

Identification of larvicidal activity of medicinal plants against *Aedes aegypti* (Diptera: Culicidae)

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Santos, Ellen Caroline Nobre¹

 <https://orcid.org/0000-0002-1467-1919>

Araújo, Taynara Lopes de²

 <https://orcid.org/0000-0002-0063-4981>

Lima, Andson Amorim²

 <https://orcid.org/0000-0001-6770-2793>

Rufino, Polinar Bandeira³

 <https://orcid.org/0000-0002-3711-9481>

Souza, Marcos Antônio de Oliveira²

 <https://orcid.org/0000-0002-1450-6489>

Barbosa, Genilda de Andrade¹

 <https://orcid.org/0000-0002-4550-4689>

Freitas, Jorgiane de Araújo¹

 <https://orcid.org/0000-0002-2892-6716>

Souza, Janis Lunier de⁴

 <https://orcid.org/0000-0002-8836-9456>

Mourão, Pedro Junior Pinheiro¹

 <https://orcid.org/0000-0003-0683-2484>

Rocha, Ricardo da Costa⁵

 <https://orcid.org/0000-0003-1320-7935>

Costa, Emmeron Côrrea Brasil da^{6*}

 <https://orcid.org/0000-0002-1467-1919>

¹Federal University of Acre, Postgraduate in Science, Innovation and Technology for the Amazon. Rodovia BR 364, Km 04, Distrito Industrial, CEP 69920-900, Rio Branco, AC, Brazil.

²Federal University of Acre, Center for Biological and Natural Science. Rodovia BR 364, Km 04, Distrito Industrial, CEP 69920-900, Rio Branco, AC, Brazil.

³Federal University of Acre, Postgraduate in Health Science in the Western Amazon. Rodovia BR 364, Km 04, Distrito Industrial, CEP 69920-900, Rio Branco, AC, Brazil.

⁴Municipal Health Department of Rio Branco, Division of Entomology and Chemical Blocking. Av. Ceará, 3188, Abrahão Alab, CEP 69907-140, Rio Branco, AC, Brazil.

⁵Federal University of Acre, Center for Health and Sports Sciences. Rodovia BR 364, Km 04, Distrito Industrial, CEP 69920-900, Rio Branco, AC, Brazil.

⁶Federal University of the State of Rio de Janeiro, Department of Genetics and Molecular Biology, Center for Biological and Health Sciences. Rua Frei Caneca 94, Centro, CEP 20211-040, Rio de Janeiro, RJ, Brazil.

*Correspondence: costaemm.biomed@yahoo.com.

Abstract

Aedes aegypti is the main transmitter of several arboviruses, such as dengue, the world's most widespread arbovirus. The main method of controlling arbovirus transmission is through vector control, using insecticides. But its continuous, cases of resistance occur. Thus, research is seeking new botanical insecticide alternatives, because have multiple active compounds can be applied on the vector, contributing to the control of arbovirus transmission. The aim of this study is to identify plants cultivated in the Western

Amazon, which have larvicidal activity against *A. aegypti*. Ethanolic extracts from ten plants were obtained to verify the larvicidal biopotential against third instar larvae of *A. aegypti*. The promising ethanolic extracts was *Fridericia chica*, which was submitted to liquid-liquid partition for larvicidal activity evaluation and phytochemical analysis. Among the partitions analyzed, the aqueous partition of *F. chica* showed larvicidal biopotential against *A. aegypti*, being the first report in the literature of its larvicidal activity. In the phytochemical prospection of the aqueous partition of *F. chica*, we observed the presence of catechins, condensed tannins and the flavone group, flavonols and xanthenes. These groups of metabolites have been reported in the literature as larvicidal agents.

Keywords: Aedes. Plants. Insecticides. Biochemistry. Arbovirus infections.

Introduction

The *Aedes aegypti* mosquito stands out for being the transmitter of arboviruses, such as dengue, chikungunya, yellow fever, zika^[1-3], where dengue is the most widespread disease globally in comparison to other arboviruses^[4]. *A. aegypti* is a vector with high efficiency in arbovirus transmission^[5]. Study shows that mosquitoes can transmit at the same time, to a vertebrate, more than one arbovirus, which makes this fact worrisome, since about 75.2% of the countries are suitable for the development of *A. aegypti*^[4,6].

Arboviruses whose vector is *A. aegypti* can cause mild illness to and death^[7]. It may affect neurological syndromes. In 2015, there was an outbreak of Zika in Brazil, in which newborns, from mothers infected by the virus in the first trimester, presented microcephaly^[8,9]. Some cases, of Guillain Barré syndrome in infected adults^[10,11]. While, Chikungunya virus infections is capable of afflicting polyarthralgia and arthritis, even after cure, it may persist these symptoms for months to years^[12,13].

