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Research Article

MOSQUITO OVICIDAL AND REPELLENT PROPERTIES OF *CHROZOPHORA ROTTLERI* (EUPHORBIACEAE) AGAINST *ANOPHELES STEPHENSI, AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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ABSTRACT

Present study, we evaluated the larvicidal and repellent activities of *Chrozophora rottleri* (Family: Euphorbiaceae) extract against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The ovicidal activity was determined against three mosquito species to various concentrations ranging from 50-300 ppm under the laboratory conditions. The hatch rates were assessed 48 h post treatment. The repellent efficacy was determined against three mosquito species at three concentrations viz., 1.0, 2.5 and 5.0 mg/cm^2 under the laboratory conditions. Among five solvent extracts tested, the methanol extract have most promising ovicidal activity. The methanol extract exerted zero hatchability (100% mortality) at 150 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. The methanol extract of *C. rottleri* found to be more repellency than the other extracts. A higher concentration of 5.0 mg/cm^2 provided 100% protection up to 300 min against *Cx. quinquefasciatus* and 250 min against *Ae. aegypti* and *An. stephensi*, respectively. The results clearly show that repellent activity was dose dependent. From the results it can be concluded the crude extract of *C. rottleri* was an excellent potential for controlling *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* mosquitoes.

Keywords: *Chrozophora rottleri*, Ovicidal activity, Repellent activity, *Culex quinquefasciatus, Aedes aegypti, Anopheles stephensi.*

INTRODUCTION

Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases. Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis, and *Japanese encephalitis*. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (Peng *et al.,* 1999). *Anopheles stephensi* are major malaria vectors in India. With an annual incidence of 300-500 million clinically manifested cases and a death toll of 1.1-2.7 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world's population lives in areas where malaria is endemic (Wernsdorfer and Wernsdorfer, 2003). *Aedes aegypti* is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. 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, dengue haemorrhagic fever and dengue shock syndrome, or with unusual manifestations

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such as central nervous system involvement. About two-fifths of the world's populations are now at risk of catching dengue according to the World Health Organization (Pancharoen *et al.,* 2002). *Culex quinquefasciatus* acts as a vector for filariasis in India. Human filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries (Udonsi, 1986). *Lymphatic filariasis* caused by *Wuchereria bancrofti* and transmitted by mosquito *Cx. quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *Cx. quinquefasciatus* infects more than 100 million individuals worldwide annually (Rajasekariah *et al.,* 1991). *W. bancrofti* is the most predominant filarial nematode, which is usually characterized by progressive debilitating swelling at the extremities, scrotum, or breast (elephantiasis) in an infected individual (Myung *et al.,* 1998).

Chemical insecticide has been used indiscriminately during the past few decades to control the insects. This has produced serious repercussions such as insect resistance, mammalian toxicity, bio accumulation and environment damage (Klein, 1976). Toxic chemicals are responsible for contamination of food chain and pollution of the environment. In larval mosquito control application of insecticide in ponds, well and other water bodies may cause health hazards to human and larvivorus fishes. Constant application of organophosphates such as temephos and fenthion and insect growth regulator such as diflubenzuron and methoprene are generally used for the control of mosquito larvae (Yang *et al.,* 2002). Mosquito repellent using people complained of ill health effect and some time required medical treatment (Sharma, 2001). These problems have highlighted the need for the development of new strategies for selective mosquito control. Phytochemicals are advantageous due to their eco-safety, target-specificity, not development of resistance, reduced number of applications, higher acceptability and suitability for rural areas. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programmes. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, methanol, chloroform, benzene, ethyl acetate and acetone, etc., depending on the polarity of the phytochemical. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, While other interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimuli thus acting as repellent or attractant (Markouk *et al.,* 2001). More than 1005 plant species are found to possess insecticidal properties, 384 contain antifeedants, 297 contain repellents, and 27 contain attractants and possess growth inhibitors (Jayaraj, 1993). All these indicate that the plant kingdom is a vast storehouse of potentially useful chemicals for pest control.

