LARVICIDAL ACTIVITY OF LEAVES OF *BAUHINIA RACEMOSA* (LAM.) AGAINST THE LARVAE OF *ANOPHELES STEPHENSI*

Babita R. Malik [1]*, Mukesh K. Malik [1], P. Dhamodaran[1], N. Balakrishnan[2]

1. Dept. of Pharmacognosy, J.S.S. College of Pharmacy Rockland, Ooty-643 001, T.N., India.
2. National Institute of Communicable Diseases, Southern India branch Conoor-643101, T.N., India.

*Corresponding author: Tel No: +919305730760, +919634401029.
Email: tinaindia771@yahoo.co.in
tinaindia771@gmail.com

Summary

Aim of this work was to study the larvicidal activity of leaves of *Bauhinia racemosa* (Lam.) against the larvae of *Anopheles stephensi.*
A preliminary laboratory trial was undertaken to determine the efficacies of petroleum ether and ethyl acetate extracts of dried leaves of *Bauhinia racemosa* (Lam.) belonging to the family Caesalpiniaceae at various concentrations against the late third or early fourth instar larvae of *Anopheles stephensi* by following the WHO guidelines.
The results suggests that hundred percent mortality of petroleum ether and ethyl acetate extracts of *Bauhinia racemosa* (Lam.) was observed at 300ppm and 200ppm while ethanolic extract was inactive up to 300ppm. No control mortality was observed
The result suggests the use of the plant in insect control as an alternative method for minimizing the noxious effect of some pesticide compounds on the environment. Thus the extracts of leaves of *Bauhinia racemosa* may deliver promising more selective and biodegradable agent.

Key words: - *Anopheles stephensi,* Larvicidal activity, Leaves of *Bauhinia racemosa* (Lam.)

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.[1] Several numbers of species belonging to genera *Anopheles, Culex, Aedes* and vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, Dengue, and yellow fever.

Thus one of approaches for control of these mosquitoes borne diseases is the interruption of disease transmission by killing or preventing mosquito bites.[2] Herbal products which proven potential as insecticides or replicants can play an important role in the interruption of the transmission of mosquitoes 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 insecticides of choice against mosquitoes but also become the major weapon for mosquito control. [3]

But the extensive use of synthetic organic insecticides during the last decades 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 use with minimum care by individual and communities in specific situations.[4]

The plant *Bauhinia racemosa* (Lam.) is described in Ayurveda and Siddha, as a potent drug used in diarrhoea, malaria, urinary disorders, and also used to expel intestinal worms. Deciduous tree with bilobed leaves. Flowers white, in racemosa. Pod falcate, turgid. Found in almost all districts of India, Sri Lanka, China.[5, 6, 7]

Materials and Methods

Collection Of Plant Material

The plant was collected during flowering stage in the month of July-August from Nilgiris. Then their identification was established with the aid of an expertise botanist Dr. S. Rajan and compared with herbarium sheets of the authentic sample.

Many of defensive components are biodegradable with non-residual effect on the biological environment hence; an attempt has been made in present investigation to identify plant with potential to control vector mosquitoes.

Extraction

The dried leaves of *Bauhinia racemosa* (Lam.) plant was powdered and extracted in soxhlet with petroleum ether 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 to WHO for the evaluation of new larvicidal agents.[8] 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 ± 2ºC with a photoperiod of 12 hours light and dark and 80± 10% RH. A brewer's yeast powder mixed with an equal quantity (w/w) of ground dog biscuit is used in laboratory as a food for larvae.

The late third or early fourth instar larvae were collected according to larval size and degree of chitinization of respiratory siphon.[9] Different concentrations of the extracts were prepared in 1ml of acetone for each experiment. All experimental exposure was done in 500ml glass beaker in triplicate. 25 larvae were collected with a pasture pipette, placed on a filter paper for removal of excess of water and placed in 250ml dechlorinated tap water containing various concentration of crude extracts. Three controls in triplicate were setup, one with acetone (1ml), the other with distilled water (250ml).

The beakers were covered with muslin cloth to avoid to entry of any foreign material. Sufficient control was also kept for each extracts. The observed mortality (Crude mortality) was recorded at 24 hours of exposure to test solution. From this crude mortality, percentage crude mortality was obtained. Subsequently control 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 recorded during the experiment so we have not used of Abbot's formula.

Statistical Analysis

LC_{50} and LC_{90} 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.[10] All analysis was carried out using the (Statistical Package Social Science) SPSS software, version 13.0.

Results and Discussion

Six different concentrations of test solution ranging from 50-300 ppm for Petroleum ether extract and five different concentrations of test solution ranging from 40-200 ppm for Ethyl acetate extract were subjected to 24 hr bioassay using early 4^{th} instar larvae of *Anopheles stephensi*. The estimated LC_{50} and LC_{90} values (95% confidence intervals) with Petroleum ether extract were 149.3 (121.2-175.3) and 254.3 (220.5-316.0) and Ethyl acetate extract were 104.0 (95.2-112.5) and 176.1 (163.3-193.1) respectively. The median lethal concentrations suggest that the Ethyl acetate extract is only 1.4 times more efficacious than Petroleum ether extract. The difference in efficacy between extracts is not statistically significant (95% confidence interval for LC_{50} do not overlap). The results of susceptibility of larvae for the extracts were given in table I.

