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Research Article

LABORATORY EVALUATION OF *PEDALIUM MUREX* L. EXTRACTS ON THE SOUTH EAST INDIA DISEASE VECTOR MOSQUITOES (DIPTERA: CULICIDAE)

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ABSTRACT

Laboratory activities of crude (hexane, ethyl acetate, chloroform and methanol) extract of *Pedalium murex* have been assessed in the present study. The fourth instars larvae were showing to concentrations (50-250 ppm) of methanol, chloroform, ethyl acetate and hexane leaf extracts of *P. murex* plant. The mortality was evidenced after 24 hrs exposure and LC_{50} and LC_{90} were determined. The ovicidal activity was resolute against different mosquito species to different concentration variety from 60–360 ppm lower than the laboratory condition. The repellent activity of *P. murex* extracts tested at concentration 3.5 mg/cm^2 was appraised in a net cage (45cm × 30cm × 45cm) containing 100 blood ravenous female mosquitoes of *Ae. aegypti* and *Cx. quinquefasciatus.* The current investigation discovered that the LC_{50} values methanol extract of *P. murex* against *Cx. quinquefasciatus* followed by *Ae. aegypti* larvae were 111.66 and 127.08 mg/L respectively. Highest concentrations 300 and 360 ppm of extract exhibited 100% ovicidal activity. It demonstrated that repellency depends on the potency of the 3.5 mg/cm^2 provided 100% protection up to 200 and 240 min against *Cx. quinquefasciatus* and *Ae. aegypti* respectively. The repellent action of methanol extract *P. murex* to be the majority effectual and the maximum activity was observed at 3.5 mg/cm^2 concentration given 100% protection up to 200 and 240 min against *Cx. quinquefasciatus* and *Ae. aegypti*. The results evidently demonstrate that larvicidal, ovicidal and repellent activity was dosage reliant. The highest larvicidal, ovicidal and repellent activity against *Cx. quinquefasciatus* was gated with methanol extract of *P. murex.*

Keywords: *Pedalium murex , Aedes aegypti, Culex quinquefasciatus,* Larvicidal, Ovicidal, Repellent activity.

INTRODUCTION

Mosquitoes are dangerous vectors of deadly pathogens and parasites, which may it as epidemics or pandemics in the increasing world population of human and animals (Mehlhorn *et al.,* 2012; Benelli, 2015). Mosquitoes symbolize a historic danger to human physical condition as of their competence to vector pathogens that reason diseases that make dejected millions of people universal (WHO, 2010). Mosquito-borne diseases intimidate the lives and livelihoods of millions of people worldwide (Townson *et al.,* 2005). Mosquitoes compose a major public health problem as vectors of serious human like dengue fever, Japanese encephalitis, filariasis, malaria, yellow fever and chikungunya cause substantial mortality and morbidity among people living in tropical and subtropical zone (Jang *et al.,* 2002). *Ae. aegypti* L. is commonly identified as a vector for an arbovirus accountable for dengue and chikungunya, which is rife to the Pacific island area, Africa, South Asia, and the Americas. Approximately 40% of the world's population is at the present at hazard of infectivity by dengue virus, it is approximate that amongst this population, 50-100 million are infected once a year, 5,00,000.00 cases being severe. Something like 2.5% of those affected pass away and most of them are children alive in Asia and Latin American countries (WHO, 2009a; WHO, 2012a, b). In stipulations of dengue, 2.5 billion people be alive at hazard of infectivity with one or more of the four serotypes of the virus, which cause an approximate 390 million infections a per year (Bhatt *et al.,* 2013), and the affected area has increased rapidly in the past 30 years (Guzman *et al.,* 2010). *Ae. aegypti* is the very highly anthropophilic species. It has modified to the built-up

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environment and uses synthetic containers for oviposition and development of the aquatic phases of its live cycle.Virus transmission to humans comes about through the bites of impure female mosquitoes, which are day-time feeders (Eldridge, 2005; Klowden, 2007). In 2014, estimate is impure with lymphatic filariasis parasites and more than 20 per cent of the world population is at hazard of obtaining filarial infection. In India, it is estimated that about 554.2 million people are at hazard of lymphatic filariasis illness in 243 districts (Ghosh *et al.*, 2013). Worldwide, 25 million men duffer with genital disease and over 15 million people are troubled with lymphoedema (WHO, 2014).

