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Research Article

STUDIES ON LARVICIDAL ACTIVITY OF *LEUCAS ASPERA, VITEX NEGUNDO* AND EUCALYPTUS AGAINST *CULEX QUINQUEFASCIATUS* COLLECTED FROM COOVUM RIVER OF CHENNAI, INDIA

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ABSTRACT

Crude leaf extracts of *Leucas aspera,Vitex negundo* and *Eucalyptus* were tested for their larvicidal activity against *Culex quinquefasciatus.* There are four different solvents were used (Petroleum ether, Water, Acetone and Ethyl acetate) for the preparation of crude extracts from the plant leaves. The larval mortality of second and third instar larvae *C. quinquefasciatus* after 24 hour to 72 hour of treatment were observed separately in control, 125, 250, 500, and 1000 ppm concentrations of the leaf extract. The four different solvent extract of *Vitex negundo* showed good larvicidal activity. The highest mortality percentage of mosquito larvae was observed in 1% concentration of crude extracts. No mortality was observed in control. ANOVA for mortality percentage of mosquito larvae in different concentration and different solvent shows significant different at 5% level.

Keywords:

INTRODUCTION

The mosquito is the principal vector of many of the vector borne diseases affecting human beings and other animals. Several mosquito species belonging to genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, japanese encephalitis, dengue fever, dengue haemorrhagic fever and yellow fever. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents [3]. One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centres of the vectors. This study is concerned with the using of such effective plant source against the larval of Mosquito.

MATERIALS AND METHODS

Collectio of plant leaves

The list of plant leaves included in this study was

1. *Vitex negundo (Nochi)*
2. *Eucalyptus (Thailam)*
3. *Leucas aspera (Thumbai*

Solvents Used

The solvents used for the preparation of crude extract from the plants are,

1. Petroleum ether
2. Ethyl acetate
3. Ethanol
4. Water.

Preparation of Crude Extract

The locally available plant leaves were collected from Villangadu Village near Madurandhagam, TamilNadu, INDIA. The leaves of the plant material were shade dried for about 15 days (28±2ºC). The completely dried leaves were ground and sieved to get fine powder from which the extracts were prepared [4]. The dried leaves were made to fine powder by crushing it in a mixer grinder. Each plant leaf powder was taken separately.

To each leaf powder (500gm), 1.5 litre of petroleum ether, ethyl acetate, ethanol and water solvent was added solely. It was left for around 24 hours with periodic shaking and then filtered. This procedure was repeated with fresh solvent till clear. Each solvent were filtered using whattman filter paper. The finally obtained solvents were collected in a separate container. Finally different solvent from each plant leaves were obtained. Totally 12 different solutions were obtained. Each solvent solution was kept for evaporation in Soxhlet's apparatus extraction mantle at a temperature of around 60-70°C. At regular intervals it was cooled and heated in the extraction mantle. Finally crude extracts of each plant solution was obtained.

Collection of Mosquito Larvaes

The mosquito larvae (*Culex quinquefasciatus*) were collected from Coovum River, Chennai. The larvae were collected in a container and transferred to the laboratory immediately. From these larvae, unwanted large size larvae and pupae were collected and discarded [5]. From the remaining medium sized larvae second and third instar larvae alone were collected for the larvicidal bioassay. Feed is supplied to the mosquito larvae for its growth.

Preparation 0f Various Concentrations of Crude Extracts

The crude extracts from each plant leaf was made into different concentrations of 125ppm, 250ppm, 500ppm, and 1000ppm respectively. For the bioassay study 20 larvaes were taken in a plastic container with 100ml of water. Totally 5 containers containing 20 larvaes were taken for each concentration of the crude extract and control.

Larvicidal Bioassay

Initially 12.5 mg crude extract of petroleum ether solvent of each solvent extract was taken and dissolved in 1ml of acetone in an eppendorf. Then the dissolved crude extract was mixed in container containing 20 larvae in 100ml of water. Every 24 hours the mortality rate was noted and reading was taken. The dead larvae were taken out at every 24 hours since it may leads to contamination of the water. The readings were taken for 3 days (72hours).

Then 0.25% (250ppm) concentration from each plant crude extract was introduced into containers containing larvae. Similarly for this reading was taken for every 24 hours for 3 days. Then 0.5% concentration (500ppm) of crude extract was introduced and reading was noted for every 24hrs for three days. Finally 1% concentration (1000ppm) of crude extract from each plant was introduced and reading was noted for every 24 hours for 3 days.

