



Efficacy of Phytochemicals as Sustainable Sources of Larvicidal Formulations for the Control of *Culex sitiens*

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Abstract

Mosquitoes are significant group of arthropods in terms of public health, as they spread serious human diseases, causing millions of deaths every year and the development of resistance to chemical insecticides ensuing in rebounding vectorial competence. Controlling mosquitoes at the larval stage is easy as target specificity of the larvicides used can be ensured. Phytochemicals derived from the vast diversity of plant species are important sources of environmentally safe and biodegradable chemicals, which can aid in mosquito control. The present study has been outlined to assess the feasibility of using phytochemicals, as a larvicidal agent against third instar larvae of *Culex sitiens*. Twelve species of plants belonging to Zingiberaceae (2), Asclepiadaceae, Caricaceae, Myrtaceae (2), Rutaceae (2), Calophyllaceae, Annonaceae, Euphorbiaceae, Bignoniaceae families were screened for this purpose. Aqueous leaf extracts of selected plants were prepared (0.5, 1.0, 2.0, 4.0, and 8.0 ml) and tested against mosquito larvae reared from eggs under laboratory conditions, for a period of 6 hours. Mortality percentages and LC₅₀ were calculated as per WHO protocols and standards. Of twelve plants studied, all the plant extracts infatuated significant larvicidal potential with LC₅₀ values ranging from 1.31 to 4.79 mg/ml against the third instar larvae of *Culex sitiens*. Extracts from plants like *Calotropis gigantea*, *Pimenta dioica*, *Curcuma longa*, *Polialthia longifolia*, *Saritaea magnifica*, *Ricinus communis*, *Alpinia galanga*, *Carica papaya*, *Murraya koenigii* and *Eucalyptus globulus* have the highest potential to be used as an effective larvicidal agent, signifying an ecofriendly approach for the control of mosquito vectors. Further investigations would be desired to separate and make out the constituents responsible for larvicidal properties.

Keywords: Aqueous extracts, *Culex sitiens*, mortality percentages, LC₅₀.

Introduction

Mosquitoes are the most imperious group of insects, adversely influencing the health status of human beings. They have a worldwide distribution and are involved in the transmission of many dreadful diseases affecting millions of people every year^{1,2}. They do not only transmit parasites and pathogens but they also source of allergic reaction that includes local skin and systemic sensitivity^{3,4}. Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are acting as vectors for many pathogenic organisms causing diseases like Malaria, Filariasis, and Japanese Encephalitis, Dengue fever, yellow fever etc. These diseases spread globally, causing high levels of human mortality and thereby acting as factors impeding the economic development of most of the developing countries across the world⁵⁻⁸. Two million people primarily in tropical countries are being at the risk of mosquito-borne diseases^{9,10}. To prevent mosquito-borne diseases and reduce fatality, it is necessary to stumble on a method to control the mosquito population. For this substantiation many chemical insecticides were developed and used against the vector and with significant success, but it contains some distressing drawbacks like selectiveness and non-biodegradability which leads to toxic hazards to ecosystem, environment and human health¹¹. So an alternative strategy needs to be overcome this problem. The

plant based herbal insecticides are found to more efficient, safe and best substitute for chemical insecticides. Natural products of plant origin are safe to use than synthetic insecticides. Therefore biological and ecofriendly natural resources are broad search area for the control of mosquito vectors¹². In this light, the present endeavor is designed to determine the efficiency of aqueous leaf extracts of plants belonging to varied taxonomic groups on *Culex sitiens* competent vector of Ross River Virus and Japanese encephalitis.

Material and Methods

Plant collection and processing: The selection of plants were carried out based on their local availability and reported medicinal properties. The materials were collected from the plants in field located in Botanical garden, University of Calicut. The materials were taken from healthy plants free from dust, dirt and other impurities and were fetched to the laboratory for subsequent procedure.

Preparation of extracts: The washed plant materials were chopped properly and kept in clean trays. For the preparation of extracts, approximately twenty grams (20gms) of plant material (Leaves) was taken and ground in a homogenizer using distilled water. The extract was filtered and the filtrate was made upto

1000 ml with distilled water and retained as stock solution for further experimentation. Serial dilutions of the stock solutions were prepared for assessing treatment efficiencies.

Collection and culture of mosquito species: Vector species *Culex sitiens* was selected for the present study. *Culex sitiens* commonly breeds in all impoundments, including pools, puddles, wells, ditches; as well as cement tanks, jars and cans. It is a competent vector of Ross River Virus and Japanese encephalitis¹³. This species is also involved in the transmission of filariasis (*Brugia malayi*), although only in a secondary role.

