

Larvicidal Activity of Leaf Extract From *Mauritiella armata* (Aceraceae) on *Aedes aegypti* and *Culex quinquefasciatus* (Culicidae)

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Abstract

The mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) are important vectors of several arboviruses, and are relevant public health problems. Conventional control, using chemical larvicides have selected resistant Culicidae populations and caused negative effects on the environment and human health. However, the use of plant extracts has represented a sustainable alternative for insect control. Popularly known as Xiriri, *Mauritiella armata* (Mart.) Burret (Aceraceae) is an abundant palm tree in Vereda ecosystems and has economic and social importance. In this study, the aim was to evaluate the larvicidal activity of the aqueous extract (AE) leaves of this plant on *Ae. aegypti* and *Cx. quinquefasciatus* larvae. The mortalities of larvae were analyzed after treatment with four concentrations of the extract, comparing with a negative control using mineral water. The AE promoted 100% efficacy against *Ae. aegypti* larvae at 7.9 mg/mL. The lethal concentration to promote 90% mortality of *Cx. quinquefasciatus* larvae was estimated at 30.57 mg/mL. After chromatographic analyses, flavonoids, catechin and carbohydrates were detected. AE from *M. armata* leaves presented high larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*, and represents a promising alternative to be used in vector control.

Keywords: arboviruses, plant extract, palm tree and unique health

1. Introduction

Vector-borne diseases account for 17% of the estimated global burden of all infectious diseases (WHO, 2018). *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823) are vectors of several arboviruses, which are considered a public health problem due to their increasing territorial dispersion, high capacity to adapt to artificial ecotopes and difficulty to be controlled (Donalisio, Freitas, & Zuben, 2017).

Vector control is the main form of prevention, and the elimination of breeding sites is the most effective method; however, conventional treatments with chemical larvicides select resistant populations of Culicidae (Gray et al., 2018; Atyame et al., 2019; Lopes et al., 2019). As a result, the use of plant extracts appears as a promising

alternative, ecologically and environmentally safe, and biodegradable, contributing to the reduction of negative effects on the environment and public health (Hwang et al., 2017; Hari & Mathew, 2018).

Mauritiella armata (Mart.) Burret belongs to the Arecaceae family and is popularly known as Xiriri. It is a palm tree widely distributed in the Brazilian territory, in the Vereda areas and has economic and social importance for the Vereda communities (Andrew Henderson, 1995; Martins, 2012). Although some studies have shown that plants of the Arecaceae family have a larvicidal effect against Culicidae larvae (Tayler et al., 2019, Koc et al., 2016), the phytochemical composition of *M. armata* extracts and the potential of metabolites in the control of insects however, are not known. In this perspective, the study proposed to evaluate the larvicidal activity of aqueous extract of *Mauritiella armata* leaves on *Aedes aegypti* and *Culex quinquefasciatus*.

2. Method

2.1 Plant Material

The plant material was composed of leaves of *Mauritiella armata*, from the Almescla vereda of the Pandeiros Environmental Protection Area, located in northwestern Minas Gerais, Brazil (15°22'50"S and 44°55'28"W). The desiccated leaves were deposited at the Montes Claros Herbarium: MCMG and identified as *Mauritiella armata* (Mart.) Burret (xiriri), receipt n° 5778a.

2.2 Production of Extract

Leaves of *M. armata* were selected and washed in running water, and damaged or deteriorated were discarded. Afterwards, they were dehydrated in a greenhouse with forced air circulation at 40 °C for 72 hours and ground in a feed mill. The resulting material was placed in paper bags, free from the incidence of light.

The aqueous extract (AE) was prepared by adding 500 mL of distilled water to 50 g of raw powder of the plant species, being heated in a water bath at 40 °C for 60 min. After this period, the extracts were hot filtered in a funnel with gauze and cotton and later sent to a greenhouse with forced air circulation at 40 °C until constant weight was obtained (Nery et al., 2010, Morais-Costa et al., 2015).

The subsamples of the extracts were submitted to dry matter determination at 105 °C, to calculate the tested concentrations (Patricia Cunniff, 1995). After determining the dry matter weight, the extracts were adjusted to the concentrations to be tested.

