

## Larvicidal Efficacy of *Jasminum* sp. (Oleaceae) Flower Extracts against the Dengue and Chikungunya Vector *Aedes aegypti* L. (Diptera: Culicidae)

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### Abstract

Dengue and chikungunya are transmitted by *Aedes aegypti* and for controlling these diseases, the vector mosquito has to be controlled. Extensive use of synthetic and chemical insecticides has resulted in environmental hazards and also in development of physiological resistance among vector mosquito species. Plant products are considered to be a potential alternative approach as they are environmentally safe, target specific and biodegradable. In the present study, the crude chloroform, methanol and aqueous flower extracts of *Jasminum officinale*, *Jasminum auriculatum* and *Jasminum grandiflorum* were tested for the larvicidal efficacy against the third instar larvae of *Aedes aegypti* at concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L. Mortality was recorded after 24 and 48 h. Amongst the extracts of *Jasminum* species tested, the crude chloroform flower extract of *Jasminum grandiflorum* was found to be effective showing 100% mortality at 1000 mg/L with LC<sub>50</sub> value of 344.01 and 300.47 after 24 and 48 h respectively followed by the crude methanolic flower extracts of *Jasminum officinale* and *Jasminum auriculatum*. Further investigations are needed to elucidate the larvicidal activity of *Jasminum grandiflorum* crude chloroform flower extract against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

**Keywords:** Larvicidal efficacy; *Aedes aegypti*; *Jasminum officinale*; *Jasminum auriculatum*; *Jasminum grandiflorum*; Crude flower extracts

### Introduction

Human beings are being suffered from the menace of mosquitoes since time immemorial and it is believed that mosquitoes are ranked as the most important pests causing human health concerns, since they are responsible for transmission of dengue, dengue haemorrhagic fever, chikungunya, malaria, filarial fever and Japanese encephalitis that cause severe public health problems [1]. Vector control is an important component in a disease control programme. A myriad of methods and strategies though available, may not contribute to the total control of vectors unless they are used judiciously and in a sustained manner. The discovery and development of synthetic organic chemicals with persistent residual action not only overshadow the use of herbal products against mosquitoes, but also become the major weapon for mosquito control. But the extensive use of synthetic organic insecticides has resulted in environmental hazards, ecological imbalance, harm to humans and animals and non target organisms being affected, in addition to the physiological resistance of vectors [2]. This has necessitated the need for search and development of environmental-safe, biodegradable and indigenous method for vector control. The flora of India has a rich aromatic plant diversity with potential for development of natural insecticides for the control of mosquito and other pests [3]. Phytochemical insecticides have received much attention, in this regard, as they are considered to be more environmentally biodegradable and considered safer than synthetic insecticides [4]. Therefore, the search for such compounds has been directed extensively to the plant kingdom. Co-evolution has equipped plants with a plethora of chemical defenses against insects. Aware of this effect, mankind has used plant parts or extracts to control insects/mosquitoes since ancient time. Plant derived products have received increased attention from scientists as they are a rich source of novel natural substances possessing insecticidal properties, safer to humans and ecosystem [5]. During the last decade, various studies on natural plant products against vector mosquito indicate them as possible alternatives to chemical synthetic insecticides for mosquito control [6-13]. Therefore, in the present study, the crude flower

extracts of *Jasminum officinale*, *Jasminum auriculatum* and *Jasminum grandiflorum* were tested for the larvicidal efficacy against the third instar larvae of *Aedes aegypti*.

### Materials and Methods

#### Plant collection and extraction

Mature fresh flowers of *Jasminum officinale*, *Jasminum auriculatum* and *Jasminum grandiflorum* collected in and around Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered flowers (1 kg) each was macerated with 3 L of chloroform, methanol and distilled water for a period of 96 h each separately and filtered. The filtrate was then concentrated at reduced temperature on a rotary evaporator. The crude chloroform, methanol and aqueous flower extracts of *Jasminum officinale*, *Jasminum auriculatum* and *Jasminum grandiflorum* thus obtained were lyophilized and a stock solution of 1,00,000 mg/L prepared by adding adequate volume of acetone was refrigerated at 4°C until testing for bioassay.