Mosquito larvae, collected from controlled breeding sites maintained with coconut shells, jars and cans kept at varying distances round households were used in the present study. Collected larvae were pooled in the laboratory and subjected to species level identification using standard manual¹⁴.

In the laboratory, the larvae were transferred to enamel tray until adult emergence. After emergence, the mosquitoes were identified and species confirmed before rearing. Cyclic generations (two generations) of *Culex sitiens* were maintained and kept separately in mosquito cages in an insectary. For general rearing; mosquitoes were maintained at 26°C, 84% relative humidity, under a 16hr light and 8hr dark cycle with 1hr crepuscular period at the beginning and end of each light cycle based on the procedure of Kamaraj et al¹⁵ with slight modification. The adult mosquitoes were fed on five percent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with artificial blood feeding membrane¹⁶. Ovitrap (Petridishes with tap water lined with filter paper) was placed inside the cages for egg laying¹⁷. The eggs laid were then transferred to enamel trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1)¹⁸. The larvae on becoming pupae were collected, transferred to plastic

bowls and kept inside mosquito cages for adult emergence¹⁹

Larvicidal Bioassay: Bioassay for the larvicidal activity was carried out using WHO²⁰ procedure with minor modifications. Twenty numbers of early third instar larvae were introduced into treatment trays containing 250 ml of their natural growth media. To the treatment set, varying concentrations of the plant extracts (ie.0.5, 1.0, 2.0, 4.0 and 8.0 ml) were added from the stock solution; maintaining a relative concentration of the plant extracts as 10,20,40,80 and 160 mg/ml respectively. A control was also maintained for the treatment set. Mortality counts of larvae were monitored at regular intervals i.e.6, 12,24,48,72 and 96 hours after treatment and the control mortality as corrected using Abbott's²¹ formula when the control mortality ranged between 5-20 percent^{10,22-24}.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical analysis: The mortality percentage was corrected using Abbott's²¹ formula during the scrutiny of the larvicidal potentiality of the plant extracts. The experimental data was executed with MS Excel 2007 to find the Standard deviation and LC₅₀ using probit analysis²⁵.

Results and Discussion

The toxicity of aqueous leaf extracts of twelve species of plants belonging to various families were experienced against third instar larvae of *Culex sitiens*. Details of plants used for the present study and mortality percentage of mosquito larvae noticed are depicted in table-1. The efficacy of various leaf extracts on *Culex sitiens* exposed to 96 hours, for confirming lethality as per WHO¹⁹ standards are given Table 2. The data pertaining to LC₅₀ are depicted in figure 1 and 2.

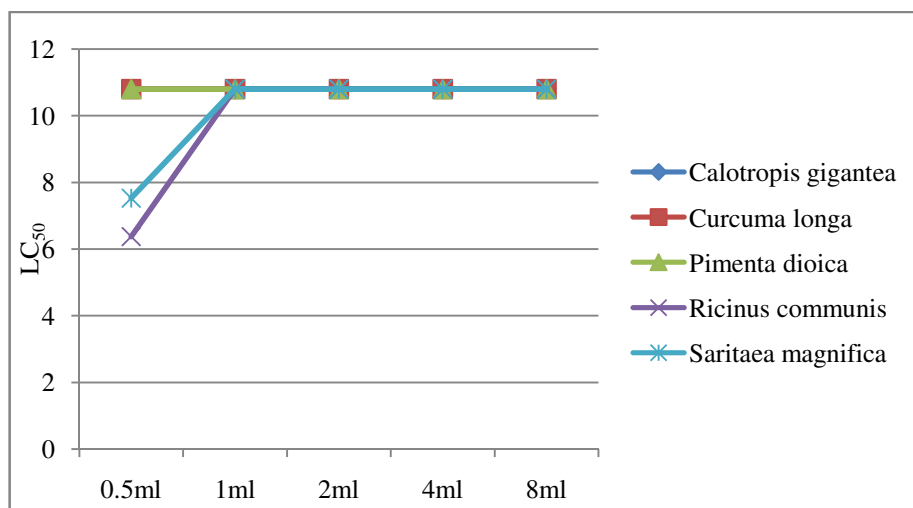


Figure-1
 LC₅₀ value of promising plant extract against *Culex sitiens*

Table-1
List of plant species used for the preparation of aqueous leaf extracts and their impact on *Culex sitiens* larvae

