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# Short Communication

# A comparative study of larvicidal activity of insecticidal plants against malaria vector mosquito *Anopheles* stephensi

\*Bachchan O. Malik, Mhasalkar P. Malik and Bachchan Nagarkar

Department of Pharmacognosy, Manipal University, Madhav Nagar, Manipal, Karnataka 576104, India. \*Corresponding author. E-mail:bomalik@gmail.com

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The aim of this work was to compare larvicidal activity of insecticidal plants against malaria vector mosquito *Anopheles stephensi*. The larvicidal activity of *Lantana camara* Linn. and *Bauhinia racemosa* Lam., extracted in petroleum ether, chloroform and ethyl acetate were tested against mosquito larvae of *A. stephensi*. Late third or early fourth instar larvae were used for the screening. These extracts were used for determining the larvicidal activity using World Health Organization (WHO) method for evaluation of new larvicidal agents. The petroleum ether extract of *L. camara* showed highest larvicidal activity in comparison to petroleum ether extract of *B. racemosa*, and ethyl acetate extract of *B. racemosa* showed highest larvicidal activity in comparison to chloroform extract of *L. camara* against the mosquito vector *A. stephensi*. No mortality was observed in control. The result suggests the use of the plants in insect control as an alternative method for minimizing the noxious effect of some pesticide compounds on the environment. Thus, the extracts of whole plant of *L. camara* Linn. and leaf extracts of *B. racemosa* Lam. may deliver promising, more selective and biodegradable agents.

**Key words:** Lantana camara, Bauhinia racemosa, Anopheles stephensi.

## INTRODUCTION

Mosquitoes are one of the most medicinally significant vectors and they transmit parasites and pathogens which continue to have devastating impact on human beings (Maheswaran et al., 2008). Several numbers of species belong to genera *Anopheles, Culex, Aedes* and vectors for the pathogens of various diseases like malaria, filaria, Japanese encephalitis, dengue, and yellow fever. Thus, one of the approaches for control of these vector-borne diseases is the interruption of disease transmission by killing mosquitoes or preventing mosquito bites (Das, 1989). Herbal products which have proven potential as insecticides or replicants can play an important role in the

interruption of the transmission of mosquito-borne diseases both at the individual and community level. However the discovery, development and use of synthetic organic insecticidal chemicals with persistent residual action not only over shadowed the use of herbal products as insect-cides of choice against mosquitoes but also become the major weapon for mosquito control (Sakthivadivel and Daniel, 2008). The extensive use of synthetic organic insecticides during the last decade has resulted in environmental hazards and also in the development of physiological resistance in most vector species. This has necessitated the need for research and development of environmentally safe, biodegradable,

low cost indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations (Singh et al., 2006). The plant *Lantana camara* Linn. (Verbenaceae) and *Bauhinia racemosa* Lam. (Caesalpiniaceae) are described in Ayurveda and Siddha, as a potent drug against a variety of ailments. These plants are widely distributed and cultivated in various parts of India (Yoganarasimhan, 2000; Varier, 2006; Prajapati et al., 2007; Ivan, 1999; Singh and Himadri, 2005; Raveendra and Martin, 2006).

#### **MATERIALS AND METHODS**

### Collection of plant material

The plants were collected during flowering stage in the month of July to August from Nilgiris. Then, their identification was established with the aid of an expertise botanist and by making comparison with herbarium sheets of the authentic sample. Many of the defensive components are biodegradable, with non-residual effect on the biological environment hence; an attempt has been made in the present investigation to identify plants with the potential to control vector mosquitoes.

#### Extraction

The plant *L. camara* Linn. and *B. racemosa* Lam. were powdered and extracted in soxhlet, with petroleum ether, chloroform and ethyl acetate. The extracts were concentrated under reduced pressure to a semisolid mass. These extracts were used for determining the larvicidal activity against mosquito larvae.