For the control of arbovirus transmission, studies have been carried out to produce vaccines. However, there are certain limitations, such as the efficiency in immune responses against the four dengue serotypes is still an issue^[14,15]. Therefore, the main method for controlling arbovirus transmission is to prevent the development and spread of the vector, by applying synthetic insecticides against larvae and mosquitoes^[16,17]. Although, with the continuous use of insecticides, cases of resistance have been reported^[18-20].

Thus, to reduce the incidence of these diseases, the epidemiological surveillance has applied synthetic insecticides to control the vector, but with their continuous use cases of resistance have arisen^[21,22]. For this reason, many researches are looking for control alternatives for *A. aegypti* by means of plant metabolites^[23-25]. Since botanical insecticides can be composed of multiple active compounds, which makes the emergence of resistance difficult^[26], in addition to being biodegradable and specific for the target species^[27].

Studies have shown the effectiveness of several plant species cultivated in the Amazon against *A. aegypti* larvae, such as *Tapura amazonica*, *Piper aduncum*, *Piper tuberculatum*, *Simaba polyphylla* and *Acmella oleracea*^[25,28], that can be an alternative for mosquito control, in order to interrupt the development phase and, consequently, the transmission of arbovirus. In some cases, plants are known to have larvicidal activity against *A. aegypti*, such as *Tagetes patula*, our positive control in the larvicidal bioassays. Thus, the objective of this work is to identify plants species cultivated in the Western Amazon that have insecticidal activity against *A. aegypti*.

Material and Methods

Obtaining plant material

Ten plants used in popular medicine and located in Acre state, were collected in the mornings of May to September between the years 2017 to 2019. The plant materials were identified used morphological identification keys of each family, incubated for in an oven at 40°C until brittle (on average, 3 days drying time) and triturated. Then, the technique of exhaustive maceration was adopted using 99% ethanol, to be later filtered, evaporated using a Quimis® rotary evaporator at a temperature of 55°C and lyophilized, to obtain the ethanolic extracts (TABLE 1).

TABLE 1: Plants collected for larvicidal activity evaluation.

Scientific name	Family	Used part	Popular name	Exsiccate number
<i>Alternanthera brasiliana</i>	Amaranthaceae	Leaf	Penicilin	UFACPZ: 20740
<i>Kalanchoe pinnata</i>	Crassulaceae	Leaf	Leaf of fortune	UFACPZ: 20741
<i>Senna alata</i>	Fabaceae	Leaf	Sickle pod	UFACPZ: 20739
<i>Euphorbia tirucalli</i>	Euphorbiaceae	Aerial part	Pencil cactus	UFACPZ20440
<i>Fridericia chica</i>	Bignoniaceae	Leaf	Cricket wine	UFACPZ: 20562
<i>Jatropha gossypifolia</i>	Euphorbiaceae	Leaf	Bellyache bush	UFACPZ20558
<i>Momordica charantia</i>	Cucurbitaceae	Leaf	Momordica	UFACPZ20561
<i>Morinda citrifolia</i>	Rubiaceae	Leaf	Noni	UFACPZ20557
<i>Plectranthus barbatus</i>	Lamiaceae	Leaf	Coleus barbatus	UFACPZ20560
<i>Tagetes patula</i>	Asteraceae	Aerial part	Mexican marigold	UFACPZ20445

The ethanolic extracts that manifested larvicidal activity were submitted to liquid-liquid partition, using the solvents hexane, dichloromethane and ethyl acetate, where the initial solution used was methanol and distilled water (1:10).

Obtaining *A. aegypti* larvae

To obtain *A. aegypti* larvae, it was necessary to collect eggs of the species near residences located in ten selected neighborhoods of Rio Branco, Acre: Loteamento Joafrá, Mocinha Magalhães, Cruzeiroinho, Santa Helena, Santa Cecília, Universitario, Vila Betel, Conjunto Esperança, Laélia Alcântara and Rui Lino.

The method adopted for collection was by means of ovitraps, containing a 0.04% brewer's yeast-based attractant solution and a eucatex palette^[29]. The ovitraps were installed for ten days, in the peridome, with the solution and palette changed on the fifth day of installation.

After this period, the pallets were taken to the Intermediate Phytotherapeutic Laboratory of the Federal University of Acre (UFAC) for identification and counting of *A. aegypti* eggs, where these eggs were stored in a closed styrofoam box for a maximum period of two months until hatching for the creation and

maintenance of the F0 generation. Subsequently, the pallets containing *A. aegypti* eggs were placed in a container with distilled water for hatching, for subsequent identification of the larvae by the identification keys described by Consoli and Oliveira^[30].

