

Larvicidal activity of leaf crude extract of *Vitex negundo* against malaria and filariasis vectors



Zoology

KEYWORDS: Larvicidal, *Vitex negundo*, *Anopheles stephensi*, *Culex quinquefasciatus*.

P. Rajiv Gandhi

Ph.D., Research Scholar PG and Research Department of Zoology, Auxilium College (Autonomous), Gandhi Nagar - 632 006, Vellore District, Tamil Nadu, India.

R. Regina Mary*

Assistant Professor, PG and Research Department of Zoology, Auxilium College (Autonomous), Gandhi Nagar - 632 006, Vellore District, Tamil Nadu, India.

ABSTRACT

The present study was carried out to evaluate the Larvicidal activity of crude Methanol, Ethyl acetate and acetone extracts of the leaf of *Vitex negundo* were tested against the fourth instar larvae of the malaria, *Anopheles stephensi* Liston; and filariasis vector, *Culex quinquefasciatus*, Say (Diptera: Culicidae). The larval mortality was observed after 24 h of exposure. However, the highest larval mortality was found in methanol extract of *V. negundo*, against the larvae of *A. stephensi* and *C. quinquefasciatus* (LC₅₀=94.81 and 88.83 ppm and LC₉₀=171.91 and 161.206 ppm and χ^2 = 3.307 and 5.288) respectively. This is an ideal eco-friendly approach for the control of the malaria vector, *A. stephensi* and the lymphatic filariasis vector, *C. quinquefasciatus*.

1. Introduction

Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (Fradin and Day, 2002). Vector control is a serious concern in developing countries like India. Every year, a large of the population in the world is affected by one or more vector-borne diseases. The mosquitoes are potential vectors of many diseases, including malaria, filariasis, dengue, brain fever, etc. There is an urgent need to check the proliferation of the population of vector mosquitoes in order to reduce vector-borne diseases by appropriate control methods (Kuppusamy and Murugan, 2009).

Anopheles stephensi are major malaria vectors in India. With an annual incidence of 300-500 million clinically manifested cases and a death toll of 1.1-2.7 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world's population lives in areas where malaria is endemic (Wernsdorfer and Wernsdorfer, 2003). *Culex quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard et al., 2003). The harmful effects of chemicals on non-target populations, ever-growing resistance to chemical insecticides along with the recent resurgence of different mosquito borne diseases have induced scientists to explore alternative, simple, sustainable methods of mosquito control (Gandhi et al., 2016). Synthetic insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance, and harm to mammals. Hence, there is a constant need for developing biologically active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimizing the accumulation of harmful residues in the environment. Natural products are generally preferred because of their less harmful nature to nontarget organisms and due to their innate biodegradability.

Vitex negundo L. (Verbenaceae) commonly known as nirkundi chiefly occurring throughout India (Watt, 1972; Gupta et al., 2005). Though almost all parts of *V. negundo* are used, the leaves and the barks are the most important in the field of medicine. The decoction of leaves is considered as tonic, vermifuge and is given with pepper in catarrhal fever (Chandramu et al., 2003). The leaves of this plant have been shown mosquito repellent effects (Hebbalkar et al., 1992). In the present study, we reported the different solvent extracts of *V. negundo* would be useful in promoting research aiming at the development of new agent for mosquito larvicidal (*A. stephensi*, and *C. quinquefasciatus*) activity.

2. Materials and Methods

2.1. Materials

The healthy leaves of *Vitex negundo* Linn. (Verbenaceae) were collected from Thandrampet Village, Thiruvannamalai district, Tamil Nadu, (12°9'15"N, 78°56'48"E) India and the taxonomic identification was made by Dr. C. Hema, Department of Botany, Arignar Anna Govt. Arts College for Women, Walajapet, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference. Methanol, Ethyl acetate and acetone was purchased from Sigma-Aldrich, India.

2.2. Preparation of plant extracts

The dried leaves (500 g) were powdered mechanically using a commercial electrical stainless steel blender and extracted with methanol (2,000 ml; Qualigens), ethyl acetate (1,200 ml; Fine) and acetone (1,000 ml; Qualigens), in a Soxhlet apparatus separately until exhaustion. The aqueous and hot water extracts were prepared as per the procedure of Chowdhury et al. (2008). The extract was concentrated under a reduced pressure of 22-26 mmHg at 45°C and the residue obtained was stored at 4°C.