It is believed that insect resistance likely to occur less because many botanicals contain multiactive principles. The pest control principles include properties of insecticide, antifeedant, repellent, chemosterilant, attractant, juvenile and anti-juvenile hormone, moulting and antimoulting hormone, nematicide, rodenticide, fungicide and bactericide (Rajkumar and Jebanesan, 2004; Govindarajan, 2010b,c,). Sivagnaname and Kalyanasundaram (2004) studied the methanolic extracts of the leaves of *Atlanta monophylla* that were evaluated for mosquitocidal activity against immature stages of three mosquito species, *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* in the laboratory. Vasudevan *et al.* (1989) noted that ovicidal activity of castor oil extracted from castor seeds against mosquitoes *An. stephensi, Cx. fatigans* and *Ae. aegypti.* Prakash (1992) stated that ovicidal action of certain chitin synthesis inhibitors diflubenzuron, penfluron and bay SIR 8514 against mosquitoes, *Cx. quinquefasciatus, Ae. aegypti, An. stephensi* and *An. culicifacies*. Ovicidal effects of the seed extract of *Atriplex canescens* was reported against *Cx. Quinquefasciatus* (Ouda *et al.,* 1998). Su and Mulla (1998) reported that the ovicidal activity of neem products *Azadirachtin* against mosquitoes *Cx. tarsalis* and *Cx. quinquefasciatus.*

Rajkumar and Jebanesan (2004) studied that ovicidal activity of *Solanum trilobatum* leaf extract against *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*. Yit *et al.* (1985) reported benzene and methanol extracts of *Artemisia vulgaris* has been repellent activity against *Ae. aegypti*. The aerial parts of the plant *Kleina pendula* was used in Somalia for the repellent and insecticidal properties; the *Zanthoxylum armatus, Z.alatum, Azadirachta indica* and *Curcuma aromatica* were possess repellent properties against mosquitoes (Das *et al.,* 2000); the repellency effect of three plants viz., fever tea (*Lippia javanica*), rose geranium (*Pelargonium reniforme*) and lemongrass (*Cymbopogan excavatus*) against laboratory reared *An. arabiensis* mosquitoes (Govere *et al.,* 2000). *Chrozophora rottleri* have been using in traditional medicine by native medical practitioners for the treatment of various diseases in Saudi Arabia, Pakistan and India (e.g. against jaundice and purifying blood). In India and Sudan, powdered stems or whole plants are applied to wounds to improve healing. In Nepal, juice of the fruit is given in cases of cough and colds, purifying agent (leaf) and laxative (seed), having bioactive components. The fruits yield a purplish blue dye, which is used in East Africa to dye mats. The leaves are very much beneficial in treating skin diseases and also used as a depurative agent. The seeds are used as cathartic like Ghodtapde and credited with purgative properties (Meena and Rao, 2010; Manandhar and Manandhar, 2002; Singh *et al.,* 2010; Ganga Rao *et al.,* 2012).The aim of the present study was to determine the effect of ovicidal and repellent activities of the plant leaf extract of *C. rottleri* against the malaria (*An. stephensi*), dengue (*Ae. aegypti*) and filariasis (*Cx. quinquefasciatus*) vector mosquitoes.

MATERIALS AND METHODS

Collection of Plants

Fully developed leaves of the *C. rottleri* were collected from Nilgiris, Western Ghats (11° 10'N to 11° 45' N latitude and 76° 14'E to 77° 2' E longitude), Tamil Nadu State, India. The identity was confirmed at the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. Voucher specimens were numbered and kept in our laboratory and are available upon request.

Extraction

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material (3.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with five different solvents namely benzene, hexane, ethyl acetate, chloroform and methanol individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. The *C. rottleri* with five different solvents yielded 84.60, 107.38, 99.37, 114.96 and 136.28 gm of crude residue respectively. Standard stock solutions were prepared at 1% by dissolving the residues with ethanol. From this stock solution, different concentrations were prepared and these solutions were used for ovicidal and repellent activity.

Test Organisms

Laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with Parafilm as membrane for 4 h. *Ae. aegypti* feeding was done from 12:00 p.m. to 4:00 p.m. and *An. stephensi* and *Cx. quinquefasciatus* were fed during 6:00 p.m. to 10:00 p.m. A membrane feeder with the bottom end fitted with Parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C were maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28±2 °C, 70–85 % relative humidity, with a photo period of 12-h light and 12-h dark.