Pedalium murex is a little herb disseminated in India, Ceylon, tropical Africa and Mexico. *P. murex* generally called Gukhru in India belong to the family Pedaliaceae, is disseminated in the coastal of south India. Many Indian medicinal plants demonstrate beneficial special effects against mosquito diseases. Ayuerveda, an ancient system of the Indian medicine, several plants that are useful in the treatment of mosquito diseases. *P. murex* consists of ash, small amount of resin, greenish fatty oil and alkaloids. The fruits ethanolic extract has nephro protective activity (Shelke *et al.*, 2009). Fruit contains a mucilaginous alkaloids, resin, fat and gum. Cukaric acid, caffeic acid, ferulic acid, daucosterol, vanilic acid, ursolic acid, hepatatriacontonic acid and sitosterol; Stem contains a saponins, herman, phytosterols, carbohydrares and tannins; Leaves contains a flavonoids, steroids, alkaloids, saponins, proteins and resins were isolated from this plant of *P. murex* were reported. *P. murex* plant parts are habitually and extensively make use of for the treatment of a variety of ailments of humans and stock *P. murex* leaves have been used in completely curing gonorrhea and dysurea (Rajashekar *et al.*, 2012). It has also been assessed for its antipyretic activities, analgesic, and antioxidant activity (Thangadurai Chitra *et al.*, 2013). Mosquitocidal properties of medicinal plant are being ever more reported from dissimilar parts of the world.

Laboratory effects were carried out in the current study to trial the potentiality of the leaf extracts of *P. murex* in controlling the *Ae. aegypti* and *Cx. quinquefasciatus*.

MATERIAL AND METHODS

Collection of medicinal plant

Completely developed leaves of *P. murex* were collected from Velankanni (L. 10º40’49.09”N and L. 79º50’58.91”E), Nagapattinam District, Tamil Nadu, India, and washed methodically, blotted and shade dried. It was genuine by plant taxonomist from the Department of Botany, Annamalai University. A coupon specimen is the deposited at the herbarium of plant Phytochemistry division, Department of Zoology, Annamalai University, Tamil Nadu, India.

Preparation of extracts

The healthy leaves were cleaned with sterile distilled water, shade dried, and thinly ground. the finely ground leaf powder (500 g/ solvent) was extracted with hexane, ethyl acetate, chloroform and methanol using Soxhlet extraction apparatus, and the extraction was continued till visibly no further extraction is possible (by observing the colour of the extracted portion). The extracts are detached using a rotary vacuum evaporator to gather the crude extract and stored at 4ºC. Normal stock solutions were readied at 1% by dissolving the remainders in ethanol. Starting this stock solution, various concentrations were readied and this solution is used for larvicidal activity.

Mosquitoes rearing

The mosquitoes, *Ae. aegypti* and *Cx. quinquefasciatus* were procured from the Centre for Research in Medical Entomology (ICMR), Viruddhachalam, nurtured in the laboratory, Department of Zoology, Annamalai University. The larvae were feed on dog biscuits and yeast powder in the 3:1 ratio. Adults were giving with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28±2) ºC, 70%-85% Relative Humidity (RH), with a photo period of 14 h light, 10 h dark.

Larvicidal activity

The larvicidal activity of plant crude extract was assessed as per the protocol before described by WHO (2005). As of the stock solution, six dissimilar test concentrations (50, 100, 150, 200, and 250 ppm) were readied and experimental against the freshly moulted (0–6 h) IV instar larvae of *A. aegypti* and *C. quinquefasciatus*. The test medium (500 ml plastic cups) was prepared by adding 1 ml of suitable dilution of test concentrations and varied with 249 ml of dechlorinated water to make up 250 ml of experiment solution. The larvae were fed with dry yeast powder on the water surface (50 mg/l). The control test was also run parallel with each replicate. For each test, five replicates were maintained at a time. A smallest amount of 25 larvae per concentration was utilized for all the experiments. The larval mortality was experiential and evidenced after 24 h post-treatment. Percent mortality was prospered for control mortality by means of probit analysis (Abbot, 1925).

Ovicidal activity

Valuation of the plant extracts for ovicidal activity was carried out by following the method of Su and Mulla (1998). Eggs were showing to dissimilar concentrations varying from 60 to 360 ppm. The most wanted concentrations of the test solutions were reached by adding 1.0 ml of an appropriate stock solution to 99 ml of tap water. Each eggs raft containing 100 eggs of *C. quinquefasciatus* and hundred eggs of *A. aegypti* were exposed to each dose of extract for 48hr. counting of eggs was done under a microscope. DSMO served as control. Four replicates for each concentration were maintained. After 24 hrs of incubation, the egg rafts or eggs exposed to each concentration were transferred to distilled water cups. The hatch rates were calculated by the following formula.

