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

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ABSTRACT

Hexane, diethyl ether, dichloromethane and ethyl acetate extracts of twenty five plant species, including fourteen varieties of leaves, five varieties of whole plants, two varieties of barks and one variety of flower, fruit, seed and root were tested for ovicidal activity against *Culex quinquefasciatus* at 1000 ppm. During preliminary screening, significant ovicidal activity was observed in all four solvent extracts of *Cleistanthus collinus, Hydrocotyle javanica, Leucas aspera, Murraya koeingii, Sphaeranthus indicus* and *Zanthophyllus limonella*. Solvent crude extracts of plants showing maximum ovicidal activity were selected and treated at 500 ppm. The percentage of egg hatchability significantly reduced in different solvent extracts of above mentioned plants and eggs treated with different plant extracts varied from 26.59 per cent in diethyl ether extract of *Murraya koeingii* to 82.61 per cent in hexane extract of *Leucas aspera*.

Introduction

Human beings have suffered from the activities of mosquito since time immemorial and it is believed that mosquitoes are ranked as the most important human health pests (Jaswanth *et al*., 2002). The control of mosquito at the immature stage is necessary and efficient in integrated mosquito management because during the immature stages, mosquitoes are immobile (Rutledge *et al*., 2003; Elimam *et al*., 2009). Though continuous application of synthetic chemicals such as temephos, fenthion, diflubezuron and methoprene (Dame *et al*., 1998) are effective, the repeated use of these products for mosquito control has gradually contaminated the ecosystem, increase in development of resistance among the mosquitoes (Rivero *et al*., 2010), and has also caused undesirable effects on non target organisms (Tariq *et al*., 2007). With increased public awareness on the adverse effects of synthetic insecticides on the environment, alternatives that are environmentally safe and ecologically compatible have assumed considerable importance. These problems have highlighted the need for the bioassay guided development of bioinsecticides for mosquito control with the use of enriched plant extracts which would be safe to non target organisms, degrading after sometime, having different action mechanisms and the development of resistance in mosquitoes is limited. In addition to application as general toxicants against mosquitoes, these products also have the potentials as adulticides, repellents, ovipoistion deterrents, growth and morphological disruptors (Isman, 2006; Arnason and Bernards, 2010).

Botanicals are considered to provide safer alternatives. Botanical sources have provided a variety of phytochemicals which have a wide range of benefits ranging from pharmaceutical products to insecticides. Pesticides derived from botanical sources are natural products, which are mainly secondary metabolites and include various alkaloids, terpenoides, phenolics and other secondary chemicals. The plant extracts can be used for the purpose of controlling vectors and insect pests and have a number of advantages over the conventional chemical insecticides. The major advantage is that phytochemicals are more easily degraded in nature. Environmental hazards due to chemicals of plant origin are also limited. India possesses the diversity of plants species, which provide a range of natural products. Many secondary chemicals from different plant species have been known for their pharmacological and insecticidal properties. During the last decade, various studies on natural plant products against vector mosquito indicate them as possible alternatives to chemical synthetic insecticides (Mittal and Subbarao, 2003; Rajkumar and Jebanesan, 2005; Promsiri *et al*., 2006; Elimam *et al*., 2009). Therefore, the present screening study was carried out to evaluate the ovicidal activity of twenty five plant extracts against the filarial vector *Culex quinquefasciatus*.

Materials and Methods

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 are 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.

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 were sieved using kitchen strainer. One kilogram of

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powdered plant material was sequentially extracted with hexane, diethyl ether, dichloromethane and ethyl acetate for a period of 72 hours 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 4^0C in a refrigerator. Standard one per cent stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 100 ml of acetone.

Establishment of filarial vector *Culex quinquefasciatus*

Culex immatures collected in open drains in Chennai, Tamil Nadu, 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 *Culex quinquefasciatus* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of 27 $\pm 2^0$C and a relative humidity of 70-80 per cent was 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.

Ovicidal bioassay

The method of Su and Mulla (1998) was adopted for the ovicidal bioassay and twenty five plant extracts were screened against the eggs of *Culex quinquefacsiatus* at 1000 ppm. Based on the preliminary experiment, the effective plant extracts were further tested against the eggs of *Culex quinquefasciatus* at a concentration of 500 ppm. The percentage of egg hatching and mortality were recorded. The number of eggs hatched in control and treatments were recorded and the percentage of ovicidal activity was calculated. The experiment was conducted at room temperature 30 ±2˚C and relative humidity 75 ±5 per cent. Five 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. A total of three trials were carried out. The data obtained were subjected to angular transformation before two way analysis of variance and least significance difference (LSD) test was used to separate mean significant difference within the plant extracts tested.

$$\text{Per cent ovicidal activity} = \frac{\text{\% of eggs hatched in control} - \text{\% of eggs hatched in treated}}{\text{\% of eggs hatched in control}} \times 100$$

Statistical analysis

Two way analysis of variance was worked to find out the significances of the treatments. The treatments were separated by least significance difference (LSD) at p = 0.05 level.

Results

Twenty five plant extracts were screened for their ovicidal activity against the eggs of *Culex quinquefasciatus* (Table 2). During preliminary screening significant ovicidal activity was observed in all four solvent extracts of *Cleistanthus collinus, Hydrocotyle javanica, Leucas aspera, Murraya koeingii, Sphaeranthus indicus* and *Zanthoxylum limonella*. Solvent crude extracts of plants showing maximum ovicidal activity were selected and treated at 500 ppm. The percentage of egg hatchability significantly reduced in different solvent extracts of above mentioned plants and eggs treated with different plant extracts varied from 26.59 per cent in diethyl ether extract of *Murraya koeingii* to 82.61 per cent in hexane extract of *Leucas aspera* (Table 3). The per cent data collected were angular transformed and subjected to two-way analysis of variance. Among the solvent extracts and within the plants tested, showed statistical significance (Table 3).