Name of plant	Family	Condition at which 100% larval mortality noticed
<i>Alpinia galanga</i>	Zingiberaceae	12hours in 80mg/ml
<i>Calotropis gigantea</i>	Apocynaceae	12hours in 10mg/ml
<i>Carica papaya</i>	Caricaceae	12 hours in 80mg/ml
<i>Curcuma longa</i>	Zingiberaceae	48 hours in 10 and 24 hours in 20mg/ml
<i>Eucalyptus globulus</i>	Myrtaceae	96 hours in 40 and 72 hours in 80mg/ml
<i>Glycosmis pentaphylla</i>	Rutaceae	50% mortality at 96hrs 8ml
<i>Mesua ferrea</i>	Calophyllaceae	72 hours in 80mg/ml
<i>Murraya koenigii</i>	Rutaceae	12 hours in 80mg/ml
<i>Pimenta dioica</i>	Myrtaceae	12 hours in 20mg/ml
<i>Polialthia longifolia</i>	Annonaceae	12hours 20mg/ml
<i>Ricinus communis</i> ,	Euphorbiaceae	24hours 20mg/ml
<i>Saritaea magnifica</i>	Bignoniaceae	12hours 160mg/ml and 24hours 20mg/ml

Table-2
The Efficacy of leaf extracts to the third instar larvae of *Culex sitiens*

Plants	Concentration of the extract (ml in 250 ml of growth medium)						Mean ± S.D
	Control	0.5	1	2	4	8	
<i>Alpinia galanga</i>	0	95	98.33	86.67	100	100	29.33±16.89
<i>Calotropis gigantea</i>	0	100	100	100	100	100	50±0
<i>Carica papaya</i>	0	26.67	55	100	100	100	27.83±18.25
<i>Curcuma longa</i>	0	100	100	100	100	100	43.33±8.17
<i>Eucalyptus globulus</i>	0	0	95	100	100	100	22.25±2.77
<i>Glycosmis pentaphylla</i>	0	0	0	0	0	56.67	13.64±11.82
<i>Mesua ferrea</i>	0	75	85	75	100	100	20.15±4.13
<i>Murraya koenigii</i>	0	0	0	46.67	100	100	18±15.14
<i>Pimenta dioica</i>	0	100	100	100	100	100	45.67±8.67
<i>Polialthia longifolia</i>	0	33.33	95	100	100	100	37.13±15.78
<i>Ricinus communis</i> ,	0	70	100	100	100	100	31.33±4
<i>Saritaea magnifica</i>	0	91.67	100	100	100	100	36.11±7.03

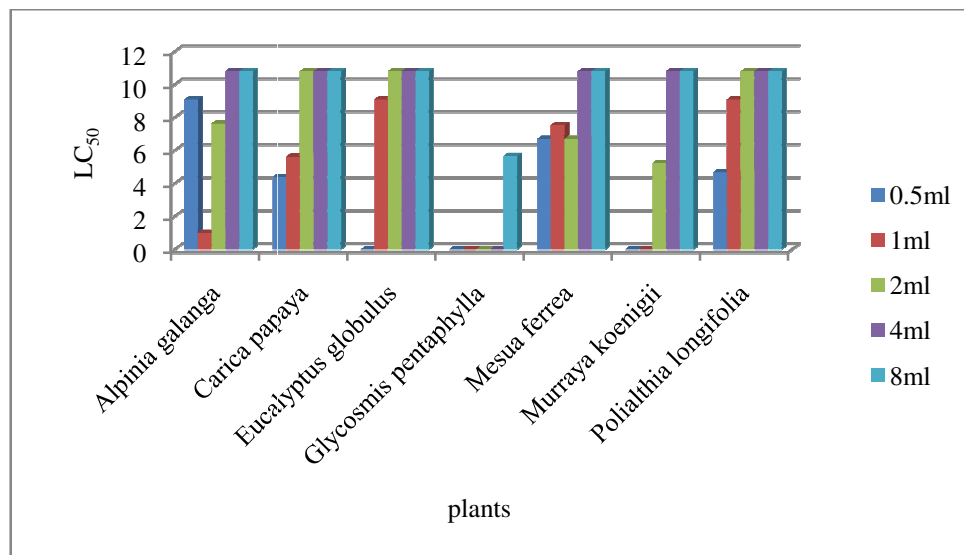


Figure-2
LC₅₀ value of plant extract against *Culex sitiens*

Discussion: Mosquitoes are vectors for the pathogens of various diseases like malaria, Filariasis, Japanese encephalitis, Dengue, yellow fever, Chikungunya etc that causes high levels of morbidity and mortality¹⁹. So, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or averting mosquitoes from biting human beings. The wide spread use of synthetic organic insecticides during the last five decades has upshoted in environmental hazards and development of resistance in the major vector species^{26,27}. This has demanded the search for herbal preparations that do not produce any adverse effects in the nontarget organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control^{6,28,29}. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive and are readily available throughout of the world²⁶. In fact, many researchers have reported the effectiveness of plant extracts and isolated components tested against different species of mosquito^{28,30,31}.

