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ORIGINAL ARTICLE

Ovicidal and Oviposition Deterrent Activities of Medicinal Plant Extracts Against *Aedes aegypti* L. and *Culex quinquefasciatus* Say Mosquitoes (Diptera: Culicidae)

Appadurai Daniel Reegan*, Munusamy Rajiv Gandhi, Micheal Gabriel Paulraj, Savarimuthu Ignacimuthu

Division of Vector Control, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India.

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Abstract
Objectives: To evaluate the ovicidal and oviposition deterrent activities of five medicinal plant extracts namely *Aegle marmelos* (Linn.), *Limonia acidissima* (Linn.), *Sphaeranthus indicus* (Linn.), *Sphaeranthus amaranthoides* (burm.f), and *Chromolaena odorata* (Linn.) against *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes. Three solvents, namely hexane, ethyl acetate, and methanol, were used for the preparation of extracts from each plant.
Methods: Four different concentrations—62.5 parts per million (ppm), 125 ppm, 250 ppm, and 500 ppm—were prepared using acetone and tested for ovicidal and oviposition deterrent activities. One-way analysis of variance (ANOVA) was used to determine the significance of the treatments and means were separated by Tukey's test of comparison.
Results: Among the different extracts of the five plants screened, the hexane extract of *L. acidissima* recorded the highest ovicidal activity of 79.2% and 60% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Similarly, the same hexane extract of *L. acidissima* showed 100% oviposition deterrent activity at all the tested concentrations against *Cx. quinquefasciatus* and *Ae. aegypti* adult females.
Conclusion: It is concluded that the hexane extract of *L. acidissima* could be used in an integrated mosquito management program.

1. Introduction

Mosquitoes are medically important insects and are considered major public health pests [1]. Mosquitoes transmit many dreadful diseases to humans and other vertebrates; therefore, they have been declared "Public Enemy Number One" [2]. Mosquitoes belonging to the genera *Aedes* and *Culex* are transmitting dengue, dengue

*Corresponding author. Present address. NVBDCP, ROHFW, Govt. of India, Besant Nagar, Chennai, India.
E-mail: danielreegan85@gmail.com

hemorrhagic fever, yellow fever, chikungunya, Japanese encephalitis, and filariasis [3,4]. Mosquito bites cause allergic responses including local skin reactions and systemic reactions such as angioedema and urticaria [5]. Tropical areas are more vulnerable to mosquito-borne diseases and the risk of contracting arthropod-borne illnesses is increased due to climate change and intensifying globalization [6].

It is imperative to control mosquitoes in order to prevent mosquito-borne diseases and improve public health. *Aedes aegypti* is the primary vector of dengue, dengue hemorrhagic fever, and chikungunya. Dengue fever is endemic in south-east Asia including India, Bangladesh, and Pakistan [7]. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease such as dengue hemorrhagic fever and dengue shock syndrome or with unusual symptoms such as central nervous system involvement [8,9]. *Culex quinquefasciatus* is an important vector of lymphatic filariasis in tropical and subtropical regions. It is a pantropical pest and urban vector of *Wuchereria bancrofti* [10] and is possibly the most abundant house mosquito in towns and cities of tropical countries. According to [11], about 90 million people worldwide are infected with *W. bancrofti,* and 10 times more people are at risk of being infected. In India alone, 25 million people harbor microfilaria (mf) and 19 million people suffer from filarial disease manifestations [12].

In recent years, mosquito control programs have suffered a setback because mosquitoes are developing resistance to synthetic chemical insecticides such as organochlorides, organophosphates and carbamates and insect growth regulators such as methoprene, pyriproxyfen, and diflubenzuron [13−16]. Moreover, many organophosphates and organochlorides adversely affect the environment and damage biological systems [17]. These side effects of synthetic chemicals prompted many researchers to find environment-friendly alternatives for mosquito management. Literature reveals sufficient amounts of work on the mosquito control potential of plant extracts and plant essential oils [18−25].

