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BOUGAINVILLEA SPECTABILIS WILD., A POTENTIAL LARVICIDAL AGENT TO CONTROL THE FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* SAY.

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ABSTRACT Larvicidal property of *Bougainvillea spectabilis* Wild. leaf extracts were studied against the filarial vector *Culex quinquefasciatus* Say, Methanol and Acetone extracts of the leaf possess larvicidal properties and estimated 24 hr LC_{50} were 358.8 and 56.9 ppm respectively. The less active methanol fraction **of *Bougainvillea spectabilis*** was subjected to column fractionation and the **n- Hexane: Ethyl acetate, 5:5** column fraction show an enhanced larvicidal property than that of crude methanol extract. The crude methanol extract produced a mortality of 96.67 % at 700 ppm whereas the **n- Hexane: Ethyl acetate, 5:5** column fraction of methanol extract produced 96.67 % mortality at 200 ppm.

KEYWORDS : ***Bougainvillea spectabilis***, *Culex quinquefasciatus*, LC50, Larvicidal property

INTRODUCTION

Insecticides which are obtained from plants and plant derived products attract more attention for Integrated Vector Management programs owing to many reasons. Plants have evolved for over millions of years that have equipped them with plenty of chemical defense in the form of secondary metabolites to defend themselves from insect attack [1]. Till date, more than 2000 plant species have known to be introduced in biological pest control programmes and among these, the secondary metabolites/ products of some 344 species have been reported with significant activity against mosquitoes [2]. The plant families Asteraceae, Labiatae, Solanaceae, Oocystaceae, Cladophoraceae, Rutaceae and Meliaceae are well known for their larvicidal, adulticidal and repellent activities against different species of mosquitoes[3]. The plant based compounds like phenolics, terpenoids and alkaloids have proved their activity as repellents, antifeedents, oviposition deterrents, growth inhibitors, moulting hormones, anti-moulting hormones, juvenile hormone mimics, as well as attractants, which are responsible for interfering with the biological activity of the insect pests/vectors [4]. Mosquitoes are the most important insect vectors which spread major life threatening diseases such as dengue, chikungunya, malaria, fialariasis, Japanese encephalitis etc. Continuous application of synthetic insecticides for the control of these insect vectors has resulted in various issues in different magnitudes such as development of resistance in target species, deposition of residues in the environment and has led to bio magnification and moreover, contributed to environmental pollution which ultimately affects public health. Hence the scientific world now focuses on a less toxic, environmentally safe and cost effective alternatives in plant based formulations for the control of these insect vectors. The present study envisages to carry out search for the mosquitocidal activity of the leaf extract of *Bougainvillea spectabilis* Wild. on the filarial vector *Culex quinquefasciatus* Say.

Materials and Methods

Collection and Screening of Plants

The leaves of the plant, *Bougainvillea spectabilis* Wild were collected and utilised for the present study. *Bougainvillea spectabilis* Willd. is referred to as "Paper Flower" because its bracts are thin and papery with various colour range from purple or magenta and white to orange. It is native to Brazil, Argentina, Bolivia, Peru and Chubut Province. Literatures provided the information regarding the medicinal properties of *B. spectabilis* such as antidiabetic, antiviral, antibacterial, hepatoprotective and it possess insecticidal properties also. Traditionally it is used to cure the diseases like Diarrhea, sore throat, cough, stomach disorder, and hepatitis[5]. The plant is native to South America, Peru, Brazil and Argentina. It is a woody vine or shrub, reaching 15 to 40 feet. It has a thorny and pubescent stems. Leaves are heart-shaped and flowers are generally small, white and inconspicuous, highlighted by several brightly coloured modified leaves with varying in colour, ranging from white, red, mauve, purple-red, or orange. Fruit is small, inconspicuous, dry and elongated achene.

Test Organism- *Culex quinquefasciatus* Say

Culex quinquefasciatus taxonomically comes under the member of *Culex pipiens* species complex. It is commonly known as 'Southern House Mosquito. It is considered as the primary vector *Wuchereria bancrofti,* a nematode that causes lymphatic filariasis. It also transmits a malarial parasite of bird *Plasmodium relictum* and is the principle vector in Hawaii. Female *Cx. quinquefasciatus* is the definitive host for malarial parasite as it harbors the sexual cycle.

Maintenance of Laboratory Culture of *Cx. quinquefasciatus* Say

Developmental stages of *Culex quinquefasciatus* were collected from open drains from Calicut, Kerala State, India, brought to the laboratory and maintained at 27±2°c and 75-85% relative humidity, fewer than 14:10h light and dark cycles. The larvae were kept in plastic or enamel trays containing tap water and fed by a diet of fine powder of dog biscuits and Brewer's yeast in the ratio of 3: 1 respectively. The pupae were kept inside the standard emergence cages. After emergence the mosquitoes were identified and species confirmed before rearing. The adults were fed by 10% sucrose solution and additional blood meal was provided (immobilized quail) to adult females to facilitate the development of egg. A bowl containing water kept in the emergence cages to facilitate oviposition. The eggs laid were removed from the cage and after hatching, the larvae were reared in the laboratory at room temperature.