Conclusion

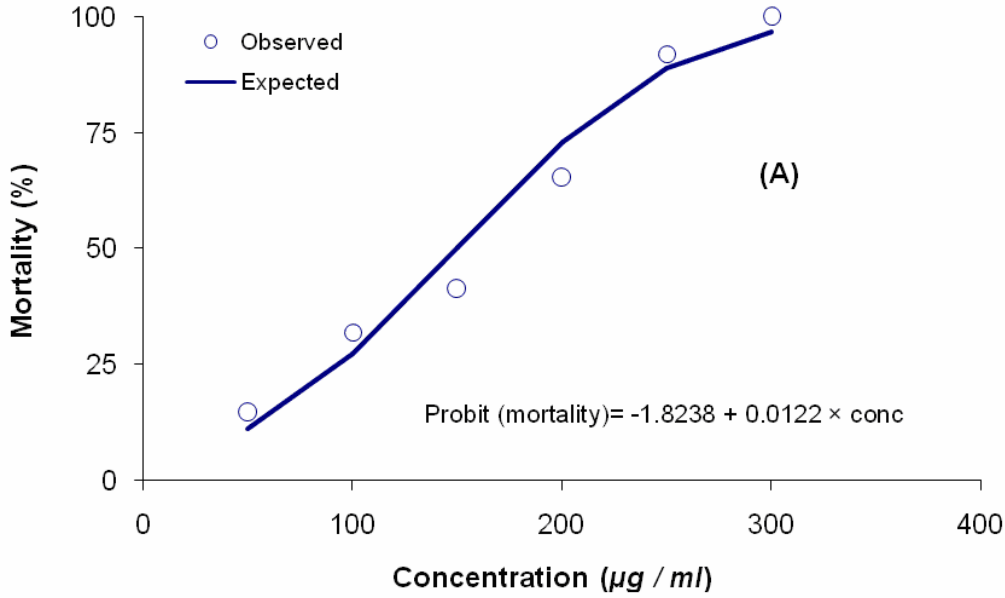
The use of the plants in insect control offers a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. Moreover, these results could be useful in the search for newer, more selective and biodegradable larvicidal natural compounds.

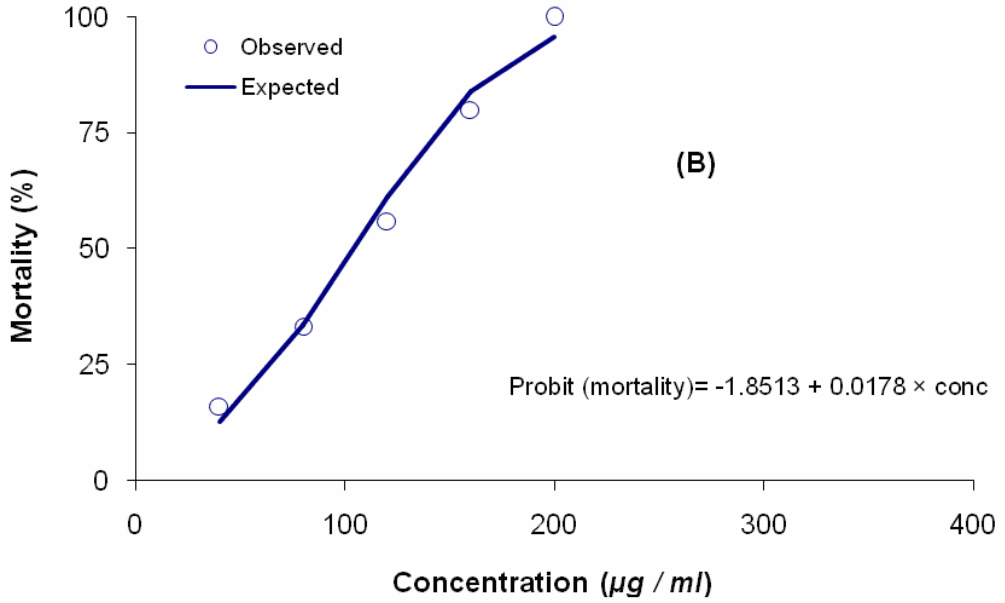
Table 1- Observed and expected mortality of *Anopheles stephensi* larvae exposed to *Bauhinia racemosa* with Petroleum ether and Ethyl acetate extracts. Expected mortality is based on probit regression analysis.

Conc. (µg/ml)	No. of Larvae		Mortality (%)		Expected Mortality			Probit(mortality) = a + b x conc	χ^2 D.F.P value	LC_{50} (95 % CI)	LC_{90} (95%CI)
	Exposed	Dead	Crude	Corrected	Probit	Dead	%				
Petroleum ether extract											
50	75	11	14.7	14.7	-1.21	8.4	11.2	-1.8238+ 0.0122 x conc	χ^2 = 9.7 D.F.=4 P=0.046	149.3 (121.2-175.3)	254.3 (220.5-316.0)
100	75	24	32.0	32.0	-0.60	20.5	27.3				
150	75	31	41.3	41.3	0.01	37.7	50.2				
200	75	49	65.3	65.3	0.62	54.8	73.1				
250	75	69	92.0	92.0	1.23	66.7	89.0				
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	-1.8513+ 0.0178 x conc	χ^2 = 5.9 D.F.=3 P =0.11	104.0 (95.2-112.5)	176.1 (163.3-193.1)
80	75	25	33.3	33.3	-0.43	25.1	33.5				
120	75	42	56.0	56.0	0.28	45.9	61.2				
160	75	60	80.0	80.0	1.00	63.0	84.1				
200	75	75	100.0	100.0	1.71	71.7	95.6				

D.F. = Degrees of freedom

Figure 1- Relation 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.





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