The present study was undertaken to evaluate the ovicidal and oviposition deterrent activities of five medicinal plant extracts namely *Aegle marmelos* (Linn.), *Sphaeranthus indicus* (Linn.), *Sphaeranthus amaranthoides* (burm.f), *Limonia acidissima* (Linn.), and *Chromolaena odorata* (Linn.) against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes.

2. Materials and methods

2.1. Collection of plant material

The matured leaves of each plant were collected from Chennai, Tirunelveli and surrounding areas in Tamil Nadu, India and the plant species were authenticated by a Botanist at Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India. The voucher specimens (ERI-LA-MOS-210-214) of each plant species were deposited in the herbarium of the institute. The collected leaves were shade-dried for 5 days and coarsely powdered using an electric blender.

2.2. Preparation of solvent extracts

Crude extracts were prepared from the powdered leaves of each plant by a sequential extraction method using hexane, ethyl acetate, and methanol solvents (Fisher Scientific and Himeddia, Chennai, India). Leaf powder (1 kg) of each plant was soaked in 3 L of hexane for 48 hours with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper, concentrated in a rotary evaporator (Medica instruments Mgf.Co. Sl.No:EV11.JF.012), and finally dried in vacuum. The residue was soaked in other solvents consecutively and extracted. All the crude extracts were stored at 4°C in air-tight glass vials in the dark until used.

2.3. Test mosquitoes

The mosquito life stages used in this study were obtained from the Entomology Research Institute, and they were free of exposure to pathogens, insecticides, or repellents. The rearing conditions were: 28 ± 1°C; 70−75% relative humidity (RH); and 11 ± 0.5-hour photoperiod [26].

2.4. Ovicidal assay

Ovicidal activity was studied following the method of Elango et al [27]. Twenty five freshly laid eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were separately exposed to four different concentrations, namely 62.5 parts per million (ppm), 125 ppm, 250 ppm, and 500 ppm, prepared using acetone. Each concentration was replicated five times. Control (acetone in water) was maintained separately and egg mortality was observed under the microscope. Azadirachtin (10 ppm) and temephos (10 ppm) were used as positive controls for comparison with five replications each. The percent ovicidal activity was assessed at 120 hours post-treatment using the following formula:

$$\text{Percent ovicidal activity}: \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$$

2.5. Oviposition deterrent assay

The oviposition deterrent activity was assessed using earlier reported methods [27,28] with slight modifications. Ten blood-fed females of *Ae. aegypti* and *Cx. quinquefasciatus* (10 days old, 2 days after blood feeding) were transferred to separate cages (45 cm × 45 cm × 45 cm) made of mosquito net with a

muslin socket on the front side for access. In each cage, four plastic bowls holding 200 mL of tap water were placed in opposite corners of each cage; one bowl was treated with the test material (extract), two bowls were used for positive control (temephos and azadirachtin), and the other one served as control. The concentrations used were 62.5 ppm, 125 ppm, 250 ppm, and 500 ppm. Each concentration was replicated five times. Sucrose solution (10%) was provided to the adult as feed throughout the study period. Experiments were carried out at room temperature (28 ± 1°C; RH: 70−75%) for a period of 72 hours. After 72 hours, the number of eggs laid in each bowl was counted and recorded. The percent effective repellency (ER) for each concentration was calculated using the following formula:

$$\text{Effective repellency(ER)}(\%) = \frac{\text{NC} - \text{NT}}{\text{NC}} \times 100(\%)$$

where NC is the number of eggs in the control, and NT is the number of eggs in the treatment.