The larvicidal activity of different crude solvent (hexane, ethyl acetate, and methanol) extracts of plants like *Aristolochia indica*, *Cassia angustifolia*, *Diospyros melanoxylon*, *Dolichos biflorus*, *Gymnema sylvestre*, *Justicia procumbens*, *Mimosa pudica*, *Zingiber zerumbet* against adult and early fourth instar larvae of *Culex quinquefasciatus* and *Culex gelidus* were experimented by Kamaraj et al³². Vahitha et al^{33,34} worked out the larvicidal efficacy of methanol leaf extracts of *Pavonia zeylanica* and *Acacia ferruginea* were tested against the late third instar larvae of *Culex quinquefasciatus* with LC50 values of 2,214.7 and 5,362.6 ppm, respectively.

The toxicity of crude extracts from the *Ricinus communis* against fourth instar larvae *Anopheles arabiensis* and *Culex quinquefasciatus*. The LC₅₀ values of 2nd, 3rd and 4th instar

larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* are 403.65, 445.66 and 498.88ppm, 1091.44, 1364.58 and 1445.44ppm respectively were reported by Elimam et al³⁵. Singh et al^{36,37} reported that the LC₅₀ values of crude aqueous and hexane extracts of *Momordica charantia* against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* mosquito larvae are 0.50, 1.29, and 1.45% (aqueous extracts) and 66.05, 96.11, and 122.45ppm (hexane extracts), respectively. The monotonous observations carried out by various researchers in the field of mosquito control by plants.

In the present endeavor the effect of the leaf extracts of *Alpinia galanga*, *Calotropis gigantea*, *Carica Papaya*, *Curcuma longa*, *Eucalyptus globulus*, *Glycosmis pentaphylla*, *Mesua ferrea*, *Murraya koenigii*, *Pimenta dioica*, *Polialthia longifolia*, *Ricinus communis* and *Saritaea magnifica* were tested against the third instar larvae of *Culex sitiens* at varying concentrations. All plant extracts showed utmost larvicidal effects after 96 hours. However, the highest larval mortality was found with leaf extracts of *Calotropis gigantea* (50±0), *Curcuma longa* (43.33±8.17), *Pimenta dioica* (45.67±8.67), *Saritaea magnifica* (36.11±7.03) and *Ricinus communis* (31.33±4) against the third instar larvae of *Culex sitiens* (table-2).

The present investigation revealed that the aqueous extract of *Calotropis gigantea* (Leaf) was the most efficient, divulging a substantial LC₅₀ value of 10.8 mg/ml. But the leaf extract of *Glycosmis pentaphylla* was least efficient with an LC₅₀ value of 5.66 mg/ml (figure-1 and 2). It was also noticed that the extracts made from *Calotropis gigantea*, *Curcuma longa*, *Pimenta dioica*, *Ricinus communis* and *Saritaea magnifica* obsessed important larvicidal potential with LC₅₀ values ranging from 1.01 to 10.8 mg/ml (Figure 1). Other extracts (*Alpinia galanga*, *Carica papaya*, *Mesua ferrea*, *Murraya koenigii*, *Polialthia longifolia*, and *Eucalyptus globulus*) showed only moderate

toxicity against *Culex sitiens* larvae (figure-2).

Conclusion

A Study has been undertaken to assess the efficiency of phytochemicals derived from selected plants as potential and sustainable sources of larvicidal agents for the management of mosquito vectors. Twelve plant species belonging to Zingiberaceae (2), Asclepiadaceae, Caricaceae, Myrtaceae (2), Rutaceae (2), Calophyllaceae, Annonaceae, Euphorbiaceae, Bignoniaceae, families were screened for this purpose. Aqueous leaf extracts of selected plants were prepared (0.5, 1.0, 2.0, 4.0 and 8.0 ml) and tested against mosquito larvae reared from eggs under laboratory conditions, for a period of 96hours. Mortality percentages and LC₅₀ were calculated as per WHO protocols and standards. Extracts from plants like *Calotropis gigantea*, *Pimenta dioica*, *Curcuma longa*, *Polialthia longifolia*, *Saritaeta magnifica*, *Ricinus communis*, *Alpinia galanga*, *Carica papaya*, *Murraya koenigii*, and *Eucalyptus globulus* were noted to be lethal to the larvae. It can be concluded that these plants can be treated as source of phytochemicals for the control of mosquitoes. Isolation of the active components from these plants substantiates useful in the development of safer bio-insecticides in future.

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