 [b]
150	26.869 ± 0.95 [c]
75	11.223 ± 1.21 [d]
50	10.457 ± 1.07 [d]

Vertically, values (% AA) followed by the same letter do not differ significantly for ANOVA (p <0.05)

It was observed that the extract at lower concentrations obtained 93.421% of antioxidant activity, being necessary to reduce the concentrations tested to determine the inhibitory concentration 50% (IC 50%). The concentration of the

solution was reduced to 5 mg/mL to 1 mg/mL (1000 µg/ mL) and this solution at concentrations solutions were made at 500, 250, 150, 75 and 50 µg/mL, the results are Table 2.

Using the SPSS software was observed that the results are in congruence with the largest linear regression through it was possible to obtain the IC50% 326.85 mg / ml of the EBE *Pseudoxandra cuspidata*, which was established by linear regression, where R^2 determination coefficient was 0.992 as shown in Figure 2.

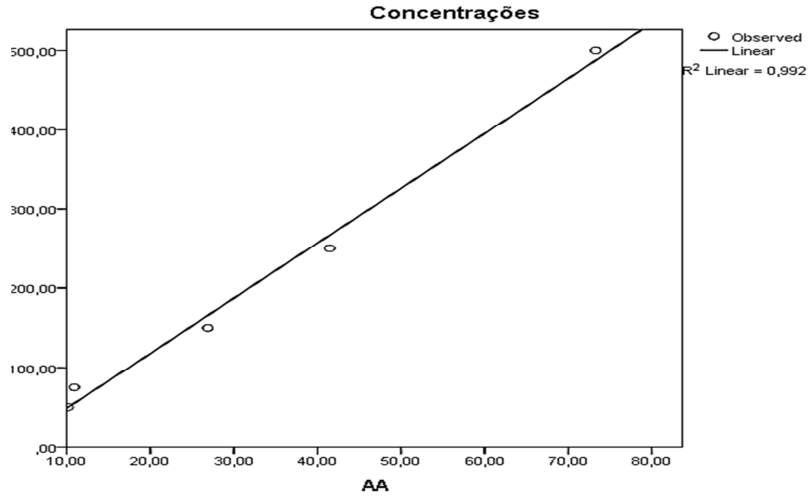

Figure 2 - Relationship between the tested concentrations and antioxidant activity in% of EBE *P. cuspidata*

Larvicidal Activity of crude ethanoic extract *P. cuspidata*

In the survey assessed the potential of the EBE *Pseudoxandra cuspidata* regarding the larvicidal activity against strains of *Aedes aegypti*. During larvicidal activity observed the contact extract (EBE) at different concentrations during the 24 and 48 hours, characterizing acute or delayed larvicidal action. In Table 3 shows the relationship of mortality with contractions.

Table 3 - Correlation of mortality and concentrations within 24 and 48 hours EBE P. cuspidate

Mortality %					
Concentrations	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
24 Hours	0.85	8.47	15.94	36.10	69.71
48 Hours	17.06	32.03	47.73	64.80	84.08

To determine LC50% during the first 24 hours of exposure was performed analyzing the relationship between the data obtained from the mortality (%) x concentration (ppm) in SPSS program in Probit graph to determine the lethal concentration that causes death 50% of the population (LC50). Significant differences were observed between the means in ANOVA test and confirmed by the Tukey test, where $p < 0.05$ and the results are in the range of 95% confidence interval logo are statistically significant. The CL50% of EBE for 24 hours was 437.79 ppm, established through regression Probit, as can be seen in Figure 2.

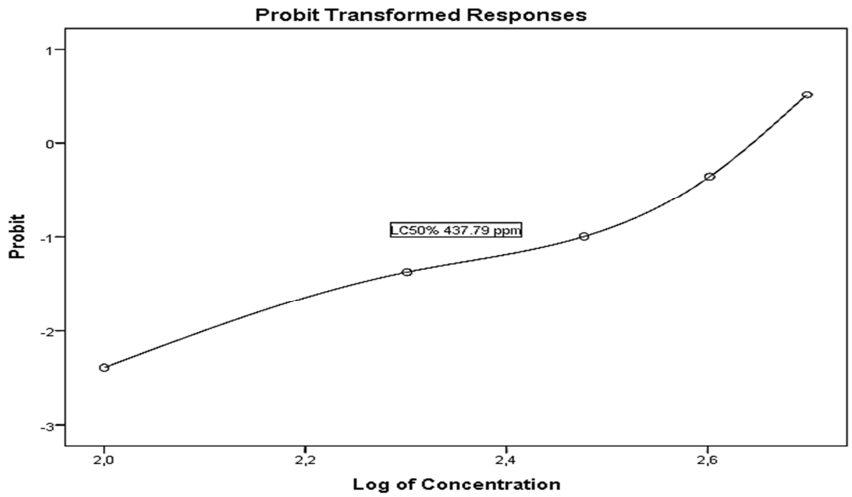

Figure 2 - Relationship regression of probit mortality (%) log concentration in 24 hours