Statistical Analysis

The data recorded from the assay tests were analyzed by probit analysis based on the statistical package for social sciences (SPSS) version 15. Analysis of variance (ANOVA) which was carried using the statistical package for social sciences (SPSS) for windows version 15.

RESULTS AND DISCUSSIONS

Preparation of Crude Extract

The crude extract of *Vitex negundo (Nochi), Eucalyptus (Thailam) and Leucas aspera (Thumbai)* was extracted from soxhlet's apparatus [6]. The different concentration of 125 ppm, 250ppm, 500ppm and 1000ppm were prepared with three plant crude extract. Thus 12 solutions were prepared for larvicidal activity.

Preparation of Bioassay Container

60 container containing 20 larvae each were taken of respective concentration, marking a control for each extract. The mortality rate of larvae was noted for every 24 hours for three days. The mortality of Larvae were tabulated and statistically analyzed.

Mortality percentage of mosquito larvae treated in *Eucalyptus* sp crude extract in different solvent

Table 1 showed the mortality percentage of mosquito larvae treated in different solvent. 1% crude extract of ethyl acetate and ethanol showed 100% mortality followed by water (97%), while 0.125% crude extract of ethyl acetate, ethanol and water 25% of mortality were observed[7]. ANOVA for mortality percentage of mosquito larvae in different concentration and different solvent shows significant different at 5% level (Table II) (Refer Graph 1).

Table 1: Mortality percentage of mosquito larvae treated in *Eucalyptus* sp crude extract in different solvent

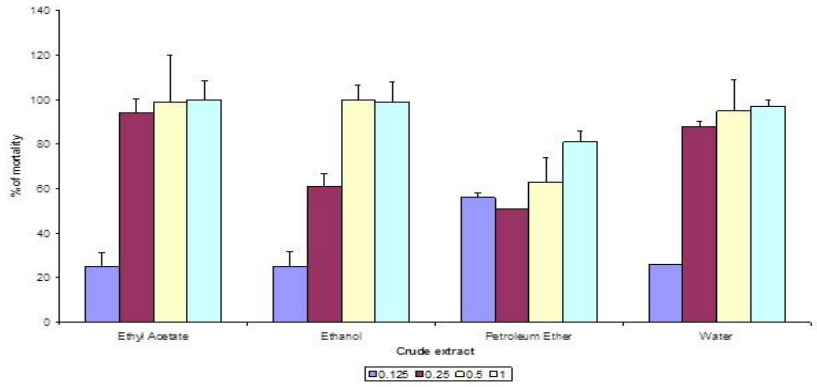
Concentration (%)	Ethyl acetate	Ethanol	Petroleum ether	Water
0.125	25.00±6.12 ^{a}	25.00±6.12 ^{a}	56.00±21.03 ^{ab}	26.00±8.22 ^{a}
0.250	94.00±6.51 ^{b}	61.00±5.47 ^{b}	51.00±6.51 ^{a}	88.00±9.08 ^{b}
0.500	99.00±2.23 ^{b}	100.00±.00 ^{c}	63.00±10.95 ^{ab}	95.00±5.00 ^{b}
1	100.00±.00 ^{b}	100.00±2.23 ^{c}	81.00±13.87 ^{b}	97.00±2.74 ^{b}
Control	0 ^{c}	0 ^{d}	0 ^{c}	0 ^{c}
LC_{50}	0.15	0.19	0.13	0.16

Different superscripts in the same column shows significantly different at 5% level

Table 2: Anova for mortality percentage of mosquito larvae treated in Eucalyptus in different solvent

Parameters		Sum of Squares	Mean Square	F	Sig.
Ethyl acetate	**Between Groups**	19905.000	6635.000	312.235	.000*
	Within Groups	340.000	21.250		
Ethanol	**Between Groups**	19203.750	6401.250	353.172	.000*
	Within Groups	290.000	18.125		
Petroleum ether	**Between Groups**	2583.750	861.250	4.320	.021*
	Within Groups	3190.000	199.375		
Water	**Between Groups**	17225.00	5741.667	125.845	.000*
	Within Groups	730.00	45.625		

*** - significantly different at P < 0.05**



Graph 1: Mortality percentage of mosquito larvae treated in Eucalyptus crude extract in different solvent

Anova followed by Tukey's test performed that 0.125% concentration of ethyl acetate and water solvent showed significantly different when compared to other concentration. In petroleum ether solvent, 0.125 and 0.500 % concentration were no significant different to 0.250 and 1% concentration at P > 0.05 level.

