Artigo Original

ESTUDO LARVICIDA EM *AEDES AEGYPTI* (LINNAEUS, 1762), CITOTÓXICO E FITOQUÍMICO DO EXTRATO ETANÓLICO BRUTO DE *MIKANIA LINDLEYANA* DC.

LARVICIDAL STUDY IN AEDES AEGYPTI (LINNAEUS, 1762), CYTOTOXIC AND PHYTOCHEMICAL OF THE CRUDE ETHANOLIC EXTRACT OF MIKANIA LINDLEYANA DC.

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Resumo

Palavras-chave: atividade biológica, biocida, sucuriju.

Mikania lindleyana é uma planta pertencente à família Asteraceae, conhecida popularmente como Sucuriju, é uma trepadeira de grande porte, perene utilizada na medicina popular como anti-inflamatória, analgésica, cicatrizante, diurética, anti-hipertensiva no tratamento contra varizes e acne. As plantas desta família apresentam-se como potenciais biocidas no controle do *Aedes aegypti*. O objetivo desta pesquisa foi avaliar a atividade larvicida contra *Aedes aegypti*, o estudo fitoquímico e de citotoxicidade do extrato etanólico bruto de *Mikania lindleyana*. A triagem fitoquímica do extrato etanólico bruto revelou saponinas, azulenos e depsídeos, esses metabólitos secundários que podem justificar algumas atividades biológicas da espécie vegetal. A atividade larvicida apresentou CL_{50} de 146,32 $\mu g.mL^{-1}$, o extrato foi capaz de causar a morte de indivíduos na concentração de 100 $\mu g.mL^{-1}$ em 48 horas de contato com as larvas. O extrato contra *Artemia salina* não foi capaz de causar a morte de nenhum metanáuplio nas concentrações testadas. Esta pesquisa indicou a presença dos metabólitos secundários saponinas, azulenos e dépsidos no extrato etanólico bruto, indicou atividade larvicida contra larvas de Aedes aegypti provavelmente causada pela presença de saponinas e não apresentou citotoxicidade contra *Artemia salina* indicando baixa toxicidade do extrato.

ABSTRACT

Key words: biological activity, biocide, sucuriju

Mikania lindleyana is a plant belonging to the Asteraceae family, popularly known as Sucuriju, it is a large vine, perennial used in folk medicine as anti-inflammatory, analgesic, healing, diuretic, antihypertensive in the treatment against varicose veins and acne. Plants of this family present themselves as potential biocides in the control of *Aedes aegypti*. The objective of this research was to evaluate the larvicidal activity against *Aedes aegypti*, the phytochemical and cytotoxicity study of the crude ethanolic extract of *Mikania lindleyana*. The phytochemical screening of the crude ethanolic extract showed saponins, azulenes and depsides, these secondary metabolites that may justify some biological activities of the plant species. Larvicidal activity showed an LC50 of 146.32 $\mu g.mL^{-1}$, the extract was able to cause the death of individuals in the concentration 100 $\mu g.mL^{-1}$ in 48 hours of contact with the larvae. The extract against *Artemia salina* was not able to cause the death of any metanáuplium in the tested concentrations. This research indicated the presence of secondary metabolites saponins, azulenes and depsides in the crude ethanolic extract, indicated larvicidal activity against *Aedes aegypti* larvae probably caused by the presence of saponins and did not show cytotoxicity against *Artemia salina* indicating low toxicity of the extract.

INTRODUCTION

Aedes aegypti (Linnaeus, 1762) is the vector of human viral diseases of epidemiological interest such as Dengue, Chikungunya, Yellow Fever and Zika. Dengue is one of the most prevalent human arboviruses and responsible for 100 million annual infections, putting half the world's population at risk, its transmission occurs in more than 120 countries, mainly in the tropical and subtropical regions. Chikungunya, another virus transmitted by the arthropod, has caused more

than 2.5 million infections in the last decade and spreads in the Americas after emerging in Europe, imposing new challenges on health systems. The burden of Yellow Fever disease has been significantly reduced due to large-scale vaccination programs in the 20th century, but there are estimates of 51,000-380,000 serious cases in Africa per year, which point to the persistent difficulty in fully controlling this virus (KRAEMER et al., 2015; RODRIGUES, 2022). The spread of these diseases occurs by combinations of factors: increased urbanization, migration, international travel and difficulty in controlling the vector (WHITEHORN; FARRAR; 2010).

The development of culicid resistance to chemical insecticides and its toxicity motivate the search for new substances in the genetic heritage of the Amazon. The use of natural products in the formulation of new biocides as an alternative to control the vector of these diseases has been valued due to the composition of the natural agents being bioavailable and degradable because they act in new mechanisms of action (COSTA et al., 2005).