The use of Probit aims is to predict the likelihood of an event for any value of stress in the experimental group (at different concentrations), especially for him to make a binary analysis (life and death) as is the case with the larvicidal potential study.

To obtain the LC 50% within 48 hours added to the same period of waiting, just for the larvicidal activity extended were tested. The LC50% value calculated by Probit regression of EBE was 270.85 ppm as shown in Figure 3.

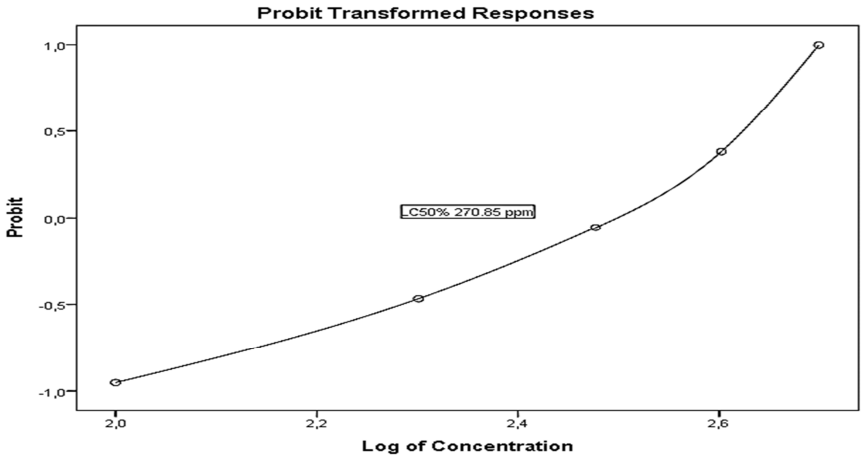

Figure 3 - mortality rate ratio with increasing concentration in 48 hours

It was observed that the EBE increased its larvicide action with 24 hours increased contact with the larvae, featuring prolonged larvicidal action. The presence of alkaloids in its constitution may be presumed as responsible for the larvicidal activity because they possess interactivity with the nervous system [10,11]. In other studies where the majority of plant species composition was alkaloid action larvicide was proven to *Culex quinquefasciatus* [20].

According to Cheng criteria (2003) [22], the sample is considered potential larvicidal when their LC50% is below 100 ppm within 48 hours, but it takes into account pure fractions or essential oils with high content purified and low contaminant substances or purpose of substances not shown, soon to plant extracts should review the criteria for characterization of the biocidal potential. At the moment the most affordable and economically viable control of arboviruses is the elimination of the vector through natural biocidal products, elimination of breeding. The biggest problem in relation to chemical insecticides that is caused by use over the production of resistant and/or high rate of adaptability strains, a super-tough vector creates a higher viability for transmission of the virus [17, 23].

The search for alternative methods to be used for controlling the Aedes is in evidence, and these methods should provide efficiency, be economically viable, biodegradable and selective. Among the main botanical insecticides are highlighted by the Brazil hold great biodiversity, making the potential in this area of research.

Through the observed results can highlight the antioxidant action against the radical DPPH, other methods of analysis of antioxidant activity should be tested so that it can be established which can involve mechanism of action, and thus demonstrate that the inhibition of oxidative activity can be by primary (a natural product) or synergistic action of various compounds (such as alkaloids). While fighting oxidative stress and free radicals coming from the same EBE also fights intermediate stages of *Aedes aegypti*.

CONCLUSION

From the results obtained, it is concluded that the EBE from stem bark of Pseudoxandra cuspidata has in its chemical constitution promising secondary metabolites with antioxidant action. The extract showed potential activity larvicide in *Aedes aegypti*, having necessity explore others biological activities and isolation of substances of extract for characterization of the chemical profile.

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