2.6. Statistical analysis

The mean values and standard deviations were calculated from replication data. One-way analysis of variance (ANOVA) was used to determine the significance of the treatments and means were separated by Tukey's test of multiple comparisons using SPSS software (version 11.5; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Ovicidal activity results

Among the different extracts of the five plants screened, the hexane extract of *L. acidissima* recorded the highest ovicidal activity of 79.2% and 60% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Tables 1 and 2). The hexane extract of *A. marmelos* recorded moderate ovicidal activity of 53.6% and 48.8% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Tables 1 and 2). The ethyl acetate extract of *C. odorata* recorded 42.4% and 13.6% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. The other two plant extracts showed much less ovicidal activity. The positive control azadirachtin recorded ovicidal activity of 95.2% and 92.8% at 10 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Temephos recorded 46.4% and 44% at 10 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Tables 1 and 2). Overall, the ovicidal activity was higher against *Cx. quinquefasciatus* eggs than *Ae. aegypti* eggs.

3.2. Oviposition deterrent activity results

Among the five plant extracts screened, the hexane extract of *L. acidissima* showed 100% oviposition

Table 1. Percent ovicidal activity of crude extracts against *Culex quinquefasciatus* eggs.

			Concentration (ppm)			
Mosquito species	Plant	Treatment	62.5	125	250	500
Culex quinquefasciatus	*Aegle marmelos*	Hexane	7.1 ± 2.08^{b}	14.4 ± 6.06^{b}	22.4 ± 2.19^{b}	53.6 ± 2.19^{b}
		Ethyl acetate	6.4 ± 2.19^{bc}	9.6 ± 2.19^{bc}	20.0 ± 2.82^{bc}	43.2 ± 1.78^{c}
		Methanol	3.2 ± 1.78^{bcd}	7.2 ± 1.78^{c}	14.4 ± 2.19^{cd}	28 ± 2.82^{ef}
	Limonia acidissima	Hexane	17.6 ± 2.19^{a}	36 ± 2.82^{a}	56.8 ± 3.34^{a}	79.2 ± 3.34^{a}
		Ethyl acetate	0^{cd}	0.8 ± 1.78^{d}	1.6 ± 3.57^{f}	4.0 ± 2.82^{hi}
		Methanol	4 ± 2.82^{bcd}	8.8 ± 3.34^{c}	18.4 ± 2.19^{bcd}	39.2 ± 1.38^{cd}
	Sphaeranthus indicus	Hexane	3.2 ± 1.78^{bcd}	7.2 ± 3.34^{c}	13.6 ± 2.19^{cd}	25.6 ± 2.19^{f}
		Ethyl acetate	2.4 ± 2.19^{bcd}	7.2 ± 1.78^{c}	14.4 ± 2.19^{cd}	29.6 ± 2.19^{ef}
		Methanol	1.6 ± 2.19^{cd}	4.8 ± 1.78^{cd}	6.4 ± 2.19^{ef}	15.2 ± 3.34^{g}
	Sphaeranthus amaranthaides	Hexane	3 ± 2.73^{bcd}	7.8 ± 3.03^{c}	16 ± 4.48^{bcd}	33 ± 2.73^{de}
		Ethyl acetate	4 ± 2.82^{bcd}	8 ± 2.82^{c}	15.2 ± 1.78^{cd}	30.4 ± 2.19^{ef}
		Methanol	4 ± 2.82^{bcd}	9.6 ± 2.19^{bc}	16 ± 2.82^{bcd}	32.8 ± 3.34^{de}
	Chromolaena odorata	Hexane	3.2 ± 3.34^{bcd}	6.4 ± 2.19^{c}	12.8 ± 4.38^{de}	24.8 ± 3.34^{f}
		Ethyl acetate	4 ± 2.82^{bcd}	9.6 ± 2.19^{bc}	19.2 ± 3.34^{bcd}	42.4 ± 2.19^{c}
		Methanol	0^{cd}	0^{d}	4 ± 2.82^{f}	9.6 ± 2.19^{gh}
		Control	1.6 ± 2.19^{cd}	0.8 ± 1.78^{d}	0.8 ± 1.78^{f}	0^{i}
		Azadirachtin (10 ppm)	95.2 ± 1.78			
		Temephos (10 ppm)	46.4 ± 3.57			

Data are the mean ± standard deviation (SD) of five replicates. Means were separated by Tukey's test of multiple comparisons, one-way analysis of variance (ANOVA). ppm = parts per million. $p \leq 0.5$, level of significance. Results with same letters in the column are not significantly different.

