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Mosquito Larvicidal Activity of Leaf Extract of *Annona squamosa* against Dengue Vector, *Aedes albopictus*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The *Aedes albopictus* mosquito serves as a vector for various diseases affecting both humans and livestock. Current mosquito control strategies primarily rely on synthetic insecticides, which, despite their effectiveness, pose significant challenges such as insecticide resistance, environmental pollution, and adverse effects on human health. Plant-based products offer a promising alternative as they generally exhibit fewer adverse effects on ecosystems. In this study, we investigated the larvicidal efficacy of *Annona squamosa* leaf extracts using aqueous, ethanol, and hexane solvents against third-instar larvae of *Aedes albopictus*. The results revealed varying LC50 values for the third instar larvae: 2790.318 ppm (aqueous extract), 11775.170 ppm (ethanol extract), and 20417.320 ppm (hexane extract). Notably, the hexane extract exhibited the highest larvicidal activity, particularly after 24 hours of exposure. These findings underscore the potential of plant-derived compounds, particularly those extracted using hexane, as effective agents for mosquito control.

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1. INTRODUCTION

"Mosquitoes belong to the family Culicidae in the class Insecta. They are responsible for transmitting the most important vector-borne diseases, including malaria, lymphatic filariasis, Japanese encephalitis, dengue, and yellow fever" [1]. "They are common pests in every corner of the globe, having evolved to adapt to virtually any climatic conditions" [2]. "The majority of mosquito control programs target the larval stage in their breeding sites. Medicinal plants are a rich source of alternative agents for mosquito control because they contain bioactive chemicals that act against a limited number of species, including specific target insects, and are eco-friendly. Several plant extracts and isolated botanicals from certain plant families have been evaluated for their promising larvicidal activity, with no hazardous effects on the ecosystem" [3]. "Approximately 3500 species of mosquitoes have been described worldwide, with relatively few of them being significant vectors of human diseases. However, the worldwide problem of mosquito-transmitted diseases is quite severe" [4]. "The only efficacious approach to minimizing the incidence of these diseases is to eradicate and control mosquito vectors, mainly by applying insecticides to larval habitats and educating the public" [5]. "Aedes albopictus is responsible for transmitting many arboviruses to laboratory animals and birds, and it also transmits chikungunya and dengue fever to humans" [6,7]. "The incidence and geographical distribution of dengue fever have increased dramatically over the past 30 years. It is estimated that 2.5 billion of the world's population are at risk, with 100 million cases arising annually, including 500,000 cases of dengue hemorrhagic fever (DHF). In 2011, a dengue outbreak occurred in Punjab, with 20,500 confirmed cases and 304 reported deaths"[8].

"The most effective approach to minimizing the incidence of these diseases is to eradicate and control mosquito vectors, primarily by applying insecticides to larval habitats and educating the public" [9]. However, the control of vector populations using synthetic insecticides has led to insecticide resistance and has negative effects on humans and the environment. Therefore, the development of eco-friendly control tools is crucial for public health [10,11]. More attention has been focused on the search for eco-friendly compounds that could serve as alternatives to synthetic insecticides. In pursuit of this objective, numerous studies on the efficacy of phytochemicals against mosquito vectors have been conducted worldwide, demonstrating their larvicidal, pupicidal, adult emergence inhibition, and repellent properties [12]. Thus, phytochemicals can be utilized as alternatives to synthetic insecticides or in conjunction with other insecticides within integrated vector control programs. Consequently, research into alternative methods utilizing plants for the development of environmentally safe, biodegradable insecticides for mosquito control is encouraged. Botanical insecticides may emerge as viable alternatives to synthetics in the future, given their relative safety, ease of degradation, and widespread availability in many parts of the world [13].

Annona squamosa is a small deciduous or semi-evergreen tree in the plant family Annonaceae, renowned for its fruit known as custard apple. This study aims to assess and compare the larvicidal activity of aqueous, ethanol, and hexane extracts of *A. squamosa* against the third instar larvae of the dengue vector *A. albopictus*.

2. MATERIALS AND METHODS

2.1 Preparation of *A. squamosa* Leaf Extract

Fresh *A. squamosa* leaves were collected. The leaves were shade dried, powdered in a blender, sifted and stored in an air-tight bottle.