Ovicidal Activity

Ovicidal activity was assessed by the slightly modified method of Su and Mulla (1998). The egg raft/eggs of *Cx.quinquefasciatus, An. stephensi* and *Ae.aegypti* were collected from Vector Control Laboratory, Annamalai University, India. The different leaf extract diluted in the appropriate solvent to achieve various concentrations ranging from 50 to 300 ppm. Eggs of these mosquito species (100 nos.) were exposed to each concentrations of leaf extract. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

$$\% \text{ of egg hatchability} = \frac{\text{Number of hatched larvae}}{\text{Total no. of eggs/egg raft}} \times 100$$

Repellent Activity

The repellent study was following the method of World Health Organization (2009). Three-day-old blood-starved female *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* mosquitoes (100) were kept in a net cage (45 cm × 30 cm × 45 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of volunteer, only 25 cm^2 dorsal side of the skin on each arms was exposed and the remaining area covered by rubber gloves. The crude extract was applied at 1.0, 2.5 and 5.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. The time of the test dependent on whether the target mosquitoes day-or night biters. *Ae. aegypti* was tested during the day time from 07.00 to 17.00h, while *Cx. quinquefasciatus* and *An. stephensi* were tested during the night from 19.00 to 05.00h. The control and treated arm were introduced simultaneously in to the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arm in to the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood; making out a 5 minutes protection. The percentage of repellency was calculated by the following formula.

$$\% \text{ Repellency} = [(Ta - Tb)/Ta] \times 100.$$

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

RESULTS

The leaf extract of *C. rottleri* have been studied for use as natural insecticides instead of organic phosphorous materials or other synthetic agents. Results on the ovicidal and repellent effects of leaf extract was reported in the present study, confirm their potential for control of the mosquito populations. Table I shows the mean per cent hatchability of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*. All the five solvent extracts tested, the methanol extract have most promising ovicidal activity. The methanol extract exerted zero hatchability (100% mortality)

at 150 ppm for *Cx. quinquefasciatus, Ae. Aegypti, An. stephensi. Cx. quinquefasciatus* eggs were more susceptible to the *C. rottleri* leaf extract than those of *Ae.aegypti* and *An.stephensi*. The results from the skin repellent activity of *C. rottleri* leaf extract against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* are given in Table 2,3 and4. The repellent activity was very high at the initial stage of exposure. Increase in the exposure period showed reduction in repellent activity and it depends upon the concentration of the extract and density of mosquito. The methanol extract of *C. rottleri* found to be more effective than the other extracts. A higher concentration of 5.0 mg/cm^2 provided 100% protection up to150 min against *Cx. quinquefasciatus* and 150 min against *Ae. aegypti* and *An. stephensi*, respectively. The *C. rottleri* gave the maximum protection time against *Cx. quinquefasciatus* than *Ae. aegypti* and *An. stephensi*. The results clearly show that repellent activity was dose dependent.

Table 1. Ovicidal activity of *Chrozophora rottleri* plant extracts against *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti.*

Mosquito	Name of the solvent	Percentage of egg hatch ability						
		Concentration (ppm)						
		Control	100	150	200	250	300	350
Anopheles stephensi	Hexane	100±0.0	70.2±0.6	61.7±0.8	47.3±0.4	34.8±1.9	18.4±1.2	NH
	Ethyl acetate	100±0.0	62.5±1.7	55.6±1.9	40.2±1.2	29.4±0.8	NH	NH
	Benzene	100±0.0	58.3±0.6	49.2±1.5	36.5±1.8	24.2±1.2	NH	NH
	Chloroform	100±0.0	50.2±1.8	41.8±1.2	29.3±1.5	20.6±0.4	NH	NH
	Methanol	100±0.0	42.8±0.4	34.7±0.6	23.9±1.2	NH	NH	NH
Aedes aegypti	Hexane	100±0.0	80.6±1.5	66.4±1.7	53.8±1.3	40.2±1.2	29.4±0.2	NH
	Ethyl acetate	100±0.0	69.4±0.2	59.8±1.3	46.2±1.8	35.7±1.5	24.6±0.8	NH
	Benzene	100±0.0	64.3±0.6	56.7±0.8	39.5±1.2	28.3±0.4	NH	NH
	Chloroform	100±0.0	60.9±0.8	47.3±0.6	34.8±1.7	23.6±1.2	NH	NH
	Methanol	100±0.0	49.5±1.2	39.2±0.8	27.6±0.2	NH	NH	NH
Culex quinquefasciatus	Hexane	100±0.0	85.2±1.8	73.5±1.7	59.1±1.2	45.6±1.9	34.8±0.4	22.4±1.2
	Ethyl acetate	100±0.0	75.4±1.2	63.8±0.4	50.4±0.6	37.7±0.8	25.3±0.6	NH
	Benzene	100±0.0	69.5±0.4	57.2±1.8	44.9±1.2	32.8±0.6	20.7±1.2	NH
	Chloroform	100±0.0	64.9±1.9	52.3±1.2	39.5±0.2	28.2±1.7	17.6±0.8	NH
	Methanol	100±0.0	56.8±0.6	45.2±0.4	28.7±0.8	19.3±1.2	NH	NH