#### Test mosquitoes

*Aedes* immatures collected from various places in Chennai, Tamil Nadu, India were transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to

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enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified upto species level and confirmed before rearing. Cyclic generations of *Aedes aegypti* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of  $27 \pm 2^\circ\text{C}$  and a relative humidity of 70-80% were maintained in the insectary. The adult mosquitoes were fed on ten per cent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with laboratory reared albino mice. Ovitrap were placed inside the cages for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

### Larvicidal bioassay

Standard WHO [14] protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. *Aedes aegypti* immature particularly early third instar larvae were obtained from laboratory colonized mosquitoes of  $F_1$  generation. From the stock solutions, concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L were prepared. Twenty healthy larvae were released into each 250 ml glass beaker containing 200 mL of water and test concentration. Mortality was observed for 24 and 48 h after treatment. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of acetone to distilled water. Distilled water served as untreated control. The larval per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula [15]. SPSS 11.5 version package was used for determination of  $LC_{50}$  and  $LC_{90}$  values [16]. One way ANOVA followed by Tukey's test was performed to determine the difference in larval mortality between concentrations.

### Results

Results of the larvicidal effects of crude flower extracts of *Jasminum* species against *Aedes aegypti* are presented in Tables 1 and 2. Among the plant species and extracts tested, the crude chloroform flower of *Jasminum grandiflorum* was found to be effective followed by the crude methanol flower extracts of *Jasminum officinale* and *Jasminum auriculatum*. One hundred per cent mortality was observed in *Jasminum grandiflorum* crude chloroform flower extract at 1000 mg/L at 24 hours (Tables 3 and 4). The crude chloroform flower extract *Jasminum grandiflorum* was found to be effective and promising with  $LC_{50}$  values of 344.01 and 300.47 mg/L after 24 and 48 h respectively (Table 5).

### Discussion

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting counter measures such as developmental of novel insecticides [17]. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. Mosquito control approaches based on synthetic insecticides have created many problems like insecticide resistance [18]. Natural products of plant origin with insecticidal properties have been tried in the recent past in order to control a variety of insect pests and vectors. This has necessitated the need for a research and development of environmentally safe, biodegradable indigenous method for vector control. Many researchers have reported on the effectiveness of plant extract against mosquito larvae [19-21]. Phytoextracts are emerging as potential mosquito control agents, and can be used successfully in mosquito management programmes [22]. The results of the present study indicate the crude chloroform extract of *Jasminum grandiflorum* flowers to possess larvicidal activity against *Aedes aegypti*.

Plant species	Solvents	Concentration (mg/L)									
		UC	TC	62.5	125	250	500	1000	2000	4000	8000
<i>Jasminum officinale</i>	Chloroform	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	1.00 ± 1.00 <sup>b</sup>
	Methanol	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>	1.33 ± 1.15 <sup>ab</sup>	3.66 ± 1.15 <sup>b</sup>	3.66 ± 2.08 <sup>b</sup>
	Aqueous	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.66 ± 1.15 <sup>a</sup>
<i>Jasminum auriculatum</i>	Chloroform	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.33 ± 0.57 <sup>ab</sup>	0.33 ± 0.57 <sup>ab</sup>	0.66 ± 0.57 <sup>abc</sup>	1.33 ± 0.57 <sup>abc</sup>	1.66 ± 1.52 <sup>abc</sup>	2.00 ± 0.00 <sup>bc</sup>	2.33 ± 0.57 <sup>c</sup>
	Methanol	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	1.00 ± 1.00 <sup>a</sup>	4.00 ± 2.64 <sup>b</sup>	4.00 ± 1.00 <sup>b</sup>
	Aqueous	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.66 ± 0.57 <sup>ab</sup>	1.00 ± 1.00 <sup>ab</sup>	1.33 ± 0.57 <sup>ab</sup>	1.33 ± 0.57 <sup>ab</sup>	1.66 ± 0.57 <sup>ab</sup>	2.00 ± 1.00 <sup>b</sup>	2.00 ± 0.00 <sup>b</sup>
<i>Jasminum grandiflorum</i>	Chloroform	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	6.66 ± 0.57 <sup>b</sup>	7.00 ± 1.00 <sup>b</sup>	7.33 ± 2.08 <sup>b</sup>	10.66 ± 2.08 <sup>c</sup>	20.00 ± 0.00 <sup>d</sup>	20.00 ± 0.00 <sup>d</sup>	20.00 ± 0.00 <sup>d</sup>	20.00 ± 0.00 <sup>d</sup>
	Methanol	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>	1.00 ± 1.00 <sup>a</sup>	6.33 ± 3.21 <sup>b</sup>	6.33 ± 0.57 <sup>b</sup>
	Aqueous	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	1.33 ± 0.57 <sup>ab</sup>	1.66 ± 0.57 <sup>ab</sup>	2.00 ± 1.00 <sup>b</sup>