Table 2. Percent ovicidal activity of crude extracts against *Aedes aegypti* eggs.

Mosquito species	Plant	Treatment	Concentration (ppm)			
			62.5	125	250	500
Aedes aegypti	*Aegle marmelos*	Hexane	6.4 ± 1.78^{ab}	13.6 ± 2.19^{b}	26.4 ± 5.21^{a}	48.8 ± 4.38^{b}
		Ethyl acetate	1.6 ± 2.19^{cd}	5.6 ± 3.57^{c}	10.4 ± 5.36^{b}	24.8 ± 4.38^{c}
		Methanol	4 ± 2.82^{bc}	7.2 ± 1.78^{c}	11.2 ± 4.38^{b}	24.8 ± 1.34^{c}
	Limonia acidissima	Hexane	8 ± 2.82^{a}	17.6 ± 2.19^{a}	29.6 ± 2.19^{a}	60 ± 2.82^{a}
		Ethyl acetate	2.4 ± 2.19^{cd}	5.6 ± 1.19^{c}	11.2 ± 1.78^{b}	19.2 ± 3.34^{cd}
		Methanol	0^{d}	0.8 ± 1.78^{e}	4 ± 2.82^{cd}	6.4 ± 1.34^{fg}
	Sphaeranthus indicus	Hexane	0^{d}	1.6 ± 2.19^{de}	4.0 ± 2.82^{cd}	8.8 ± 3.34^{ef}
		Ethyl acetate	0^{d}	0^{e}	0^{d}	3.2 ± 1.78^{fg}
		Methanol	0^{d}	0^{e}	0.8 ± 1.78^{cd}	2.4 ± 3.57^{fg}
	Sphaeranthus amaranthaides	Hexane	0^{d}	0^{e}	2.4 ± 3.57^{cd}	4.8 ± 4.38^{fg}
		Ethyl acetate	0^{d}	0^{e}	0^{d}	0^{g}
		Methanol	0^{d}	0.8 ± 1.78^{e}	1.6 ± 2.19^{cd}	3.2 ± 1.78^{fg}
	Chromolaena odorata	Hexane	0^{d}	0^{e}	0.8 ± 2.19^{cd}	3.2 ± 4.38^{fg}
		Ethyl acetate	1.6 ± 3.57^{cd}	4.8 ± 3.34^{cd}	6.4 ± 2.19^{bc}	13.6 ± 2.19^{de}
		Methanol	0^{d}	0^{e}	0^{d}	1.6 ± 2.19^{g}
		Control	0^{d}	00.8 ± 1.78^{e}	00.8 ± 1.78^{cd}	1.6 ± 2.19^{g}
		Azadirachtin (10 ppm)	92.8 ± 3.34			
		Temephos (10 ppm)	44 ± 2.82			

Data are mean ± standard deviation (SD) of five replicates. Means are separated by Tukey's test of multiple comparisons, one-way analysis of variance (ANOVA). ppm = parts per million. $p \leq 0.5$, level of significance. Results with same letters in the column are not significantly different.

deterrent activity at all the tested concentrations against *Cx. quinquefasciatus* and *Ae. aegypti* adult females (Tables 3 and 4). At 500 ppm concentration, the hexane extract of *A. marmelos* recorded 76.74% and 71.79% oviposition deterrent activity against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Tables 3 and 4). The ethyl acetate extract of *S. amaranthoides* recorded 22.31% and 20.48% oviposition deterrent activity at 500 ppm concentration against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. The extracts of *S. indicus* and *C. odorata*

Table 3. Percent oviposition deterrent activity of crude extracts against *Culex quinquefasciatus* adult females.