## **Biological assay**

Larvicidal activity was evaluated in accordance with World Health Organization (WHO) for the evaluation of new larvicidal agents (WHO, 1985). The larvae of Anopheles stephensi was obtained and reared from the neonates in National Institute of Communicable Diseases, Southern India branch field station located at Mettupalayam (District Coimbatore of Tamil Nadu State), at 28 ± 2°C with a photoperiod of 12 h light and dark, and 80 ± 10% relative humidity. A brewer's yeast powder mixed with an equal quantity (w/w) of ground dog biscuit was used in the laboratory as food for the larvae. The late third or early fourth instar larvae were collected according to larval size and the degree of chitinization of respiratory siphon (Cheng et al., 2003). Different concentrations of the extracts were prepared in 1 ml of acetone for each experiment. All ex-perimental exposure was done in 500 ml glass beaker in triplicate. Twenty-five (25) larvae were collected with a pasture pipette, placed on a filter paper for removal of excess of water and placed in 250 ml dechlorinated tap water containing various concentration of crude extracts. Three controls in triplicate were setup. one with acetone (1 ml), the other with distilled water (250 ml).

The beakers were covered with muslin cloth to avoid entry of any foreign material. Sufficient control was also kept for each extracts. The observed mortality (crude mortality) was recorded at 24 h of exposure to test solution. From this crude mortality, percentage crude mortality was obtained. Subsequently, controlled mortality, if any, was recorded and percentage crude mortality was obtained. The percentage crude mortality was corrected by using Abbot's formula. The corrected probit mortality and expected mortality was also obtained but no control mortality was recorded during the experiment, so Abbot's formula was not used.

# Statistical analysis

Lethal concentration (LC50 and LC90) values and their 95% confidence limits were estimated by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each extract (Finnely, 1971). All analysis was carried out using the statistical package for social sciences (SPSS) software, version 13.0.

#### **RESULTS AND DISCUSSION**

Seven different concentrations of test solution ranging from 50 to 350 ppm for petroleum ether extract and six concentrations of test solution ranging from 50 to 300 ppm for chloroform extract for *L. camara* Linn. and six different concentrations of test solution ranging from 50 to 300 ppm for petroleum ether extract and five different concentrations of test solution ranging from 40 to 200 ppm for ethyl acetate extract were subjected to 24 h bioassay, using late 3rd or early 4th instar larvae of A. stephensi. Based on observations made in the 24 h bioassay studies among the different plant extracts, the petroleum ether extract of L. camara Linn. was more potent than petroleum ether extract of B. racemosa Lam. and ethyl acetate extract of B. racemosa was more potent than chloroform extract of L. camara Linn. for vector mosquito and were identified as efficient against them. The results from the A. stephensi larvicidal bio-assav using different extracts of two different plants, the most active extract against late third or early fourth instar larvae of A. stephensi, were the petroleum ether extract of L. camara and Ethyl acetate extract of B. racemosa. The results of susceptibility of larvae for the extracts were given in Tables 1 and 2; and Figures 1 and 2.

# Conclusion

The use of the plants in insect control offers a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. Moreover, these results could be useful in the search for newer, more selective and biodegradable larvicidal natural compounds.

**Table 1.** Observed and expected mortality of *Anopheles stephensi* larvae exposed to *Lantana camara* with petroleum ether and chloroform extracts. Expected mortality is based on probit regression analysis.

Concentration	No. of Larvae		Mortality (%)		Expected mortality			Probit (mortality)		LC <sub>50</sub>	LC90
(µg/ml)	Exposed Dead		Crude Corrected		Probit Dead %		%	= a + b × concentration	$\chi^2$ , DF, P-value	(95% CI)	(95% CI)
						Petroleum e	extract				
50	75	17	22.7	22.7	-0.80	15.8	21.1		$\chi^2$ =1.56, DF=5, P=0.9	126.7 (112.0-139.7)	248.9 (231.2-271.8)
100	75	30	40.0	40.0	-0.28	29.3	39.1				
150	75	44	58.7	58.7	0.25	44.8	59.8				
200 250	75 75	56 67	74.7 89.3	74.7 89.3	0.77 1.30	58.5 67.7	78.0 90.3	-1.3270+0.0105×conc.			
300	75	73	97.3	97.3	1.82	72.4	96.6				
350	75	75	100.0	100.0	2.35	74.3	99.1				
						Chloroform	extract				
50	75	8	10.7	10.7	-1.95	1.9	2.6				
100	75	9	12.0	12.0	-1.16	9.2	12.3				
150 200	75 75	13 44	17.3 58.7	17.3 58.7	-0.37 0.42	26.7 49.7	35.5 66.2	-2.7408+0.0158×conc.	$\chi^2$ =39.9, DF=4, P<0.005	173.8 (120.2-235.3)	255.0 (205.5-435.0)
250	75	73	97.3	97.3	1.21	66.5	88.7				
300	75	75	100.0	100.0	2.00	73.3	97.7				

DF = degrees of freedom, conc. = concentration, CI = confidence interval.