Discussion

One of the greatest drawbacks of some chemical insecticides is their persistence in the environment and promoting the development of resistance in the insect. Development of resistance is more a function of frequency of use and persistence. Consequently, there is a need for alternative insecticides, which are effective, but with fewer side effects and rapid degradation, reducing the likelihood of resistance development (Nivsarkar *et al*., 2001). Vector control has experienced a paradigm shift over time as public health officials have come to better appreciate the potential applications of natural products in the mission of disease control. Plants can provide safer alternatives for modern deadly poisonous synthetic chemicals. Plants have been used since ancient times to repel or kill blood-sucking insects in the human history and, even now, in many parts of the world people are practicing plant substances to repel or kill the mosquitoes and other bloodsucking insects. In the present investigation, six plants have been identified for the control of vector mosquitoes.

The ovicidal efficacy of the present study was compared well with earlier reports. Pushpalatha (1997) reported decreased hatchability of *Culex quinquefasciatus* eggs against *Annona squamosa* seeds. The seed extract of *Atriplex canescens* showed complete ovicidal at 1,000 ppm concentration in eggs of *Culex quinquefasciatus* (Ouda *et al*. 1998). The methanolic crude leaf extracts of *Pemphis acidula* exhibited 100 per cent ovicidal activity against *Culex quinquefasciatus* at 500 ppm (Samidurai *et al*., 2009). Mortality of 100 per cent with ethyl acetate extract of *Aegle marmelos* and methanol extracts of *Aegle marmelos*, *Andrographis lineata* and *Cocculus hirsutus* were exerted at 1,000 ppm against the eggs of *Anopheles subpictus* (Elango *et al*., 2009). No hatchability was observed in hexane and chloroform extracts of *Andrographis lineata*, *Andrographis paniculata*, and hexane extract of *Tagetes erecta* at 1,000 ppm against the eggs of *Culex tritaeniorhynchus* (Elango *et al*., 2010). Rajkumar and Jebanesan (2004) studied ovicidal activity of *Moschosma polystachyum* leaf extract against *Culex quinquefasciatus* and observed 100 per cent egg mortality at 100 ml/l. Mullai and Jebanesan (2006) reported complete ovicidal activity (100 per cent mortality) at 300 ppm for methanol, benzene, petroleum ether, and ethyl acetate extracts of *Citrullus pubescens* against *Culex quinquefasciatus*. The benzene extracts of *Citrullus vulgaris* exerted 100 per cent mortality at 250 ppm against *Anopheles stephensi* and *Aedes aegypti*, (Mullai *et al*., 2008). The crude ethanol extract of *Imperata cylindrica* leaf decreased the hatchability of *Culex quinquefasciatus* at 100 ppm (Mohsen *et al*., 1995).

In ovicidal activity, exposure to freshly laid eggs was more effective than to the older eggs. It has been shown that the age of the embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin to *Culex quinquefasciatus* (Miura *et al*., 1976). Govindarajan *et al*. (2008) also reported that the younger age groups of egg rafts or eggs showed poor hatchability rate when exposed to higher concentrations of extract, and older age groups of egg rafts or eggs showed high hatchability rate when exposed to lower concentrations of extract. Crude extracts or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, further studies on the identification of the active principles involved and their mode of action and field trials are usually needed to recommend any of these plant materials as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

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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 nux-vomica* 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 ovicidal activity againstCulex *quinquefasciatus*

No.	Plants tested	Hexane	Diethyl ether	Dichloro methane	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 nux-vomica*	-	++	-	-
23	*Syzygium cumini*	-	-	-	-
24	*Vitex negundo*	+	-	-	++
25	*Zanthoxylum limonella*	++	+	+	+

+++Ovicidal activity above 75%; ++Ovicidal activity between 50-75%
+Ovicidal activity between 25-50%; -Ovicidal activity below 25%

Table 3: Per cent ovicidal activity of potential plant extracts against *Culex quinquefasciatus* at concentration of 500 ppm

Plants	Hexane	Diethyl ether	Dichloro methane	Ethyl acetate
Cleistanthus collinus	42.31[a] ±6.76 (40.57)	32.07[a] ±5.09 (34.88)	70.37[d] ±6.18 (56.98)	81.06[c] ±6.28 (64.60)
Hydrocotyle javanica	82.08[c] ±3.76 (64.90)	37.52[a] ±9.07 (37.76)	55.86[c] ±9.55 (48.33)	27.50[a] ±5.78 (31.67)
Leucas aspera	82.61[c] ±3.03 (65.35)	31.54[a] ±6.22 (34.14)	38.41[ab] ±4.97 (38.29)	74.46[c] ±4.32 (59.60)
Murrya koeingii	65.27[b] ±5.31 (53.85)	26.59[a] ±6.64 (30.98)	78.02[d] ±1.22 (62.03)	40.43[b] ±6.87 (39.47)
Sphaeranthus indicus	43.91[a] ±7.50 (41.50)	30.21[a] ±4.14 (33.34)	28.47[a] ±6.30 (32.20)	78.04[c] ±5.40 (62.03)
Zanthoxyllum limonella	58.47[b] ±8.07 (49.84)	37.82[a] ±3.21 (37.94)	30.65[a] ±6.61 (33.58)	48.04[b] ±8.75 (43.85)

Per cent corrected values of five replicates ±standard deviation.
Values in parentheses are angular transformed.
Within the column different alphabets were statistically significant (P<0.05) by LSD.