UC: Untreated control; TC: Treated control. Values are mean of three replicates of three trials ± standard deviation. Different superscript alphabets within the column indicate statistical significant difference in larval mortality between concentrations at  $P < 0.05$  level by one way ANOVA followed by Tukey's test.

**Table 1:** Larvicidal activity of crude flower extracts of *Jasminum* species against *Aedes aegypti* at 24 h.

Plant species	Solvents	Concentration (mg/L)									
		UC	TC	62.5	125	250	500	1000	2000	4000	8000
<i>Jasminum officinale</i>	Chloroform	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>ab</sup>	2.00 ± 1.00 <sup>b</sup>
	Methanol	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.33 ± 0.57 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 1.00 <sup>a</sup>	1.33 ± 0.57 <sup>a</sup>	1.33 ± 1.15 <sup>a</sup>	5.33 ± 1.52 <sup>b</sup>	19.66 ± 0.57 <sup>c</sup>	20.00 ± 0.00 <sup>c</sup>
	Aqueous	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	1.00 ± 1.00 <sup>a</sup>	1.33 ± 1.52 <sup>a</sup>	1.66 ± 1.15 <sup>a</sup>	1.66 ± 0.57 <sup>a</sup>	2.00 ± 0.00 <sup>a</sup>	2.00 ± 1.00 <sup>a</sup>	2.00 ± 1.00 <sup>a</sup>	2.33 ± 0.57 <sup>a</sup>
<i>Jasminum auriculatum</i>	Chloroform	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.66 ± 0.57 <sup>ab</sup>	1.33 ± 0.57 <sup>ab</sup>	1.66 ± 0.57 <sup>abc</sup>	1.66 ± 0.57 <sup>abc</sup>	2.00 ± 1.00 <sup>abc</sup>	3.00 ± 1.00 <sup>bc</sup>	4.00 ± 1.00 <sup>cd</sup>	6.00 ± 2.00 <sup>d</sup>
	Methanol	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>ab</sup>	0.33 ± 0.57 <sup>abc</sup>	0.33 ± 0.57 <sup>bcd</sup>	0.33 ± 0.57 <sup>bcd</sup>	0.66 ± 0.57 <sup>bcd</sup>	2.33 ± 0.57 <sup>cd</sup>	8.66 ± 3.78 <sup>cd</sup>	17.00 ± 1.00 <sup>d</sup>
	Aqueous	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.66 ± 0.57 <sup>ab</sup>	1.66 ± 0.57 <sup>abc</sup>	2.00 ± 0.00 <sup>bcd</sup>	2.33 ± 0.57 <sup>bcd</sup>	2.33 ± 0.57 <sup>bcd</sup>	2.66 ± 0.57 <sup>cd</sup>	2.66 ± 1.15 <sup>cd</sup>	3.66 ± 0.57 <sup>d</sup>
<i>Jasminum grandiflorum</i>	Chloroform	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	7.33 ± 0.57 <sup>b</sup>	8.66 ± 0.57 <sup>bc</sup>	11.33 ± 2.30 <sup>bc</sup>	11.66 ± 1.52 <sup>c</sup>	20.00 ± 0.00 <sup>d</sup>	20.00 ± 0.00 <sup>d</sup>	20.00 ± 0.00 <sup>d</sup>	20.00 ± 0.00 <sup>d</sup>
	Methanol	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	1.33 ± 0.57 <sup>a</sup>	1.66 ± 0.57 <sup>a</sup>	2.66 ± 0.57 <sup>a</sup>	3.00 ± 0.00 <sup>ab</sup>	3.33 ± 1.52 <sup>ab</sup>	3.33 ± 0.57 <sup>ab</sup>	7.66 ± 4.61 <sup>b</sup>	15.33 ± 1.52 <sup>c</sup>
	Aqueous	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	1.33 ± 1.15 <sup>ab</sup>	1.66 ± 0.57 <sup>abc</sup>	1.66 ± 0.57 <sup>abc</sup>	1.66 ± 0.57 <sup>abc</sup>	2.00 ± 0.00 <sup>abc</sup>	2.66 ± 0.57 <sup>bc</sup>	3.00 ± 1.00 <sup>bc</sup>	3.66 ± 1.52 <sup>c</sup>