Larvicidal Bioassay (Lc_{50})

Bioassay for the estimation of larvicidal activity was carried out using WHO protocol,[6] with slight modification adopted for the present study. Larvicidal bioassay of the leaf extracts in acetone and methanol were tested against freshly hatched first instar larvae of *Cx. quinquefasciatus* using desired concentrations. From the 1% stock solution, different concentrations were prepared and applied on the glass beakers containing 100ml of saline solution. Ten healthy First instars larvae were released in each glass beaker and mortality was observed after 24hrs of exposure at each concentration. Triplicates for each dose and both solvent and water controls were maintained. The control mortality was corrected using Abbott's formula [7].

$$\text{Percent mortality} = \frac{\text{\% mortality in treated - \% mortality in control}}{\text{100 - \% mortality in control}} \times 100$$

Lethal Concentration to kill 50% of the treated population (LC_{50}) of the extract was then calculated using the probit analysis made by Finney[8].

Fractionation of the crude extract using Column Chromatography

Column chromatography was conducted according to the protocol Harwood and *Moody*[9] with slight modifications. Column of size 50cm x 2.5cm were used for the study. The bottom of the column was plugged with little cotton to prevent the adsorbent pass out, and prepared slurry of silica gel 60- 120 mesh size with n-hexane into a homogenous suspension (Stationary phase) and poured gently into the column, set aside for 10 minutes and used. Opened the stop cock and allow some solvent to drain out until the layer of solvent should cover the adsorbent. The dry powder of crude methanolic leaf extracts of *B. spectabilis* was loaded on to the top of silica slurry. The column was eluted with solvents (Non- polar X Polar) like n-hexane: Ethyl acetate 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 with increasing polarity respectively. The coloured bands/ different fractions travel down the column as the compound was eluted. The eluted fractions were collected by changing the glass beakers according to the coloured

bands. The different column fractions were evaporated to dryness and prepared 1% stock solution and stored in the refrigerator.

Result: Percentage yield of plant extracts

Percentage yield of the extracts of leaves were prepared by Soxhlet extraction methods using acetone and methanol and are summarized in the Table 1. *B. spectabilis* leaf extracts in acetone and methanol yielded 9% and 10.85% respectively.

Table 1 – Data on Percentage yield obtained for the different extracts of the *B. spectabilis*

Name of plants	Plant parts used	Solvents used	% Yield
Bougainvillea spectabilis	Leaves	a. Acetone	9.00
		b. Methanol	10.85

Larvicidal Bioassays

In the preliminary screening, crude methanolic and acetonic extracts of B. spectabilis leaves were tested for their potential larvicidal toxicity on first instar larvae of Cx. quinquefasciatus. Crude methanolic extract of B. spectabilis exerted 100% larval mortality at 900ppm, and 96.67% mortality at 150ppm on acetonic extract (table 2).

Table 2 - Percent mortality of Crude Methanolic and Acetonic extracts of *Bougainvillea spectabilis* against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Extracts	Concentration (ppm)	Mortality (%)	Corrected %	P-Value
1	Methanol	100	23.33 + 3.33	23.33	0.0069*
2		300	40.00 + 5.77	40.00	
3		500	53.33 + 6.67	53.33	
4		700	96.67 + 3.33	96.67	
5		900	100.00 + 0.00	100.00	
1	Acetone	5.0	16.67 + 3.33	16.67	0.0065*
2		10	30.00 + 0.00	30.00	
3		50	46.67 + 3.33	46.67	
4		100	66.67 + 3.33	66.67	
5		150	96.67 + 3.33	96.67	

The values are expressed as mean ± SD for 10 animals (n=10) per group

*** Statistically Significant at P < 0.05 with Control experiments.**

On the basis of preliminary screening on larvicidal toxicity, the different extracts were subjected to detailed investigations and 24 hr LC_{50} and LC_{90} were estimated and the data are provided in table 3. LC_{50} for *B. spectabilis* leaf extracts when treated with I instar larvae of *Cx. quinquefasciatus* after 24 hr exposure is estimated as 358.8 and 56.9 ppm for methanol and acetone extracts respectively. Corresponding LC_{90} values are 698.3 and 139.2 ppm for *B. spectabilis* leaf extract

Table 3: 24 hr LC_{50} and LC_{90} (ppm) and related statistics of the selected plant extracts tested against first instar larvae of *Culex quinquefasciatus*