2.3 Chromatographic Analysis of the Extract

Quotas (1.0 mg) of the plant extract was measured in a conical glass and then dissolved in 60 µL of pyridine and 100 µL of BSTFA (N,O-bis(trimethylsilyl) = trifluoroacetamide) containing 1% of chlorotrimethylsilane. The reaction mixture was heated at 60 °C for 30 min. Of the solution obtained, only 1 µL was injected into the CG-MS, and the procedure was performed in triplicate.

Chromatographic analyses were performed in an Agilent Technologies gas chromatograph (GC 7890A) equipped with an electron impact ionization detector (CG-MS) and a DB-5MS capillary column (Agilent Technologies, 30 m long × 0.25 mm internal diameter × 0.25 µm film thickness). Helium (99.9999% purity) was used as the trailing gas at a rate of 1 mL min⁻¹. Using an autoinjector (CTC combiPaL), 1 µL of the sample was injected into the chromatograph at a split ratio of 1:10. The split/splitless injector was kept at 290 °C. The chromatographic column, initially at 80 °C, isothermal for 5 min, was heated at a rate of 4 °C min⁻¹ to 260 °C for 10 min. After separating the compounds, the temperature was raised to 300 °C and maintained for 2 min (after running). The interface temperature was maintained at 280 °C and the ionization performed by impact of 70 eV. The m/z sweep range was from 30 to 600 Da.

2.4 Origin of Insects

Native larvae of *Culex quinquefasciatus* were provided by the Montes Claros Zoonoses Control Center (Centro de Controle de Zoonoses-CCZ) and identified in accordance with the *Cx. quinquefasciatus* surveillance guide (Ministério da Saúde, 2011). The eggs of *Aedes aegypti* came from the F4 and F5 generations of the already established closed colony, provided by the insectarium of the Insect Behavior Laboratory of the Federal Institute of Northern Minas-Campus Salinas.

2.5 Bioassays and Data Analysis

The bioassays were carried out according to the methodology described by the WHO (1981). Final third instar or early fourth instar larvae were transferred to disposable plastic containers containing 30 mL of AE or negative control containing mineral water. Tests were performed in triplicate; 20 larvae of *Ae. aegypti* and 15 *Cx. quinquefasciatus* larvae were added to each container, and the final concentrations evaluated were 10.5; 7.875;

5.25 and 2.625 mg/ml and 21.0; 15.25; 10.5 and 5.25 mg/mL, respectively. After a period of 24 and 48 hours, the tests were evaluated, which included counting the dead larvae and observing the occurrence of morphological deformations by optical microscopy.

The experiments were carried out in a completely randomized split-plot design (treatments defined as concentrations and periods as subplots). The data were subjected to analysis of variance and the means were compared using the Scott-Knott test ($p > 0.05$), using the SAEG 9.1 statistical package (2007). The concentration capable of promoting 90% larval mortality was determined by probit regression analysis ($p > 0.05$) in the same statistical program.

3. Results

3.1 Effect of Tested Aqueous Extract on Culicidae Larvae

The bioassays performed showed a significant interaction between the periods and concentrations tested ($p < 0.001$) against *Ae. aegypti* and *Cx. quinquefasciatus*, obtaining greater efficacy after 48 hours of treatment. During this period, the AE of *M. armata* leaves showed 100% efficacy in the mortality of *Ae. aegypti* larvae at ≥ 7.87 mg/mL and efficacy of 88.7% against *Cx. quinquefasciatus* larvae at 21 mg/mL (Table 1).

Table 1. Mortality of *Aedes aegypti* and *Culex quinquefasciatus* in larvae treated with aqueous extract of *Mauritiella armata* leaves after two periods

Treatments (mg/mL)	24h	48h
<i>Ae. aegypti</i>		
10.5	93.33Aa	100.00Aa
7.88	60.00Bb	100.00Aa
5.25	20.00Bc	85.00Ab
2.625	6.67Bd	21.67Ac
Mineral water	0.00Ae	0.00Ae
<i>Cx. quinquefasciatus</i>		
21.00	48.67Ba	89.00Aa
15.25	37.67Bb	75.67Ab
10.50	26.67Bc	55.67Ac
5.25	11.00Bd	48.67Ad
Mineral water	0.00Ae	0.00Ae

Note. Distinct lowercase letters in columns indicate differences between treatments, and distinct uppercase letters in rows indicate differences between periods at ($p < 0.05$).