The larvae were fed with fish food (Alcon Basic[®]) until they reached the pupal stage and then transferred to cages for mosquito breeding to obtain the F1 generation. The mosquitoes were fed with absorbent cotton dampened with 10% sucrose solution^[30] and artificial feeder Glytube^[31]. The F1 generation of eggs were stored to perform larvicidal activity assays.

Larvicide test

The larvicide test followed the recommendations of the World Health Organization^[32] and Consoli and Oliveira^[30] with adaptations. The ethanolic extracts were solubilized in dimethyl sulfoxide (DMSO) at a concentration of 250 mg/mL.

To evaluate the larvicidal biopotential of the extracts against *A. aegypti*, initial screening was performed with 100 ppm of the ethanolic extracts in 10mL of distilled water and in the presence of 20 third instar larvae. As a positive control, we used the ethanolic extract of *Tagetes patula*^[33,34], described in the literature with larvicidal activity, and as a negative control, 0.04% DMSO. The mortality rate was recorded at 24 and 48 hours. For analysis, four repetitions were performed.

Subsequently, the promising plants with larvicidal activity, their partitionates were obtained, lyophilized and solubilized in 125 mg/mL using DMSO. The partitionates were submitted to larvicidal test under the same conditions as the initial screening, and 300 ppm of each partition was used.

After performing three replicates, the data was analyzed in the statistical program GraphPad Prism version 8.0.1, where means and standard deviation were calculated. As well as being used to perform ANOVA and t-tests.

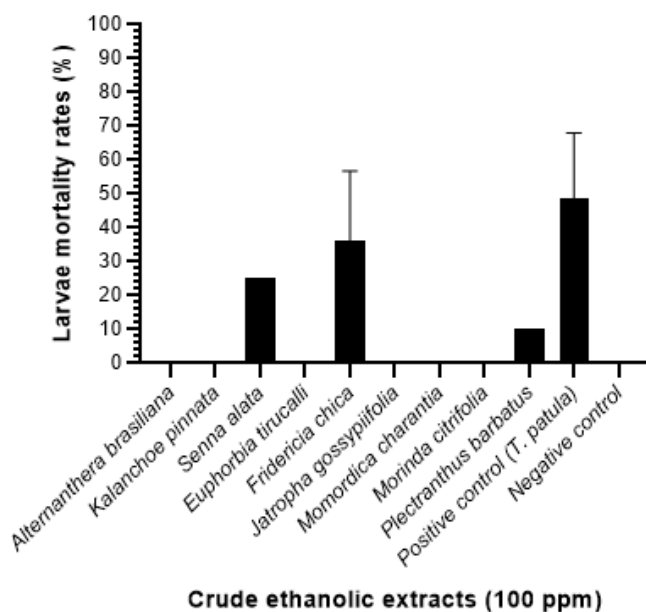
Phytochemical prospection

The phytochemical study followed the protocol described by Matos^[35]. The following classes were analyzed: tannins, phenols, saponins, reducing sugars, leucoanthocyanidins, catechins, flavanones, anthocyanidins, and flavonoids.

Results and Discussion

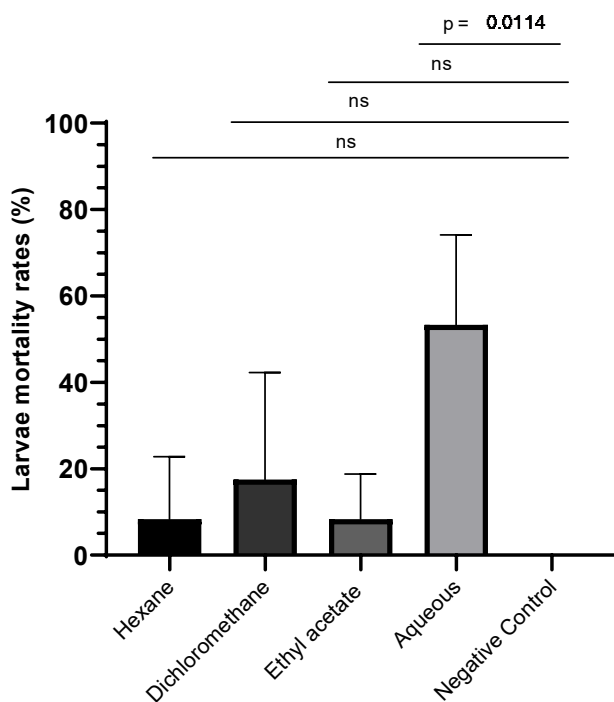
Among the ten extracts analyzed, only three plants extracts showed larvicidal activity against *A. aegypti* at the concentration 100 ppm, those that showed the best result was *F. chica* (36.2% of mortality), while the positive control (*T. patula*) was 48.7% larvae mortality up to 48 hours. While *P. barbatus* and *S. alata* showed larvicidal activity of less than 25% (**FIGURE 1**). However, larvicidal activity was observed at 24 hours in ethanolic extracts of *F. chica* (32,5% of mortality), and few deaths (3,7%) at 48 hours in your four replicates.