2.2 Collection and Maintenance of Mosquitoes

Laboratory reared III instar larvae of *Aedes albopictus* were obtained from the Insectary of the Center for Research in Medical Entomology (CRME), ICMR, Madurai. The larvae were maintained at room temperature and kept in dechlorinated tap water in an enamel bowl.

2.3 Preparation of Aqueous Extract of the Plant Material

The dried leaves of *A. squamosa*are weighted and 100g powder was soaked in 500ml distilled water for 24 hours and then filtered through filter paper and the aqueous extract is stored in bottles (to avoid degradation). These solutions were considered at stock (20%) from which various concentrations (20, 40, 60, 80, 100 ppm) were made with distilled water for evaluating its larvicidal potential.

2.4 Preparation of Ethanolic Extract of Plant Material

By percolation method powdered leaves of *A. squamosa*are weighted and 100g of the leaf powder was soaked in 500ml ethanol for 24 hours.The extract is stored at room temperature. These solutions were considered at stock from which various concentrations (20, 40, 60, 80, 100 ppm) were made with distilled water for evaluating its larvicidal activity.

2.5 Preparation of Hexane Extract of Plant Material

Powdered leaves of *A. squamosa*are weighted and 100g of the leaf powder was soaked in 500ml hexane for 24 hours. The extract is stored at room temperature. These solutions were considered at stock from which various concentrations (20, 40, 60, 80, 100 ppm) were made with distilled water for evaluating its larvicidal activity.

Percentage of mortality = (Number of dead larvae / Number of larvae introduced) ×100

2.6 Statistical Analysis

Data from all triplicates were pooled for statistical analysis. LC50 values were calculated. Chi-square and regression equation was performed to determine the difference in larval mortality between concentrations.

3. RESULTS

The larvicidal activity of aqueous, ethanol and hexane extracts of *A. squamosa* was studied against 3rd larval instar *A.albopictus* and the larvicidal activity after 24 hours and 48 hours were recorded and tabulated (Table 1, Figs. 1 and 2).

For the third instar larvae, the LC50 values are 2790.318,20417.320 and 9656.53ppm respectively for aqueous,ethanol and hexane extracts after 24 hours of exposure. The LC50 values for 48 hours of exposure are 11775.170, 6594.354 and 6568.65ppm of respectively for aqueous, ethanol and hexane extracts (Table 2).

In the present investigation it was found that petroleum ether extracts of *A. squamosa* were found to be more toxic than other solvent fractions. The minimum does of hexane extract of *A. squamosa* (9656.53) brought 50% mortality of the third instar larvae of *A.albopictus*. The regression equation for these LC50 values are Y = 0.497 X+ 2.87 (Table 3).

It was found that larvicidal activity was very high in hexane extract when compared to ethanol and aqueous solution. The results show that in all the three extracts as the concentrations increases, percentage mortality also increases.

4. DISCUSSION

Vector-borne diseases are the main concern in developing countries and *A. albopictus* is the main dengue vector responsible for transmission of dengue fever. Mosquitoes transmit serious human diseases, causing millions of deaths every year and the development of resistance to chemical resulting in rebounding vectorial capacity. Mosquitoes in the larval stage are attractive targets because mosquitoes breed in water, which makes it easy to deal with them in this habitat. The use of conventional pesticides in the water sources however introduces many risks to people and the environment. The development of insecticide resistance in populations of *A. albopictus* indicates the need for the research of safe and effective alternative measures.

The ethanol leaf extract of *A. squamosa* was found to have the most promising larvicidal activity against *Culex quinquefasciatus* larvae. The larvicidal and mosquitocidal activities of ethanolic extract of *A. squamosa* and *Centella asiatica* were effective against larvae of *An. Stephensi* [14]. The methanolic leaf extract and petroleum ether extract of seeds of *A. Squamosa* showed larvicidal activity when tested against *An. stephensi*, *Cx. quinquefasciatus* and *A. aegypti* [15].

The larvicidal activity of *A. reticulata* leaf crude extract at different concentration shows 100% mortality was observed at 5,10,25,50,100 and 200ppm concentrations of crude extract. Present results are in agreement with these findings are nearly 100% mortality was observed after 48 hours of treatment [16]. So far in the present study, it is suggested that aqueous, ethanol and hexane extracts of *A. squamosa* can be used as effective larvicidal agents against *A.albopictus* instead of synthetic insecticides which produce hazardous effect on the non-target organisms and to which the vectors have developed resistance.