Mortality percentage of mosquito larvae treated in *Vitex negundo* crude extract in different solvent

Mortality percentage of mosquito larvae treated in *Vitex negundo* crude extract in Ethyl acetate, Ethanol and Petroleum ether showed in Table III.1% concentration of crude extract prepared in Ethyl acetate, Ethanol and Petroleum ether showed 100% mortality followed by aqueous extract with 97% while the aqueous extracts of Vitex *negundo* had the least mortality with 40%. Also Aina *et al.* (2009)[2] reported that aqueous extract of *Vitex* showed 71.66% mortality, while compared to previous reports low percentage of Mortality(40%) was observed in this present study.

Table 3: Mortality percentage of mosquito larvae treated in *Vitex negundo* crude extract in different solvent

Concentration (%)	Ethyl acetate	Ethanol	Petroleum ether	Water
0.125	56.00±21.03 ^{a}	45.00±7.90 ^{a}	56.00±21.03 ^{a}	40.00±12.25 ^{a}
0.250	98.00±2.73 ^{b}	98.00±2.73 ^{b}	61.00±4.18 ^{a}	80.00±12.75 ^{b}
0.500	100.00±.00 ^{b}	100.00±.00 ^{b}	86.00±6.51 ^{b}	94.00±4.18 ^{bc}
1	100.00±.00 ^{b}	100.00±.00 ^{b}	100.00±.00 ^{b}	97.00±2.74 ^{c}
Control	0 ^{c}	0 ^{c}	0 ^{c}	0 ^{d}
LC_{50}	0.11	0.13	0.13	0.14

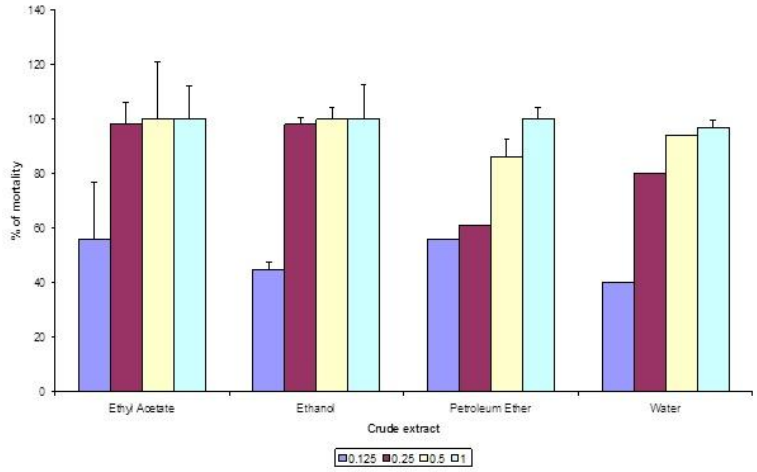
Different superscripts in the same column shows significantly different at 5% level

The mortality range of petroleum ether showed least activity when compared to other solvents at 0.25% and 0.5%.The LC 50 values shown in Table III indicated that the Ethyl acetate extract of *Vitex negundo* was the most active (0.11) followed in descending order of other solvents like 0.13 for ethanol and petroleum ether and 0.14 for aqueous extraction. ANOVA for mortality percentage of mosquito larvae in different concentration and different solvent shows significant different at 5% level (Refer Table IV and Graph 2).

Table 4. Anova for mortality percentage of mosquito larvae treated in *Vitex negundo* in different solvent

Parameters		Sum of Squares	Mean Square	F	Sig.
Ethyl acetate	**Between Groups**	7055.000	2351.667	20.904	.000*
	Within Groups	1800.000	112.500		
Ethanol	**Between Groups**	11083.750	3694.583	211.119	.000*
	Within Groups	280.000	17.500		
Petroleum ether	**Between Groups**	6503.750	2167.917	17.257	.000*
	Within Groups	2010.000	125.625		
Water	**Between Groups**	10323.750	3441.250	40.785	.000*
	Within Groups	1350.00	84.375		

*** - significantly different at P < 0.05**



Graph 2: Mortality percentage of mosquito larvae treated in vit crude extract in different solvent