NH- No hatchability.

Table 2. Repellency of different solvent extracts of *Chrozophora rottleri* against *Culex quinquefasciatus.*

Solvents	Concen-tration (mg/cm^2)	Repellency% ±SD							
		Time of post application (minutes)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	77.4±1.6	66.5±1.2	56.9±1.8
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	72.3±1.9	63.7±1.2
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	79.5±0.6	72.1±1.8
Ethyl acetate	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	83.0±1.2	76.2±0.9	63.7±1.6
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	82.3±1.9	67.5±0.4
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	87.6±1.2	79.3±1.9
Benzene	1.0	100±0.0	100±0.0	100±0.0	100±0.0	82.5±1.3	74.3±1.9	63.8±0.9	51.9±1.2
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.4±1.9	67.5±0.2	58.6±0.4
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	75.2±1.4	69.7±1.9
Chloroform	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	89.3±1.2	78.9±1.6	71.2±0.4
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	85.6±0.4	76.8±1.8
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	92.7±0.2	84.6±0.6
Methanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	92.4±1.2	82.9±1.6	74.2±0.4
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	87.6±1.2	78.5±1.8
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.3±1.9	86.4±1.2

Table 3. Repellency of different solvent extracts of *Chrozophora rottleri* against *Aedes aegypti.*

Solvents	Concen-tration (mg/cm^2)	Repellency% ±SD							
		Time of post application (minutes)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	70.6±0.4	62.8±0.6	51.4±1.2
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	74.8±1.2	69.5±1.2	56.7±1.9
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	77.6±1.9	72.3±1.2
Ethyl acetate	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	80.3±1.2	72.5±1.6	58.2±0.9
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	79.6±0.8	65.3±1.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	86.2±1.9	77.8±1.2
Benzene	1.0	100±0.0	100±0.0	100±0.0	100±0.0	74.2±1.6	66.4±1.2	57.8±0.4	43.1±1.9
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	78.6±1.8	71.5±1.6	62.7±1.9	50.5±1.2
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	80.6±1.2	74.2±0.8	68.3±0.6
Chloroform	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	81.2±0.8	73.6±1.9	60.4±1.2
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	86.7±1.5	78.7±1.2	72.8±0.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	88.4±0.6	81.6±1.8
Methanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	83.2±0.6	78..3±1.5	67.5±1.3
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	88.7±1.2	82.6±1.7	76.4±1.9
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.8±0.6	82.9±1.2

Table 4. Repellency of different solvent extracts of *Chrozophora rottleri* against *Anopheles stephensi.*

Solvents	Concen-tration (mg/cm^2)	Repellency% ±SD							
		Time of post application (minutes)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	67.2±0.4	54.6±1.2	42.3±1.9
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	72.8±1.9	63.9±1.6	48.5±1.2
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	72.5±1.7	67.2±1.9
Ethyl acetate	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	75.2±1.9	66.8±1.2	52.3±0.6
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	80.2±0.6	73.6±1.8	58.4±1.2
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	81.2±0.4	72.8±0.6
Benzene	1.0	100±0.0	100±0.0	100±0.0	100±0.0	70.6±0.9	61.7±1.2	49.8±1.9	37.3±1.2
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	75.2±1.7	65.1±1.6	58.2±1.2	44.7±1.8
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	75.8±1.9	67.3±1.8	53.5±1.2
Chloroform	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	76.4±1.3	66.8±1.2	57.3±1.9
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	82.6±1.8	72.5±0.6	67.3±1.5
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	83.2±1.8	75.6±1.7
Methanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.2±1.9	69.4±1.3	61.3±0.4
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	84.8±1.8	76.3±1.2	68.4±0.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	88.9±1.6	77.3±1.2