After 24 h of incubation, larvae subjected to AE of *M. armata* leaves showed deterioration and induction of intestinal content elimination in both vectors (Figure 1).

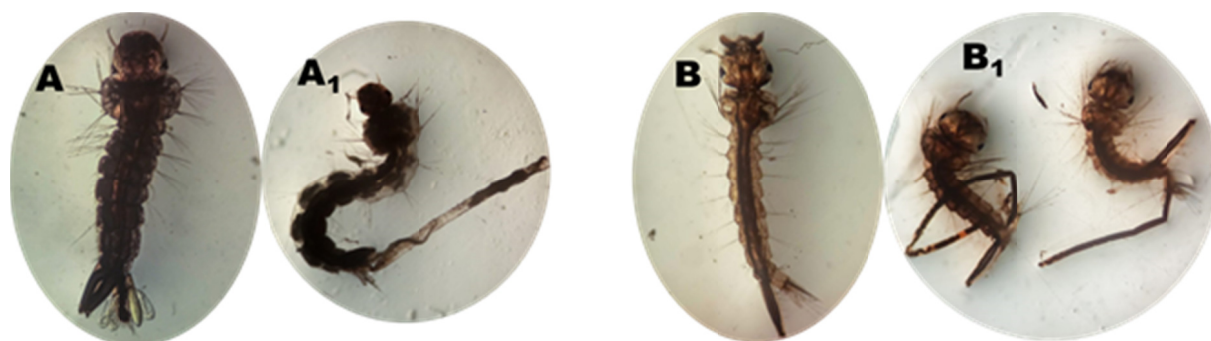


Figure 1. *Aedes aegypti* larva exposed to distilled water (A); *Ae. aegypti* larvae exposed for 48 hours to the aqueous extract of *Mauritiella Armata* (A1); Larvae of *Culex quinquefasciatus* from the control group (B) and exposed to the extract (B1). Expansion by 400 \times (objective lens: 40 \times , down: 10 \times)

For the larvae of *Ae. aegypti* it was not possible to estimate the CL_{90} , as it showed 100% mortality at low concentrations; however, the CL_{90} for *Cx. quinquefasciatus* larvae was estimated to be 30.57 mg/mL (25.57 - 39.10 mg/mL) (Figure 2).

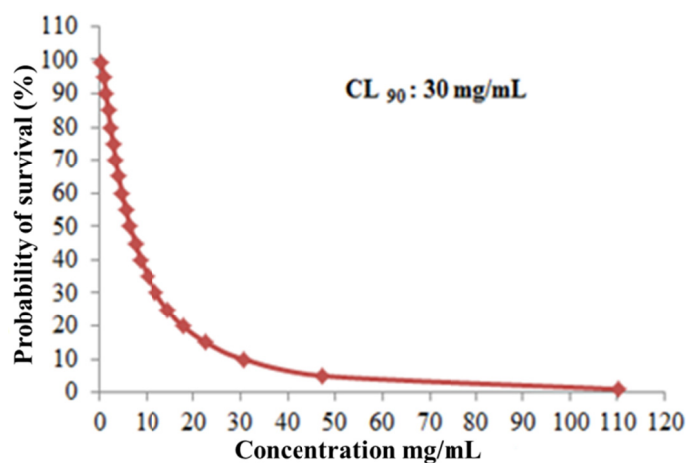


Figure 2. Probability of survival of *Culex. quinquefasciatus* larvae after 48 h of treatment with aqueous extract of *Mauritiella armata* leaves

3.2 Phytochemical Analysis

In the phytochemical analysis, carbohydrates, tannins, and acids were identified in the AE of *M. armata* leaves. The largest classes according to the size of their areas were carbohydrate- β -D-glucopyranose (18.46%) and carbohydrate-thalose (10.33%); in addition, the presence of catechin (0.05%) was observed.

Table 2. Compounds identified by gas chromatography in *Mauritiella armata* leaf extracts and their area (%) in the chromatographic profile.