Mosquito species	Plant	Treatment	Concentration (ppm)			
			62.5	125	250	500
Culex quinquefasciatus	*Aegle marmelos*	Hexane	23.09 ± 2.22^{b}	46.79 ± 1.30^{b}	56.67 ± 0.95^{b}	76.74 ± 1.02^{b}
		Ethyl acetate	8.73 ± 2.15^{c}	20.25 ± 2.13^{c}	32.87 ± 1.30^{c}	47.56 ± 1.48^{c}
		Methanol	0^{e}	4.24 ± 2.30^{f}	11.91 ± 2.22^{ef}	20.91 ± 1.65^{fg}
	Limonia acidissima	Hexane	100^{a}	100^{a}	100^{a}	100^{a}
		Ethyl acetate	2.81 ± 1.79^{d}	8.50 ± 2.39^{de}	20.68 ± 2.06^{d}	30.01 ± 1.75^{e}
		Methanol	0^{e}	5.11 ± 2.74^{f}	11.80 ± 2.14^{f}	17.74 ± 1.25^{g}
	Sphaeranthus indicus	Hexane	0^{e}	0^{g}	0^{i}	0^{i}
		Ethyl acetate	0^{e}	0^{g}	7.64 ± 1.55^{g}	11.47 ± 1.75^{h}
		Methanol	0^{e}	0^{g}	0^{i}	0^{i}
	Sphaeranthus amaranthaides	Hexane	2.06 ± 1.21^{de}	5.45 ± 1.62^{ef}	9.93 ± 2.64^{fg}	12.03 ± 1.63^{h}
		Ethyl acetate	0.32 ± 0.29^{de}	9.29 ± 2.30^{d}	15.10 ± 1.61^{e}	22.31 ± 2.59^{f}
		Methanol	0^{e}	0^{g}	4.03 ± 1.42^{h}	13.34 ± 2.07^{h}
	Chromolaena odorata	Hexane	0^{e}	0^{g}	0^{i}	0^{i}
		Ethyl acetate	0^{e}	0^{g}	0^{i}	0^{i}
		Methanol	2.48 ± 2.01^{de}	9.17 ± 1.75^{d}	22.55 ± 1.54^{d}	33.73 ± 1.88^{d}
	Azadirachtin (10 ppm)		86.29 ± 1.09			
	Temephos (10 ppm)		10.27 ± 1.75			

Data are mean ± standard deviation (SD). Means are separated by Tukey's test of multiple comparisons, one-way analysis of variance (ANOVA). $p \leq 0.5$, level of significance. ppm = parts per million. Results with same letters in the column are not significantly different.

Table 4. Percent oviposition deterrent activity of crude extracts against *Aedes aegypti* adult females.

Mosquito species	Plant	Treatment	Concentration (ppm)			
			62.5	125	250	500
Aedes aegypti	*Aegle marmelos*	Hexane	21.02 ± 2.11^{b}	45.14 ± 1.69^{b}	52.60 ± 1.77^{b}	71.79 ± 1.57^{b}
		Ethyl acetate	3.04 ± 1.54^{d}	7.20 ± 1.63^{d}	13.56 ± 2.21^{e}	31.22 ± 1.63^{d}
		Methanol	0^{e}	0^{g}	3.15 ± 1.62^{hi}	13.01 ± 1.56^{g}
	Limonia acidissima	Hexane	100^{a}	100^{a}	100^{a}	100^{a}
		Ethyl acetate	1.20 ± 0.47^{de}	6.44 ± 1.47^{de}	18.06 ± 1.23^{d}	27.71 ± 1.48^{e}
		Methanol	0^{e}	1.84 ± 0.88^{fg}	5.87 ± 2.46^{gh}	10.88 ± 2.63^{gh}
	Sphaeranthus indicus	Hexane	0^{e}	0^{g}	0^{i}	0^{k}
		Ethyl acetate	0^{e}	0^{g}	2.59 ± 1.85^{i}	4.34 ± 2.59^{i}
		Methanol	0^{e}	0^{g}	0^{i}	0^{k}
	Sphaeranthus amaranthaides	Hexane	2.27 ± 1.59^{de}	3.90 ± 2.29^{ef}	6.65 ± 1.79^{g}	8.74 ± 1.68^{h}
		Ethyl acetate	2.07 ± 1.69^{de}	4.14 ± 0.83^{ef}	10.27 ± 1.75^{f}	20.48 ± 0.83^{f}
		Methanol	0^{e}	0^{g}	0^{i}	2.61 ± 1.13^{ij}
	Chromolaena odorata	Hexane	0^{e}	0^{g}	0^{i}	0^{k}
		Ethyl acetate	0^{e}	0^{g}	0^{i}	0^{k}
		Methanol	9.93 ± 2.15^{c}	15.41 ± 2.39^{c}	25.53 ± 0.86^{c}	38.23 ± 1.73^{c}
	Azadirachtin (10 ppm)		75.21 ± 0.86			
	Temephos (10 ppm)		4.05 ± 1.36			