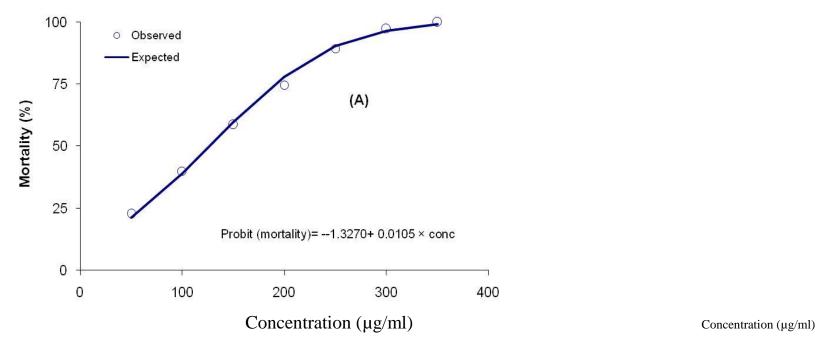
**Table 2.** Observed and expected mortality of *Anopheles stephensi* larvae exposed to *Bauhinia racemosa* with petroleum ether and ethyl acetate extracts. Expected mortality is based on probit regression analysis.

Concentration (μg/ml)	No. of Larvae		Mortality (%)		<b>Expected Mortality</b>			Probit (mortality)		LC50	LC <sub>90</sub>
	Exposed	Dead	Crude	Corrected	Probit	Dead	%	= a + b x concentration	$\chi^2$ , DF, P-value	(95% CI)	(95% CI)
						Petrole	um ether	extract			_
50	75	11	14.7	14.7	-1.21	8.4	11.2				
100	75	24	32.0	32.0	-0.60	20.5	27.3			149.3	054.0
150 200	75 75	31 49	41.3 65.3	41.3 65.3	0.01 0.62	37.7 54.8	50.2 73.1	-1.8238+0.0122×conc.	$\chi^2$ = 9.7, DF=4, P=0.046	(121.2-175.3)	254.3 (220.5-316.0)
250	75	69	92.0	92.0	1.23	66.7	89.0				

Table 2. Contd

300	75	75	100.0	100.0	1.84	72.5	96.7					
	Ethyl acetate extract											
40	75	12	16.0	16.0	-1.14	9.5	12.7					
80	75	25	33.3	33.3	-0.43	25.1	33.5			104.0	176.1	
120 160	75 75	42 60	56.0 80.0	56.0 80.0	0.28 1.00	45.9 63.0	61.2 84.1	-1.8513+0.0178×conc.	$\chi^2$ =5.9, DF=3, P=0.11	(95.2-112.5)	(163.3-193.1)	
200	75	75	100.0	100.0	1.71	71.7	95.6					

DF = degrees of freedom, conc. = concentration, CI = confidence interval.



**Figure 1.** Relationship between *Anopheles stephensi* larval mortality and concentration of *Lantana camara* with (A) petroleum ether and (B) chloroform extracts. Expected values are based on probit regression analysis. Conc = concentration.

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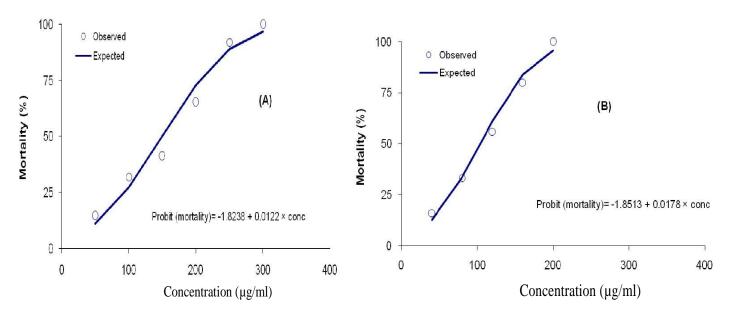


Figure 2. Relationship between *Anopheles stephensi* larval mortality and concentration of *Bauhinia racemosa* with (A) petroleum ether and (B) ethyl acetate extracts. Expected values are based on probit regression analysis. Conc = concentration.

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