DISCUSSION

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia,2001). Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. According to Bowers *et al.* (1995) the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. The mosquitocidal activities of the crude leaf extract results were also comparable with earlier reports. Su and Mulla (1998) reported that the egg rafts aged for 0, 4, 8, 12 and 24 hr were exposed to 10 ppm neem suspensions for 36 hrs and the ovicidal activity was only attained in the egg rafts deposited directly (0 hr old) in neem suspensions, not in those with ages of 4-24 hr. In this study, the exposure period also played a crucial role in causing toxicity. The ovicidal activity of *Moschosma polystachyum* leaf extract against the egg rafts of *Cx. quinquefasciatus* showed 100% mortality at 0-3 h and 3-6 h with concentrations of 125, 150, 175 and 200 mg/l12.

This ovicidal and repellent activity is comparable to previously screened plants in our laboratory using different species of mosquitoes. The leaf extract of *Cassia fistula* with different solvens viz, methanol, benzene and acetone were studied for the larvicidal and repellent activity against *Ae. aegypti*. The 24 h LC_{50} values of the extract against *Ae. aegypti* were observed at 10.69, 18.27 and 23.95 mg/l respectively. The crude extract of *C. fistula* shows significant repellency against *Ae. aegypti* (Govindarajan, 2009); the crude leaf extracts of *Pemphis acidula* were evaluated for larvicidal, ovicidal and repellent activities against *Cx. quinquefasciatus* and *Ae. aegypti*. The LC_{50} values of methanol, benzene, acetone were 10.81, 41.07, 53.22 ppm and 22.10, 43.99, 57.66 ppm, respectively. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *P.acidula* gave 100% protection up to 2.30, 4.00 and 6.45 hrs and 2.45, 4.30 and 7.0 hrs respectively (Samidurai *et al.*, 2009). The leaf extract of *Acalypha indica* with different solvents viz, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal and ovicidal activity against *An. stephensi*. The LC_{50} values are 19.25, 27.76, 23.26 and 15.03 ppm, respectively. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs (Govindarajan *et al.*, 2008); the larvicidal and repellent

activities of *Sida acuta* extract against *Cx. quinquefasciatus, Ae. aegypti* and An. *stephensi*, extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *An. stephensi* for 180 min followed by *Ae. Aegypti* (150 min) and *Cx. quinquefasciatus* (120 min) (Govindarajan, 2010a).

The insect repellent that is widely available is DEET, which has been used worldwide since 1957. DEET-based products include a plasticizer, capable of dissolving watch crystals, the frames of glasses, and certain synthetic fabrics. Continuous application of DEET causes infolding of the epidermis with fewer hairs and a thickened dermis with more vascularity (Al-Sagaff *et al.,* 2001). Venkatachalam and Jebanesan (2001) have also reported that the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Ae. aegypti* activity at 1.0 and 2.5 mg/cm^2 concentrations gave 100% protection up to 2.14±0.16 h and 4.00±0.24 h, respectively, and the total percentage protection was 45.8% at 1.0 mg/cm^2 and 59.0% at 2.5 mg/cm^2 for 10 h. The essential oil of *Zingiber officinalis* showed repellent activity at 4.0 mg/cm^2, which provided 100% protection up to 120 min against *Cx. Quinquefasciatus* (Pushpanathan *et al.,* 2008). In my study the leaf extract did not cause any such of discomfort or skin irritation to the volunteers. The finding of the present investigation revealed that the leaf extract of *C. rottleri* possess ovicidal and skin repellent activity against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*. The biological activity of the plant extract might be due to a variety of compounds in this plant. These compounds may jointly or independently contribute to cause ovicidal and skin repellent activity *against Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*.

CONCLUSION

The present findings suggest that the leaf methanol extract of *C. rottleri* have the potential for use to control mosquitoes. Further studies are in progress to evaluate the effect of purified extract on mosquitocidal activity. The purified plant metabolite of the leaf methanol extracts of *C. rottleri* may be used as environment friendly and sustainable insecticides to combat mosquitoes. In our view, biopesticides from plant origin may contribute to an effective vector control tools. These new agents should preferentially to be applied in integrated control strategies to gain maximum impact on adult mosquito populations.

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