Data are the mean ± standard deviation (SD). Means are separated by Tukey's test of multiple comparisons, one-way analysis of variance (ANOVA). $p \leq 0.5$, level of significance. ppm = parts per million. Results with same letters in the column are not significantly different.

showed the least oviposition deterrent activity at all the tested concentrations against two mosquito species (Tables 3 and 4).

4. Discussion

Over the past 5 decades, synthetic pesticides have been indiscriminately used against vector mosquitoes. As a result, side effects such as environmental pollution and toxic hazards to humans and other nontarget organisms were created. These side effects of synthetic chemicals created awareness of the need for ecofriendly and target-specific pesticides for mosquito control [29,30]. It is clearly proven that plant extracts and plant compounds are ecofriendly, target-specific, less expensive, and highly efficacious pesticides for the control of vector mosquitoes [31,32].

In the present study, the hexane extract of *L. acidissima* recorded the highest ovicidal activity of 79.2% and 60% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Previously, some investigators studied the ovicidal activity of plant extracts against mosquito eggs. Elango et al [27] reported that *Cocculus hirsutus* methanol extract caused 86% and 100% ovicidal activity at 500 ppm and 1000 ppm, respectively against *An. subpictus*. In another study, 100% ovicidal activity was recorded by a methanol extract of *Andrographis paniculata* at 150 ppm concentration in *An. stephensi* eggs [33].

Furthermore, the same hexane extract of *L. acidissima* showed 100% oviposition deterrent activity at all the tested concentrations (62.5−500 ppm) against *Cx. quinquefasciatus* and *Ae. aegypti* adult females. Previously, some investigators reported the oviposition deterrent effect of plant extracts against vector mosquitoes. Coria et al [34] reported 100% oviposition deterrent effect obtained with *Melia azedarach* L. leaf extract at 1 g/L concentration against *Ae. aegypti*. Autran et al [35] recorded the oviposition deterrent effect of essential oil obtained from leaves, inflorescence, and stem of *Piper marginatum* Jacq. Their results showed that essential oil of leaves and stems of *P. marginatum* exhibited oviposition deterrent effect on *Ae. aegypti* females at 50 ppm and 100 ppm concentration and that the number of eggs laid was significantly lower (<50%) compared to control. Similarly, Prajapati et al [36] reported that the bark oil of *Cinnamomum zeylanicum* reduced the oviposition of *Ae. aegypti* to 50% at 33.5 ppm concentration.

In conclusion, the hexane extract of *L. acidissima* was the most potent treatment against the two tested mosquito vectors. Based on these results, the hexane extract of *L. acidissima* could be used in vector mosquito control and may be further probed to isolate the active constituent responsible for the bioactivities.

Conflicts of interest

The authors do not have any conflicts of interest.

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