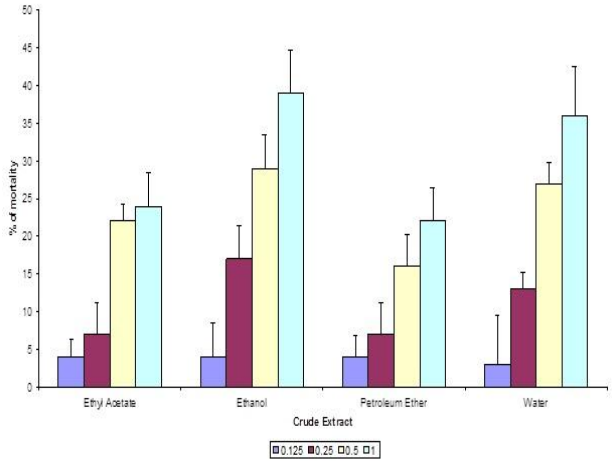
Mortality percentage of mosquito larvae treated in *Leucas aspera* crude extract in different solvent

Larvicidal effect and Mortality percentage of mosquito larvae treated in *Leucas aspera* crude extract in Ethyl acetate, Ethanol and Petroleum ether showed in Table V. The very low percentage of mortality was observed in all four solvents. The aqueous extracts of *Leucas aspera* (0.125%) had the least mortality when compare to other solvents and the same extract with 1% give the highest percentage of mortality and 1% ethanolic extract of *Leucas aspera* showed highest mortality with 39%. ANOVA for mortality percentage of mosquito larvae in different concentration and different solvent shows significant different at 5% level (also Refer Table VI and Graph 3).

Table 5: Mortality percentage of mosquito larvae treated in *Leucas aspera* crude extract in different solvent

Concentration (%)	Ethyl acetate	Ethanol	Petroleum ether	Water
0.125	4.00±2.23 [a]	4.00±4.18 [a]	4.00±2.23 [a]	3.00±4.47 [a]
0.250	7.00±4.47 [a]	17.00±4.47 [b]	7.00±4.47 [a]	13.00±5.70 [b]
0.500	22.00±2.73 [b]	29.00±4.18 [c]	16.00±4.18 [b]	27.00±4.47 [c]
1	24.00±6.51 [b]	39.00±2.23 [d]	22.00±2.73 [b]	36.00±6.52 [d]
Control	0 [c]	0 [a]	0 [a]	0 [a]
LC_{50}	3.12	1.37	4.55	1.50

Different superscripts in the same column shows significantly different at 5% level



Graph 3: Mortality percentage of mosquito larvae treated in leu crude extract on different solvent

In the present investigation, the toxicity of *Lucas aspera* was tested against two larval stages of the mosquito species *Culex quinquefasciatus*. The data were recorded and statistical data regarding LC $_{50}$. The LC_{50} value of *Leucas aspera* against second and third instars larvae of *Culex quinquefasciatus* were 3.12, 1.37, 4.55 and 1.50 ppm. No mortality was observed in control. The present findings compared with Maheswaran *et al* (2008) [1]. They observed the LC 50 values of ethanol extract of Leucas *aspera* were 63.8, 122.50, 510.0 and 326.68 ppm but the mortality range in the present study was very low.

Table 6: Anova for mortality percentage of mosquito larvae treated in *Leucas aspera* in different solvent

Parameters		Sum of Squares	Mean Square	F	Sig.
Ethyl acetate	**Between Groups**	1563.750	521.250	27.800	.000*
	Within Groups	300.000	18.750		
Ethanol	**Between Groups**	3433.750	1144.583	76.306	.000*
	Within Groups	240.000	15.000		
Petroleum ether	**Between Groups**	1023.750	341.250	27.300	.000*
	Within Groups	200.000	12.500		
Water	**Between Groups**	3213.750	1071.250	37.261	.000*
	Within Groups	460.00	28.750		

*** - significantly different at P < 0.0**

CONCLUSION

The findings of the present investigation revealed that Eucalyptus and *Vitex negundo* has good larvicidal activity against *Culex quinquefasciatus* and *Leucas aspera* showed poor mortality against the mosquito larvae. These crude extracts can be effectively used in the control of mosquitoes by replacing the chemical pesticides which cause environmental pollutions and other burdens.

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