The key to controlling these diseases is based on the management of the larval population. However, using chemical substances for vector control faces resistance and Amazonian biodiversity may be the answer to this problem. Arnason et al. (1981) isolated twenty-four polyacetylenes from plant species of the Asteraceae Bercht & J.Presl family, and tracked their UV-mediated larvicidal properties close to the *Aedes aegypti* mosquito. One of these, σ-terpenil isolated from the Tagetes genus, showed greater toxicity than DDT in a trial scaled in a simulated field situation, demonstrating its potential as a larvicide of this family.

Among the species of the Asteraceae family with little explored biocidal potential for the chemical control of *Aedes aegpti*, *Mikania lindleyana* DC stands out, flowers gathered in a congestion chapter resulting in an achene fruit, is used as anti-inflammatory, analgesic, healing and in the treatment of chronic ulcers, varicose veins, acne, diuretic and antihypertensive (VANDERLINE et al., 2012). It presents a significant potential for the fragrance industry, being found in its essential oils the compounds α-felandreno, mirceno and β-karyophylene (MAIA; ANDRADE, 2009).

The objective of this research was to evaluate the larvicidal activity against *Aedes aegypti*, the phytochemical and cytotoxicity study of the crude ethanolic extract of *Mikania lindleyana*.

METHODOLOGY

Assessment of larvicidal activity

The *Aedes aegypti* larvae used for the test came from the insectary of the Medical Entomology Laboratory of the Institute of Scientific and Technological Research of the State of Amapá (IEPA). The methodology followed the standard protocol of the World Health Organization - WHO (2009) with modifications in the test containers.

For the stock solution, 0.09 g of the crude ethanolic extract was dissolved in 85.5 mL of distilled water together with 4.5 mL of Tween 80, and for the preparation of the negative control, 350 mL of distilled water and 17.5 mL of respectively were used. Tween 80. The stock solution was diluted to the following concentrations: 100, 80, 60, 40, 20 $\mu g.mL^{-1}$ and negative control. The concentrations were tested in triplicate with 25 larvae of *A. aegypti* in the third stage (L3). After 24, and 48 hours dead larvae were counted.

Assessment of cytotoxic activity

The cytotoxicity assay against *Artemia salina* Leach was based on the technique of Araújo and collaborators (2010), and Lôbo, and collaborators (2010) with adaptations. An aqueous solution of synthetic sea salt (35.5 $g.L^{-1}$) was prepared for the incubation of 25 mg of *Artemia salina* eggs, in which they were placed in a dark environment for 24 h to hatch the larvae (nauplii), then the nauplii were exposed to artificial light in a period of 24 hours to reach the stage of metanáuplios. The stock solution was prepared containing 54 mg of crude ethanolic extract, 22.5 ml of the synthetic sea salt solution, and 4.5 mL of 5% dimethyl sulfoxide (DMSO) added to facilitate its solubilization. The metanáuplios were selected and divided into 7 groups with 10 individuals in each test tube, performed in triplicate. Each group received aliquots of the mother solution (2500, 1250, 625, 250, 25, and 2.5 µL), which was then made up to 5 mL with a synthetic sea salt solution, obtaining final solutions with the following concentrations 1000, 500, 250, 100, 10 and 1 $\mu g.mL^{-1}$. Saline solution was used to control the test. After 24 hours the number of deaths was counted. The lethal concentration that caused 50% mortality in the population (LC50) was determined using Probit analysis using SPSS® software [version 20.0; SPSS Inc., Chicago, IL, USA].

Phytochemical prospecting

In phytochemical prospecting, the presence of saponins, polysaccharides, reducing sugars, proteins and amino acids, phenols and tannins, catechins, cardiac glycosides, sesquiterpenoids, azulenes, depsides and depsones was verified in the crude ethanolic extract, through colorimetric reactions and / or by precipitation in a qualitative way according to the methodology proposed by Barbosa et al. (2001).

RESULTS AND DISCUSSION

Assessment of larvicidal activity

Table 1 shows the result obtained from the exposure of Aedes aegypti larvae to crude ethanolic extract in 24 and 48 hours. The concentration 100 $\mu g.mL^{-1}$ was able to cause the death of 50.82% of the individuals.

Table 1 - Percentage of mortality of *Aedes aegypti* larvae in different concentrations of crude ethanolic extract of *Mikania lindleyana*

	Larvicidal activity (%)	
Concentrations ($\mu g.mL^{-1}$)	**24 h**	**48 h**
Negative control	0	0
20	0.26	3.07
40	1.67	6.01
60	3.05	14.2
80	5.09	26.36
100	10.65	50.82

The larvicidal activity of the ethanolic extract of *Mikania lindleyana* showed a correction coefficient R2 of 0.996 and LC50 of 146.32 $\mu g.mL^{-1}$ for 48h of larvae exposure to the extract. According to Consoli et al. (1988) and Cheng (2003) extracts that present activity in concentrations below 100 ppm may have great biocidal potential against larvae.

As the exposure of the larvae with the extract increases, a large percentage of mortality of individuals occurs (BARRETO et al., 2006). Probably, the compounds of the extract interact and penetrate the epithelium which can cause progressive destruction in the tissue and cause the death of the larvae (SANTOS et al., 2015).