Plant/ parts	Extracts	24 hrs LC50 (LC90) ppm	Lower Fiducial Limit (LFL)	Upper Fiducial Limit (UFL)	X2	Regression	Significance
B. spectabilis leaf	Methanol	358.8 (698.3)	66.46 (514.5)	559.4 (1540.9)	24.15	y= 0.105x + 10.11 R2= 0.934	0.000*
	Acetone	56.9 (139.2)	31.14 (105.39)	84.26 (223.98)	9.663	y= 0.504x+ 19.53 R2= 0.981	0.022*

Effect of different Column fractions of selected plant extracts on I instar larvae of *Culex quinquefasciatus.*

Silica gel Column Fractions of crude methanol extract of *B. spectabilis* were tested for larvicidal activity and adult emergence with I instar larvae of *Cx. quinquefasciatus.* Apart from the nine gradients of n-hexane: Ethyl acetate (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1), the column fraction n- Hexane: Ethyl acetate (5:5) is the only fraction showed larval mortality of 96.67% at a concentration of 200 ppm. At 25, 50, 100,150 and 200 ppm of the n-Hexane: Ethyl acetate (5:5) column fraction of *B. spectabilis* produced 26.7, 36.7, 53.3, 76.7 and 96.7 % respectively after 24 hr of exposure (table 4).

Table 4 : Percent mortality of the column fraction n- Hexane: Ethyl acetate, 5:5 Methanolic extract of *Bougainvillea spectabilis* against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Concentration (ppm)	Mortality (Mean+ SE)	Corrected %	P- Value
1	25	26.67 + 3.33	26.67	0.0020*
2	50	36.67 + 3.33	36.67	
3	100	53.33 + 3.33	53.33	
4	150	76.67 + 3.33	76.67	
5	200	96.67 + 3.33	96.67	

The values are expressed as mean + SE for 10 animals (n=10) per group

*** Statistically Significant at P < 0.05 with Control experiment.**

Discussion

Recently the mosquito control programmes using synthetic insecticides face set backs due to the development of resistance in mosquitoes. The biopesticides are often considered to be important components of IVM strategies, and have received much practical attention as an alternative to synthetic insecticides(10). Phytochemicals are considered to be suitable alternatives to synthetic insecticides as they are relatively inexpensive, safe and are readily available in all parts of the world(11). More than two thousand plant species have been reported to have insecticidal properties and plant derived products have drawn increased attention as potential insect control agents and as source of new insecticides for the pesticide industry in the last few decades(12).

The larvicidal efficacies of the leaf of Bougainvillea spectabilis in the crude methanol and acetone and also column fractions observed in the present study were on par with the available literature and can be an alternative for using in the vector control programmes. It has been found to possess promising larvicidal activity with LC50 value 358.8 ppm and 56.9 ppm for the methanolic and acetone extracts. The plant extracts caused larval mortality in a dose- dependent manner (Fig 1 and 2) and the percentage of larval mortality was found to be significantly different (P < 0.05, t- test) from that of control and untreated groups.

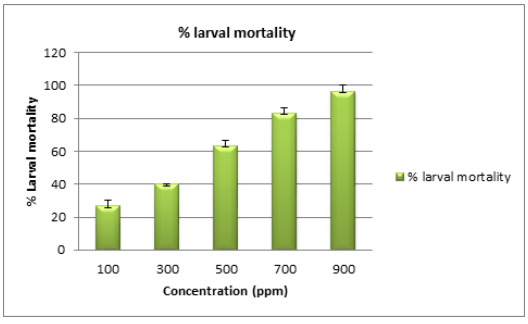

Figure 1 :Percentage mortality of Crude methanolic extract of B. spectabilis on I instar larvae of *Cx. quinquefasciatus*

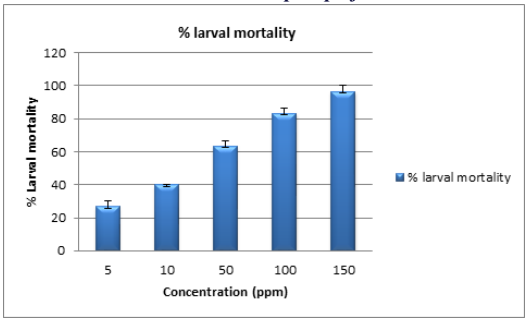

Figure 2: Percentage mortality of Crude acetonic extract of *B. spectabilis* on I instar larvae of *Cx. quinquefasciatus.*

The findings of the present study clearly demonstrates that the crude acetone extract is a promising agent to control the larvae of mosquitoes and also shows that fractionation of the methanol extract of *Bougainvillea spectabilis* leaf enhances the larvicidal efficacy and thus

offer an opportunity for developing better alternatives to rather expensive and environmentally hazardous synthetic insecticides.

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