2.3. Insect rearing

Anopheles stephensi Liston and *C. quinquefasciatus* Say (Diptera: Culicidae) larvae were collected from a stagnant water area of Melvisharam (12° 56' 23" N, 79° 14' 23" E) and identified in Zonal Entomological Research Centre, Vellore (12° 55' 48" N, 79° 7' 48" E), Tamil Nadu to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the method of (Rahuman et al., 2008a).

2.4. Larvicidal bioassay

One gram of crude extract was first dissolved in 100 ml of respective solvent (stock solution). From the stock solution, different concentrations ranging from methanol 40 to 200 ppm, ethyl acetate and acetone 50 to 250 ppm were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05%. Experiments were conducted for 24 h at room temperature (28±2°C). The larvicidal activity was assessed by the procedure of WHO (1996) with some modification and as per the method of Rahuman et al. (2000). For bioassay test, larvae were taken in five batches of 20 in 249 ml of water and 1.0 ml of the desired plant extract concentration. The numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates.

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC₅₀, LC₉₀, and other statistics at 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), and

chi-square values were calculated using the software developed by (SPSS, 2007).

3. Results and discussion

In the present study, the larvicidal activity of different solvent extracts of *V. negundo* is noted and presented in Tables 1. All the extract showed moderate and good toxic effect on larvae of *A. stephensi* and *C. quinquefasciatus* after 24 h of exposure; however, the highest mortality was found in the methanol extract of *V. negundo*, against the larvae of *A. stephensi* and *C. quinquefasciatus* (LC_{50} =94.81 and 88.83 ppm and LC_{90} =171.91 and 161.206 ppm and χ^2 = 3.307 and 5.288) and Ethyl acetate extract showed mortality against The larvae of *A. stephensi* and *C. quinquefasciatus* (LC_{50} =131.23 and 130.88 ppm and LC_{90} =248.34 and 240.41 ppm and χ^2 = 3.823 and 1.507), and acetone extract showed mortality against The larvae of *A. stephensi* and *C. quinquefasciatus* (LC_{50} =126.08 and 124.22 ppm and LC_{90} =237.62 and 228.64 ppm and χ^2 = 1.794 and 2.465), on 24 h exposure respectively. Chi-square value was significant at P<0.05 level on *A. stephensi* and *C. quinquefasciatus* larvae. (Rahuman et al., 2008b) were reported with less activity compare with the *V. negundo* extract against the larvae of *A. stephensi*, and *C. quinquefasciatus*. Thomas et al. (2004), reported that the essential oil of *Ipomoea cairica* possessed remarkable larvicidal properties as it could produce 100% mortality in the larvae of *A. stephensi*, and *C. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm.

4. Conclusion

In conclusion, an attempt has been made to evaluate the role of plant extracts in mosquito larvicidal activity. The results reported here open the possibility for further investigations on the efficacy of larvicidal properties of natural product extracts. The isolation and purification of crude extract of *V. negundo* in progress.

Table 1 Larvicidal activity of different solvent extracts against fourth-instar larvae of *A. stephensi* and *C. quinquefasciatus* on 24-h exposure.

Species	Extracts	Concentration (ppm)	% Mortality ± SD	LC ₅₀ (LCL-UCL) (ppm)	LC ₉₀ (LCL-UCL) (ppm)	χ ² (df=4)
<i>A. stephensi</i>	Methanol	40	21±2.549			
		80	36±1.581			
		120	68±2.121	94.81 (86.4-102.61)	171.91 (160.28-186.98)	3.307
		160	83±2.121			
		200	98±1.414			
	Ethyl acetate	50	17±0.707			
		100	34±2.121			
		150	65±1.414	131.23 (9.29-142.43)	248.34 (229.82-273.20)	3.823
		200	79±2.549			
		250	87±2.121			
	acetone	50	19±0.707			
		100	35±1.581			
		150	66±2.549	126.08 (114.43-136.90)	237.62 (220.38-260.48)	1.794
		200	80±3.535			
		250	91±1.414			

<i>C. quinquefasciatus</i>	Methanol	40	24±2.236			
		80	39±1.414			
		120	69±2.915	88.83 (80.64-96.36)	161.206 (150.31-175.27)	5.288
		160	88±1.581			
		200	100±0.00			
	Ethyl acetate	50	18±2.549			
		100	32±1.414			
		150	63±0.707	130.88 (119.64-141.49)	240.41 (223.28-263.07)	1.507
		200	79±2.549			
		250	91±2.121			
	acetone	50	20±2.236			
		100	33±0.707			
		150	67±1.581	124.22 (113.18-134.51)	228.64 (212.71-249.51)	2.465
		200	81±1.414			
		250	94±3.162			

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