Table 1. Larvicidal activity of different concentration of various leaf extract of *A. squamosa* againstthird instar larvae of *A. albopictus*

S. No	Dosage (ppm)	24 hours			48 hours		
		Aqueous	Ethanol	Hexane	Aqueous	Ethanol	Hexane
1	20	2.5 ± 0.25	5.5 ± 0.12	8.5 ± 0.65	4.5 ± 0.22	7.5 ± 0.65	10 ± 1.09
2	40	4.5 ± 0.35	7.5 ± 0.51	9.5 ± 0.35	6.5 ± 0.13	9.5 ± 0.95	13.5 ± 0.85
3	60	6.5 ± 0.15	8.5 ± 0.34	12.5 ± 0.25	8.5 ± 0.78	11.5 ± 0.88	15 ± 0.5
4	80	9 ± 0.11	9.5 ± 0.5	13.5 ± 0.51	9.5 ± 0.45	13 ± 0.21	17.5 ± 0.65
5	100	10.5 ± 0.15	12.5 ± 0.4	15.5 ± 0.54	11.5 ± 0.19	17.5 ± 0.5	18 ± 0.59

Table 2. LC50 and confidence intervals of different concentration of various leaf extracts of *A. squamosa* on *A. albopictus*

S. No	Hours	Aqueous			Ethanol			Hexane		
		LCL	LC50	UCL	LCL	LC50	UCL	LCL	LC50	UCL
1	24	0.019	2790.318	0.899	0.747	20417.320	1.15 2	0.594	9656.53 2	1.075
2	48	0.612	11775.170	1.087	0.471	6594.354	1.025	0.5978	6568.65 1	1.06

Table 3. Chi-square and regression for different concentration of various leaf extracts of *A. squamosa* on *A. albopictus*

S. No	Hours	Chi-square	Aqueous		Ethanol	Hexane	
			Regression Equation	Chi-square	Regression Equation	Chisquare	Regression Equation
1	24	0.02	y =0.881x+1.96	0.02	y =0.525x +2.74	0.02	y =0.535x +2.87
2	48	0.02	y =0.597x + 2.57	0.09	0.578x + 2.79	0.01	y =0.497x +3.10