UC: Untreated control; TC: Treated control. Values are mean of three replicates of three trials ± standard deviation. Different superscript alphabets within the column indicate statistical significant difference in larval mortality between concentrations at  $P < 0.05$  level by one way ANOVA followed by Tukey's test.

**Table 2:** Larvicidal activity of crude flower extracts of *Jasminum* species against *Aedes aegypti* at 48 h.

Plant species	Solvents	Larval mortality (%)									
		Concentration (mg/L)									
		UC	TC	62.5	125	250	500	1000	2000	4000	8000
<i>Jasminum officinale</i>	Chloroform	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00
	Methanol	0.00	0.00	1.65	1.65	3.30	3.30	3.30	6.65	18.30	18.30
	Aqueous	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.65	3.30
<i>Jasminum auriculatum</i>	Chloroform	0.00	0.00	0.00	1.65	1.65	3.30	6.65	8.30	10.00	11.65
	Methanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	20.00	20.00
	Aqueous	0.00	0.00	0.00	3.30	3.00	6.65	6.65	8.30	10.00	10.00
<i>Jasminum grandiflorum</i>	Chloroform	0.00	0.00	33.30	35.00	36.65	53.30	100.00	100.00	100.00	100.00
	Methanol	0.00	0.00	0.00	1.65	1.65	1.65	3.30	5.00	31.65	31.65
	Aqueous	0.00	0.00	0.00	0.00	0.00	0.00	6.65	8.30	10.00	11.65

Table 3: Per cent larval mortality of *Aedes aegypti* against crude flower extracts of *Jasminum* species at 24 h.

Plant species	Solvents	Larval mortality (%)									
		Concentration (mg/L)									
		UC	TC	62.5	125	250	500	1000	2000	4000	8000
<i>Jasminum officinale</i>	Chloroform	0.00	0.00	0.00	0.00	1.65	1.65	1.65	1.65	3.30	10.00
	Methanol	0.00	0.00	1.65	5.00	5.00	6.65	6.65	26.65	98.30	100.00
	Aqueous	0.00	0.00	5.00	6.65	8.30	8.30	10.00	10.00	10.00	11.65
<i>Jasminum auriculatum</i>	Chloroform	0.00	0.00	3.30	6.65	8.30	8.30	10.00	15.00	20.00	30.00
	Methanol	0.00	0.00	0.00	1.65	1.65	1.65	3.30	11.65	43.30	85.00
	Aqueous	0.00	0.00	3.30	8.30	10.00	11.65	11.65	13.30	13.30	18.30
<i>Jasminum grandiflorum</i>	Chloroform	0.00	0.00	36.65	43.30	56.65	58.30	100.00	100.00	100.00	100.00
	Methanol	0.00	0.00	6.65	8.30	13.30	15.00	16.65	16.65	38.30	76.65
	Aqueous	0.00	0.00	6.65	8.30	8.30	8.30	10.00	13.30	15.00	18.30