FIGURE 1: Mortality rate of ethanolic extracts against *A. aegypti* larvae at 100 ppm up to 48 hours.



Therefore, the ethanolic extract of *F. chica* was partitioned into hexane, dichloromethane, ethyl acetate and aqueous fractions, and all were tested at 300 ppm against F1 generation larvae of *A. aegypti*. All partitions showed mortality of larvae up to 48 hours and in that there are differences in the performance of the larvicidal activity of partition (p-value = 0,0151). However, when analyzed individually, only the aqueous partition of *F. chica* (p-value = 0,0114) showed larvicidal activity when compared to the negative control (FIGURE 2).

FIGURE 2: Mortality rate of *F. chica* partitions against *A. aegypti* larvae.



The mortality rates of larvae were analyzed by the One-way Anova statistical method, resulting that the performance in larvicidal action depends on the partition analyzed (p-value=0.0151). Only the aqueous partition exhibits larvicidal activity when compared to the negative control when analyzed by t test.

After these results, the phytochemical prospection of the partitions of *F. chica* was conducted. The metabolites detected in the aqueous partition of *F. chica* were condensed tannins, catechins and the flavone, flavonol and xanthone groups (**TABLE 2**). The phytochemical prospection of our positive control was not analyzed due to the low yield of its extract to be submitted to liquid-liquid partition.

TABLE 2: Phytochemical prospection of *Fridericia chica*

Metabolites	H	D	AC	AQ
Phenols	-	+	-	-
Flavones, flavonols and xanthenes	-	IR	-	+
Chalcones and aurones	-	-	+	IR
Flavanonols	+	-	IR	IR
Catechins	-	IR	-	+
Condensed tannins	+	IR	+	+

H: hexane partition; D: dichloromethane partition; AC: ethyl acetate partition; AQ: aqueous partition; IR: inconclusive result; -: negative, +: positive. Phytochemical prospection negative to anthocyanins, anthocyanidins, leucoanthocyanidins, flavanones and hydrolyzable tannins.

In our studies, the ethanolic extract of *T. patula*, the positive control, the mortality rate reached 48.7% in 48 hours at the concentration of 100 ppm. The species *T. patula* is described in the literature manifesting larvicidal activity against the genus *Aedes*, as reported its activity against larvae of *A. fluviatilis* and *A. aegypti*, whose mortality rate was 65.6% at a concentration of 100 mg/L in 24 hours and 92% at a concentration of 153.6 mg/L in 48 hours, respectively^[33,34].

The plants that demonstrated low or no activity in this research, such as *Euphorbia tirucalli*^[36] and *Plectranthus barbatus*^[37] should be further explored, since studies indicate a potential larvicidal agent against *A. aegypti*. This fact may be related to factors such as: extraction procedure, solvent used, place of collection and other factors that may cause divergence of larvicidal activity and the detection of classes of metabolites^[38].

The species *F. chica* is known for several pharmacological activities, such as antimicrobial^[39] and anti-inflammatory^[40]. And according to PubMed, Wiley Online Library, Scielo and Science Direct databases, this is the first report in the literature of the crude extract of *F. chica* with larvicidal activity against *A. aegypti*. However, recent research has shown that its essential oil demonstrated insecticidal activity on larval and pupal stages of *A. aegypti*, moreover, mosquitoes from the pupae that survived^[41]. Thus, the phytochemical prospection of *F. chica*, we can observe the presence of catechins, condensed tannins and the group of flavones, flavonols and xanthenes.

Comparing the constituents detected in the promising partitions of *F. chica*, there are investigations that demonstrate that these metabolites present insecticidal activity, such as condensed tannins, flavones and catechins extracted from several plant species against *A. aegypti*^[42-44]. There is even a study that points out

that there are morphological changes in larvae that were exposed to insecticide metabolites, such as catechins, caused lesions in the midgut epithelium and deformation of the anal papillae^[44].

Conclusion

Therefore, among the ten plants analyzed, *Fridericia chica* showed the best larvicidal biopotential against *A. aegypti*. Among these plants, this is the first report of *F. chica* extract showing larvicidal activity, and it may be a potential alternative agent in controlling the proliferation of the *A. aegypti*. However, more studies are needed to configure it as an alternative tool for the control of *A. aegypti*. Mainly, with the increasing reports of resistance to chemical insecticides usually used in urban environments.

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Conflicts of interest

The authors declare no conflicts of interest.

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Contributors

Concepção do estudo: ECNS; ECBC; RCR.

Curadoria dos dados: ECNS; TLA; AML; ECBC.

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