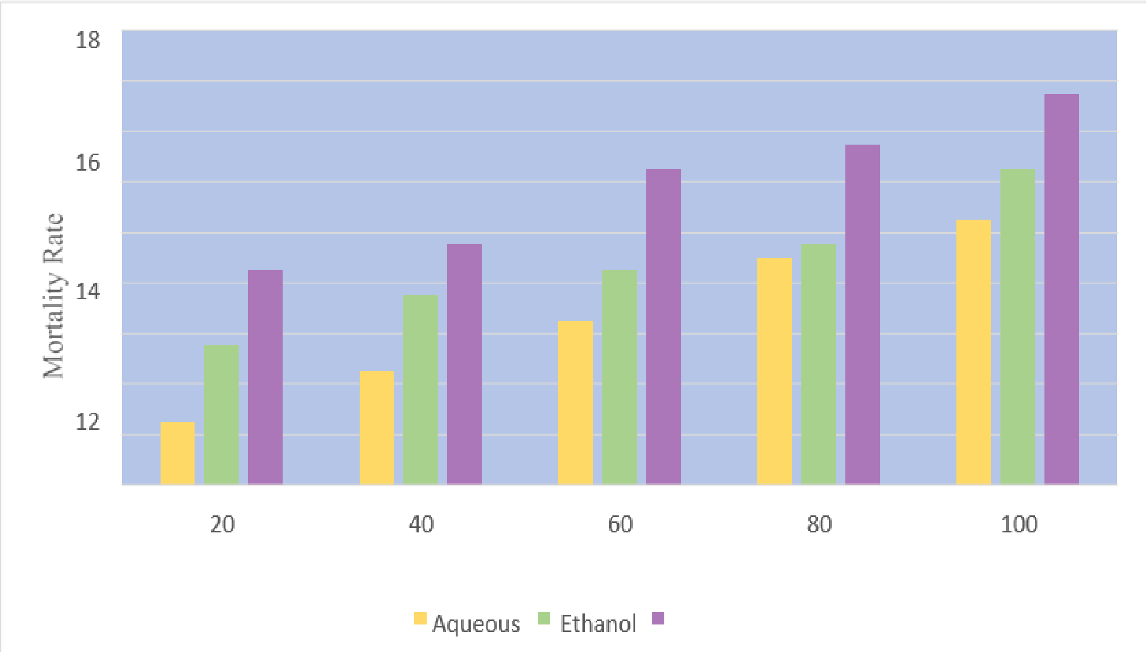

Fig. 1. Larvicidal activity of different concentration of various leaf extract of *A.squamosa* against third instar larvae of *A. albopictus* after 24 hours

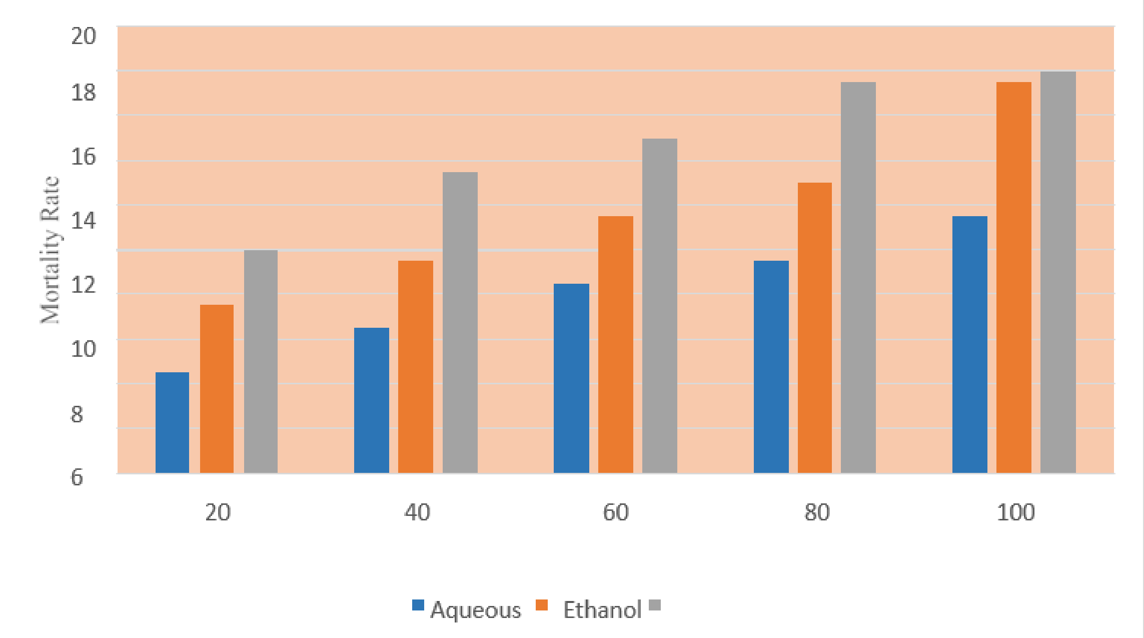

Fig. 2. Larvicidal activity of different concentration of various leaf extract of *A. squamosa* against third instar larvae of *A. albopictus* after 48 hours

5. CONCLUSION

The same trend was observed in our study as well. We evaluated the larvicidal efficacy of *A. squamosa* leaf extracts against the dengue vector, *Aedes albopictus*. Leaves of *A. squamosa* were sequentially extracted with aqueous solution, ethanol, and hexane. The larvicidal activities of these three extracts were studied in the concentration range of 20 to 100 ppm. All plant extracts exhibited moderate effects after 24 and 48 hours of exposure; however, the highest percent mortality was observed with the hexane extract, followed by ethanol and aqueous extracts, respectively. These extracts have the potential to be utilized as an eco-friendly approach for controlling the dengue vector, *A. albopictus*. The experiment revealed that larval mortality of *A. albopictus* was higher with the hexane extract compared to ethanol and aqueous extracts, and mortality increased with both time and concentration. Application of these extracts to larval habitats may yield promising results in dengue vector management programs. *A. squamosa* leaf extract could serve as a superior alternative for eradicating the mosquito population in the larval stage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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