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Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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ABSTRACT

Objective: To determine the larvicidal activity of twenty five plant extracts against *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** The larvicidal activity was determined against the third instar larvae of *Cx. quinquefasciatus* at 1 000 ppm concentration. Larval mortality was assessed after 24 and 48 h. **Results:** The hexane extracts of *Cleistanthus collinus* (*C. collinus*) and *Murraya koeingii* (*M. koeingii*) plants showed 100 percent mortality at 24 h bioassay followed by diethyl ether, dichloromethane and ethyl acetate extracts of *C. collinus*, *Leucas aspera* (*L. aspera*), *Hydrocotyle javanica* (*H. javanica*), *M. koeingii*, *Sphaeranthus indicus* (*S. indicus*) and *Zanthoxylum limonella* (*Z. limonella*) after 48 h exposure. **Conclusions:** The results indicate larvicidal activity against *Cx. quinquefasciatus* and further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also the active ingredients of the extract responsible for larvicidal activity should be identified.

1. Introduction

Man suffers extensively due to the nuisance of insect populations both in agriculture and health. In agriculture, insects affect directly on growing part of the crop and cause severe damage, resulting in revenue loss. In health point of view, insect vectors especially mosquitoes directly transmit diseases like filarial fever, malaria, dengue fever, chikungunya, *etc*[1]. Filariasis is an endemic, disabling, disfiguring disease and the filarial worm, *Wuchereria bancrofti* (*W. bancrofti*) responsible for human filariasis is carried by *Culex quinquefasciatus* (*Cx. quinquefasciatus*) which is a pantropical pest and urban vector of *W. bancrofti* and is probably the most abundant house mosquito in towns and cities of the tropical countries[2]. Interest in the control of *Cx. quinquefasciatus* lies in the fact that it acts as a vector of filarial fever, a serious public health problem in India and many developing countries. One of the strategies of the WHO in combating tropical diseases is to destroy their vectors or intermediate hosts. Since no effective vaccine is available for filarial fever, the only efficacious approach of minimizing the incidence of this disease is to eradicate and control mosquito vectors mainly by application of insecticides to larval habitats. The control of mosquito at the immature stage is necessary and efficient in integrated mosquito management because during the immature stages, mosquitoes are immobile[3].

The use of natural products for the control of insect pests offers an economically viable and eco-friendly approach, besides being harmless to beneficial insects when adopted on a large scale. Chemical pesticides have been used for several decades in controlling pests and vectors of various human diseases as they have a quick knock down effect. However, their indiscriminate use resulted in several problems such as resistance and resurgence of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affect human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environment[4,5]. Under such alarming situations, plants and plant derived products offer a tremendous advantage over synthetic pesticides in use

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as control agents for the pests and vectors of public health. In recent years, attempts are being made to identify plants, including herbs and weeds, for their insecticidal property with a view to find out suitable alternatives to replace hazardous synthetic pesticides utilized in large scale in India[6-10]. Therefore, the present study was carried out to evaluate the larvicidal property of twenty five plant extracts against the filarial vector, *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Collection of plants

A total of twenty five plants belonging to diverse families and genera were collected from Siruvani hills (near Coimbatore) Western Ghats of Tamilnadu, India. The plants were selected based on available literature, abundant availability, medicinal and insecticidal properties. The list of plants collected and utilized for the present study is presented in Table 1. Collected plants were taxonomically identified and voucher specimen deposited at Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamilnadu, India for future reference.

2..2. Extraction of plant materials

Plants collected from various families were brought to the laboratory, washed with dechlorinated water, shade dried under room temperature and the plant materials were powdered individually using an electric blender. Each powdered plant material was sieved using kitchen strainer. One kilogram of each powdered plant material was sequentially extracted with hexane, diethyl ether, dichloromethane and ethyl acetate for a period of 72 h each and then filtered. The filtered content was then subjected to rotary vacuum evaporator until solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained was stored in sterilized amber coloured bottles and maintained at 4 ℃ in a refrigerator. Standard one per cent stock solution (1 000 ppm) was prepared by dissolving 100 mg of crude extract in 100 mL of acetone.

2.3. Establishment of filarial vector Cx. quinquefasciatus

Culex immatures collected in open drains in Chennai, Tamilnadu, India were transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence the mosquitoes were identified and species confirmed before rearing. Cyclic generations of *Cx. quinquefasciatus* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of (27±2) ℃ and a relative humidity of 70-80 percent were maintained in the insectary. The adult mosquitoes were fed on ten per cent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with laboratory reared albino mice. Ovitraps were placed inside the cages for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

2.4. Larvicidal bioassay

Larvicidal bioassay of individual plant extracts was tested against third instar larvae of *Cx. quinquefasciatus*. The tests were conducted in glass beakers. Standard WHO[11] protocol with slight modifications was adopted for the study. Ten replicates and a control were run simultaneously during each trial. For control, 1.0 mL of acetone dissolved in 249 mL of dechlorinated water was used. Mosquito immature particularly early third instar larvae were obtained from laboratory colonized mosquitoes of F1 generation. Twenty healthy larvae were released in each glass beaker and mortality was observed 24 and 48 h after treatment at 1 000 ppm. A total of three trials were carried out. However, when the control mortality ranged from five to twenty per cent, the observed percentage mortality was corrected by Abbott's[12] formula.