Table 4: Per cent larval mortality of *Aedes aegypti* against crude flower extracts of *Jasminum* species at 48 h.

Plant species	Solvents	LC <sub>50</sub> (mg/L)		LC <sub>90</sub> (mg/L)	
		24h	48h	24h	48h
		<i>Jasminum officinale</i>	Chloroform	16537.93	11439.80
Methanol	12652.04		2339.06	21055.59	3738.17
Aqueous	30135.97		17013.18	55517.87	23589.38
<i>Jasminum auriculatum</i>	Chloroform	17045.78	11001.98	28289.38	20180.45
	Methanol	10593.40	5044.02	16353.63	8019.21
	Aqueous	22427.85	18829.36	38694.80	35563.24
<i>Jasminum grandiflorum</i>	Chloroform	344.01	300.47	762.68	761.07
	Methanol	8880.63	5203.02	14467.53	9963.65
	Aqueous	17839.42	15031.87	33425.77	24108.36

Table 5: Probit analysis of crude flower extracts of *Jasminum* species against *Aedes aegypti*.

The results of the present study corroborate with earlier reports of plant extracts tested against the larvae of *Aedes aegypti* viz., the dichloromethane extract of aerial parts of *Pterocaulon polystachium* (LC<sub>50</sub> 149.2 ppm) [23], ethyl acetate leaf extract of *Sphaeranthus indicus* (LC<sub>50</sub> 201.11 ppm) [24], hexane leaf extract of *Abutilon indicum* (LC<sub>50</sub> 261.31 ppm) [25], ethyl acetate leaf extract of *Leucas aspera* (LC<sub>50</sub> 483.21 ppm) [26]. Kamaraj et al. [27] have reported larvicidal efficacy of *Cassia auriculata* flower methanol extracts against the larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus*. Mathew et al. [28] reported the chloroform extract of *Nyctanthes arbor-tristis* leaves to possess larvicidal activity against the *Aedes aegypti* with LC<sub>50</sub> value of 526.3 ppm and its flower methanol extracts with 679.4 ppm. The chloroform extract of *Orthosiphon thymiflorus* exhibited larvicidal activity with LC<sub>50</sub> value of 197.91 ppm against *Aedes aegypti* [29]. The chloroform leaf extract of *Acalypha alnifolia* when tested against *Aedes aegypti* showed larvicidal activity with LC<sub>50</sub> value of 182.58 ppm [30].

The preliminary screening of plant extracts against mosquitoes is a good means of evaluating the potential mosquitocidal property present in it [13,31]. Natural insecticides of plant origin have been given importance due to their ecofriendly nature and biodegradability as a

substitute of synthetic insecticides for the control of vectors of public health importance. Plants are the chemical factories and rich source of bioactive chemicals, some of which have medicinal and pesticidal properties [32]. Different types of phytochemicals of plant either from the whole part or from the specific parts come out with solvent during chemical extraction depending on the polarity of the solvent [33-35]. The botanical extracts from the plant leaves, roots, seeds, flowers and bark in their crude form have been used as conventional insecticides for centuries. The complex mixtures of phytocompounds can be used to develop environmentally-safe vector and pest-managing agents. In conclusion, the results reported in the present study open the possibility for further investigations of the efficacy of larvicidal properties of the crude chloroform extracts of *Jasminum grandiflorum* against *Aedes aegypti* as a potential agent for combating mosquitoes. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also to identify the active ingredient(s) of the extract responsible for larvicidal activity.

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