According to Gonçalez et al. (2013) and Giacoppo (2017) the larvicidal activity of saponins occurs through complexation with cholesterol that causes disturbances in the synthesis of hormones responsible for growth regulation.

Several substances present in the bruno ethanolic extract can act synergistically increasing the toxic potential for the larvae (ROMÃO et al., 2008; SIMAS et al., 2004). In the scientific literature, studies can be found that relate saponins, a metabolite present in the extract, with the potential to interrupt larval development and inhibit food intake leading to the death of individuals (MATIAS, 2015; SOUZA; ALVARENGA; GIUSTOLIN, 2015; SINGH; KAUR, 2017).

Toxicity to *A. salina* from the ethanolic extract of *M. lindleyana*.

The toxicity assessment using *Artemia salina* is a preliminary tool to verify the toxicity of the extracts. An extract is considered toxic when the lethal dose LD_{50} is less than 100µg.mL, moderately toxic when between 100µg.mL and 500µg.mL and with low toxicity when the concentration of the extract is greater than 500µg.mL (AMARANTE et al., 2011).

Table 2 indicates the mortality accumulated in the 24 h period of cytotoxic activity in the ethanolic extract of *M. lindleyana* leaves. The ethanolic extract was not toxic to *A. salina* and did not cause the death of any individual.

Table 2 - Percentage of mortality of *A. salina* larvae in different concentrations of the ethanolic extract of *Mikania lindleyana*.

Concentrations ($\mu g.mL^{-1}$)	**Mortality %**
Controle	0
50	0
100	0
250	0
500	0
750	0
1000	0

The *Artemia salina* assay did not behave similarly to larvicidal activity. The toxicity of an extract is caused by the amount and types of compounds present (SANTOS, 2018). There are a wide variety of saponins that present themselves with different structural arrangements and depending on the type and quantity do not cause toxicity (JIANG et al.; MARINHO et al., 2018).

Many plants are used by communities for treatment and there is no concern about the possible harm of their use. Because of this context, toxicity studies are needed in other experimental models of *Mikania lindleyana* (BEDNARCZUK et al., 2010).

Phytochemical prospecting

10 phytochemical tests were performed on the ethanolic extract with positive results for depsides, azulenes and saponins (Table 03).

Table 03 - Phytochemical screening of *Mikania Lindleyana*

Metabolite	**Result**
Reducing sugars	Negative
Azulenos	Positive
Catechins	Negative
Depsideos	Positive
Phenols and tannins	Negative
Cardiac glycosides	Negative
Polysaccharides	Negative
Proteins and amino acids	Negative

Saponins	Positive
Sesquiterpenolactones	Negative

Azulenes are known as compounds that impart color to essential oils; Chamomile essential oil has this striking characteristic in a bluish color. Azulenes are unsaturated cyclic organic compounds (WANG et al., 2003). This metabolite has an antioxidant action that prevents the development of spots and degradation of epithelial tissues and for this reason they are used in the industry in beauty products. This metabolite may be one of the compounds responsible for the anti-inflammatory effect reported in ethnobotanical studies on the species (SOUSA; MATOS; MORAES, 2014).

Depsideos can be found in fungi, plants and algae, their main function in plants is to promote protection against microorganisms, insects and sunlight (IBRAHIM, 2018). This secondary metabolite has biological activities such as: antipyretic, antioxidant, anti-tumor and anti-inflammatory (MOTA; DUARTE; ALMEIDA, 2015; HONDA; VILEGAS, 1999; IBRAHIM et al., 2018). This metabolite present in the ethanolic extract of Mikania lindleyana can be related to the anti-inflammatory and antimicrobial action reported by folk medicine.

Saponins are emulsifying compounds that are made up of a part of the lipophilic molecule, and a hydrophilic part giving detergent-like properties. (GARCIA; CARRIL, 2009). In plants, saponins are metabolites that participate in the defense, and are located in places susceptible to attack by fungi, and bacteria (CUI et al., 2018). The protective activity of saponins occurs through complexation in proteins of the cytoplasmic membrane, altering its properties, and causing cell lysis (SIMÕES et al., 2007; PEDEBOS, 2011; CUI et al., 2018). This secondary metabolite of *Mikania lindleyana* can be associated with the benefits against infection declared by the traditional population in addition to possibly being related to larvicidal activity (VANDERLINDE et al., 2012).

CONCLUSION

The ethanolic extract of *Mikania lindleyana* showed a positive result for azulenes, saponins and depsides. The ethanolic extract had larvicidal activity against the larvae of *Aedes aegypti*, causing the larvae to die, with CL_{50}: 146, 32 $\mu g.mL^{-1}$,, being susceptible to the crude ethanolic extract when in contact for 48 hours, the larvicidal action was probably caused by the presence of saponins in the ethanolic extract. The extract showed no toxicity against *Artemia salina*, indicating that it can be well tolerated against biological systems.

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