Aqueous extract			
N	RT	Compounds (Area%)	n
1	7.312	2-hydroxypropanoic acid	0.18
3	8.805	L-alanine	0.06
4	9.766	Glyconic acid	0.16
11	17.909	Phosphate	1.35
12	15.035	Glycerol	0.82
13	16.381	Butanedioic acid	0.06
18	22.116	2-hydroxybutanedioic acid	0.39
28	31.343	Carbohydrate- β -D-Glycopyranosis	18.46
34	33.129	Carbohydrate- β -D-Glycopyranosis	10.68
35	33.288	Carbohydrate-galactopyranose	5.95
43	35.530	Carbohydrate-thalose	10.33
47	37.420	Carbohydrate- β -D-Galactofuranosis	1.62
48	37.900	Carbohydrate-Inositol	5.34
62	54.551	Catechin	0.05

Note. RT: Retention time (min.); N.I.: 1.

4. Discussion

Aqueous extracts from leaves appear as an alternative for vector control. In the literature, extracts of *Annona glabra*, *Bougainvillea spectabilis*, and *Saraca asoca* with larvicidal activity in larvae of *Ae. aegypti* and *Aedes*, and *Ae. Albopictus*, with LC_{50} of 5.29 mg/L and 3.02 mg/L respectively (Amarasinghe et al., 2020; Sharma et al., 2019)

stand out. Likewise, for *Cx. quinquefasciatus* AE from leaves of *Cassia didymobotrya* and *Cayratia trifolia* showed 100% mortality efficacy (Nagappan, 2012; Chakraborty et al., 2013).

The results obtained demonstrate that the AE of *M. armata* leaves presented larvicidal potential at the evaluated concentrations. In 48 h, there was a higher mortality of larvae in both insects. This fact corroborates the results of Hari and Mathew (2018), who in the same period, observed higher larval mortality of *Ae. aegypti* and *Cx. quinquefasciatus* through a combination of plant extracts. According to Santos et al. (2015), the longer the exposure time of larvae to the AE, the higher the mortality percentages, due to the absorption of toxic substances.

The control larvae of both insects showed high mobility and quick reaction to any touch. In contrast, larvae submitted to AE showed loss of mobility and morphological changes after 24 h of treatment. According to Barreto et al. (2007), the first sign of the extract's larvicidal action is the reduction of larval mobility.

Additionally, the AE from the leaves of *M. armata* caused deterioration and induction of elimination of the intestinal contents of the larvae of both vectors (Figure 2). Procopio et al. (2015), observed a similar effect on *A. aegypti* larvae exposed to the leaf extract of *Schinus terebinthifolius* (Anacardiaceae). This behavior has been reported as a defense mechanism of mosquito larvae in order to expel substances that are toxic to them (Gusmão et al., 2002). Although the larvae present this defense mechanism, our results showed that this was not enough to avoid the harmful effects of the extract, since the larvae survival rate decreased after the larvicide test.

Assessing the chemical composition of *M. armata*, Royo et al. (2019) detected significant flavonoid content in its leaves, petiole and root. Flavonoids are a diverse and abundant group among secondary metabolites in different plants (Filho, Antonio Carlos Pereira; Castro, 2019). In the study by Tayler et al. (2019), EA from leaves of *Cocos nucifera* (Arecaceae) showed antiparasitic activity against the malaria protozoan, due to the synergistic action of flavonoids with other compounds.

Diterpene extracted from *Copaifera reticulata*, and a fraction rich in catechetal tannins, extracted from *Magonia pubescens* caused the death of *Ae. aegypti* through cell destruction in the midgut (Volotto et al., 2011). In the work by Elumalai et al. (2016), the catechin isolated from *Leucas aspera* showed 100% mortality in *Ae. aegypti*, *Anopheles stephensi* and *Cx. quinquefasciatus* at a concentration of 20 ppm. The catechin, which has insecticidal properties, was identified in the phytochemical analysis of the xiriri leaf.

The aqueous extract of the leaves of *Mauritiella armata* represents a promising alternative for the control of *Aedes aegypti* and *Culex quinquefasciatus*, due to its high larvicidal effect. In addition, this study presents a simple, clear, and low-cost methodology that contributes to the valuation of ecosystem services and the promotion of unique health.

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