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

3. Results

The results of extracts of twenty five plants screened for their larvicidal activity against third instar larvae of *Cx. quinquefasciatus* are presented in Table 2. Among the twenty five plants screened, solvent extracts from *Cleistanthus collinus*, *Leucas aspera*, *Hydrocotyle javanica*, *Murraya koeingii*, *Sphaeranthus indicus* and *Zanthoxylum limonella* showed promising larvicidal effects. Hexane extracts of *Cleistanthus collinus* and *Murraya koeingii* plants showed 100 per cent mortality at 24 h. In diethyl ether, dichloromethane and ethyl acetate extracts of *Cleistanthus collinus*, *Leucas aspera*, *Hydrocotyle javanica*, *Murraya koeingii*, *Sphaeranthus indicus* and *Zanthoxylum limonella*, 100 per cent mortality was recorded after 48 h exposure (Table 3). Remaining plants also showed some larvicidal

Table 1.
List of plants collected from Siruvani hills, Western Ghats, Tamilnadu, India.

No.	Plant Name	Family	Local name	Parts used
1.	*Abrus precatorious* Linn	Papilionaceae	Kundumani	Seed
2.	*Aegle marmelos* (L) Corr	Rutaceae	Vilvam	Leaf
3.	*Alstomia scholaris* (L) R Br	Apocynaceae	Mukampalai	Leaf
4.	*Aristolochia indica* Linn	Aristolochiaceae	Karudakkodi	Root
5.	*Cassia fistula* Linn	Caesalpiniaceae	Sarakonnai	Flower
6.	*Cinnamomum zeylanicum* Breyn	Lauraceae	Sirunagapoo	Bark
7.	*Cleistanthus collinus* (Roxb) Benth	Euphorbiaceae	Oduvan	Leaf
8.	*Cymbopogon citrates* (Dc) Stapt	Poaceae	Vasanapullu	Whole plant
9.	*Drosera indica* Linn	Droceracea	Azukanni	Leaf
10.	*Evolvulus alsinoides* (L) Linn	Convolvulaceae	Vishnukarandi	Whole plant
11.	*Garcinia morella* (Gaertn) Desr	Clusiaceae	Makki	Leaf
12.	*Hydrocotyle javanica* Thunb	Apiaceae	Malaivallarai	Leaf
13.	*Ichnocarpus frutescens* (L) R Br	Apocyanaceae	Palvalli	Leaf
14.	*Lantana camara* Linn	Verbenaceae	Unnichedi	Leaf
15.	*Leucas aspera* (Willd) Link	Lamiaceae	Thumbai	Whole plant
16.	*Memecylon malabaricum* (Cl) Cong	Melastomataceae	Malamthetti	Leaf
17.	*Murraya koeingii* (L) Spreng	Rutaceae	Kariveppilai	Leaf
18.	*Ocimum americanum* Linn	Lamiaceae	Nayithulasi	Whole plant
19.	*Plumbago zeylanica* Linn	Plumbaginaceae	Neelakodaveri	Leaf
20.	*Sphaeranthus indicus* Linn	Asteraceae	Kottakkarandai	Whole plant
21.	*Strebulus asper* Lour	Moraceae	Pirayam	Leaf
22.	*Strychnos nuxvomica* Linn	Loganiaceae	Yetti	Fruit
23.	*Syzygium cumini* (L) Skeets	Myrtaceae	Neredom	Leaf
24.	*Vitex negundo* Linn	Verbenaceae	Notchi	Leaf
25.	*Zanthoxylum limonella* (Roxb) Dc	Rutaceae	Veersingapattai	Bark

Table 2.
Screening of plant extracts at 1 000 ppm concentration for larvicidal activity against *Cx. quinquefasciatus*.

No.	Plants tested	Hexane	Diethyl ether	Dichloromethane	Ethyl acetate
1.	*Abrus precatorious*	+++	−	−	++
2.	*Aegle marmelos*	−	−	−	+
3.	*Alstomia scholaris*	−	−	−	−
4.	*Aristolochia indica*	+++	−	+	++
5.	*Cassia fistula*	+	−	−	+
6.	*Cinnamomum zeylanicum*	−	−	−	+
7.	*Cleistanthus collinus*	+++	++	++	+++
8.	*Cymbopogon citrates*	++	−	−	+
9.	*Drosera indica*	−	−	−	−
10.	*Evolvulus alsinoides*	−	−	−	−
11.	*Garcinia morella*	+++	−	++	−
12.	*Hydrocotyle javanica*	++	++	++	++
13.	*Ichnocarpus frutescens*	+	−	−	−
14.	*Lantana camara*	−	−	−	+
15.	*Leucas aspera*	++	++	++	+++
16.	*Memecylon malabaricum*	−	−	−	−
17.	*Murraya koeingii*	+++	+++	++	++
18.	*Ocimum americanum*	+	−	−	+
19.	*Plumbago zeylanica*	−	−	−	+
20.	*Sphaeranthus indicus*	++	++	++	++
21.	*Strebulus asper*	−	−	−	−
22.	*Strychnos nuxvomica*	−	−	−	−
23.	*Syzygium cumini*	+	++	−	+
24.	*Vitex negundo*	+	+	−	++
25.	*Zanthoxylum limonella*	++	+++	++	+

+++100 per cent mortality at 24 h; ++100 per cent mortality at 48 h; +100 per cent mortality at 72 h; − No larval mortality.

Table 3.
Per cent larvicidal activity of potential plant extracts against *Cx. quinquefasciatus*.

Plants	Hexane		Diethyl ether		Dichloromethane		Ethyl acetate	
	24h	48h	24h	48h	24h	48h	24h	48h
Cleistanthus collinus	100.00 (90.00)	–	90.00±10.54(71.57)	100(90)	75.00±11.78(60.00)	100(90)	100.00(90.00)	–
Hydrocotyle javanica	99.00±3.16(84.26)	100(90)	92.00±9.18(73.57)	100(90)	93.00±6.74(74.66)	100(90)	97.00±6.74(80.03)	100.00 (90.00)
Leucas aspera	99.00±3.16(84.26)	100(90)	90.00±8.16(71.57)	100(90)	98.00±4.21(81.87)	100(90)	100.00(90.00)	–
Murrya koeingii	100.00 (90.00)	–	100.00 (90.00)	–	75.00±16.78(60.00)	100(90)	97.00±6.74(80.03)	100.00 (90.00)
Spheranthus indicus	99.00±3.16(84.26)	100(90)	93.00±8.23(74.66)	100(90)	99.00±3.16(84.26)	100(90)	97.00±4.83(80.03)	100.00 (90.00)
Zanthoxyllum limonella	99.00±3.16(84.26)	100(90)	100.00 (90.00)	–	97.00±4.83(80.03)	100(90)	88.00±12.29(69.73)	97.00±6.24(80.03)

Values are mean of ten replicates ± standard deviation and figures in parentheses are angular transformed.

activity but the results were not encouraging.

4. Discussion

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides. However due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought. Tikar *et al*[13] have reported the development of insecticide resistance in populations of *Cx. quinquefasciatus* against temephos, fenthion, cypermethrin, α-cypermethrin and λ-cyhalothrin indicating the need of search for safe and effective alternative safe control measures. Besides development of insecticides resistance, they are toxic to non target organisms[14]. Nowadays, mosquito control programme is focused more on the elimination of mosquitoes at larval stage with plant extracts. The advantage of targeting mosquito larvae is that they cannot escape from their breeding sites/centres until the adult stage and also to reduce the overall pesticide use in control of adult mosquitoes by aerial application of adulticidal chemicals[15-17].

A considerable number of plant derivatives have shown to be effective against mosquitoes with a safe manner. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. Identifying plant-based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the widespread insecticidal property will obviously work as a new weapon in the arsenal of insecticides and in the future may act as a suitable alternative product to fight against mosquito-borne diseases[18]. The biological activity of plant extracts might be due to various compounds, including phenolics, terpenoids, and alkaloids present in plants[19]. Pavela[20] studied the larvicidal activity of thirty one Euro-Asiatic methanolic plant extracts against the larvae of *Culex quinquefasciatus*. Likewise, Sakthivadivel and Daniel[21] screened petroleum ether extracts of sixty three insecticidal plants for larvicidal activity against *Cx. quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* of which six turned out to be potential larvicides. Nazar *et al*[22] screened one hundred coastal plant extracts against the *Cx. quinquefasciatus* larvae of which seventeen coastal plants were tested potential. The leaf extract of *Mesua ferra*[23], fruit extract of *Croton caudatus* and flower extract of *Tiliacora acuminata*[24], leaf extract of *Typhonium trilobatum*[25] and flower extract of *Tagetes erecta*[26] were found to cause larval mortality to *Cx. quinquefasciatus*. Besides these, the dichloromethane extract of *Citrullus colocynthis* whole plant, the diethyl ether extract of *Abutilon indicum* leaf, the hexane extract of *Hyptis suaveolens* aerial parts after 24 h and the *Murraya koenigii* leaf after 48 h of exposure provided hundred per cent mortality against the third instar larvae of *Cx. quinquefasciatus*[27-30].

In the present study, even though the screening of larvicidal activity was limited to one mosquito vector, *Cx. quinquefasciatus*, the present results provided six additional botanical agents which were comparable with the earlier and above mentioned screening works and these six plants might be used in purified form as alternative control strategies. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also the active ingredients of the extract responsible for larvicidal activity should be identified.

Conflict of interest statement

We declare that we have no conflict of interest.

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