$$\% \text{ Mortality} = \frac{\text{Mortality at treatment-Mortality at control}}{\text{100-Mortality at control}} \times 100.$$

Repellent activity

The repellency of the *P. murex* plant extracts tested against *Ae. aegypti* and *Cx. quinquefasciatus* were evaluated by using the percentage of protection in relation to dose method was used WHO (2009b). Three-day-old blood-starved female *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes (100) were kept in a net cage (45cm × 30cm × 45cm). The volunteer had no contact with lotion, 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 extracts were applied at 3.5 mg/cm^2 separately in the exposed area of the fore arm. The time of the test dependent on whether are the target mosquitoes day or night biters. *Cx. quinquefasciatus* are testing during the night time from 20:00 to 4:00, while *Ae. aegypti* was tested during the day time 8:00 to 16:00. The control and treated arm were introduced simultaneously in to 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.

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

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

Statistical Analysis

Mortality was recorded after 24 hrs of exposure. Values obtained were subjected to log probit regression analysis and chi-squire to obtain LC_{50} and LC_{95} values with 95% confidence limit (Finney, 1971).

RESULTS

The mosquito activity of crude plant extracts is often attributed to the complex mixture of active compounds. In the current study, the toxicity of different solvent extract of *P. murex* was experimented against *Ae. aegypti* and *Cx. quinquefasciatus* were showed in Table 1 and 2. The LC_{50} value of hexane, ethyl acetate, chloroform and methanol extracts of *P. murex* against *Ae. aegypti* were 235.52, 173.58, 221.07, 127.08 ppm and LC_{95} value were 377.72, 288.11, 364.86, 226.00 ppm (Figure 1); LC_{50} value of *Cx. quinquefasciatus* were 181.77, 150.41, 165.43, 111.66 ppm and LC_{95} value were 301.05, 253.44, 278.32, 200.01 ppm, respectively (Figure 2). The denote percent hatchability of *Ae. aegypti* and *Cx. quinquefasciatus* (Table 3). The methanol and ethyl acetate extract establish to be extra effective than the other extract against *Cx. quinquefasciatus* eggs, the 100% mortality at 300 and 360 ppm. The repellent activity of *P. murex* was established to be the majority effective for repellent activity against *Cx. quinquefasciatus* followed by *Ae. aegypti* and a superior concentration of 3.5 mg/cm^2 provided 100% protection up to 200 and 240 min against *Cx. quinquefasciatus* and *Ae. aegypti* (Table 4). From the result it can be accomplished the extracts of *P. murex* as an outstanding possible agent for controlling chosen mosquitoes species.

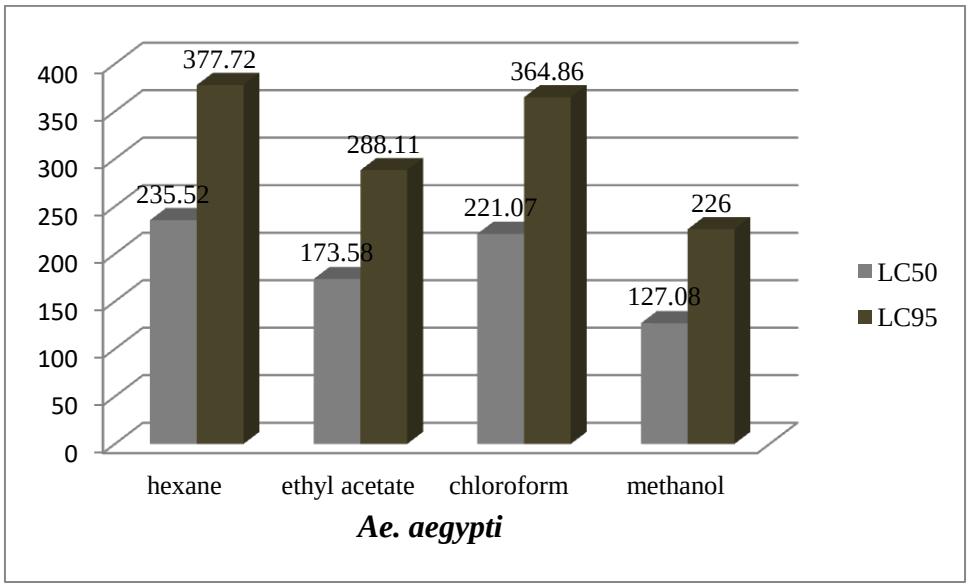

Figure 1. Graph showing the LC_{50} and LC_{95} values of *A. aegypti*.

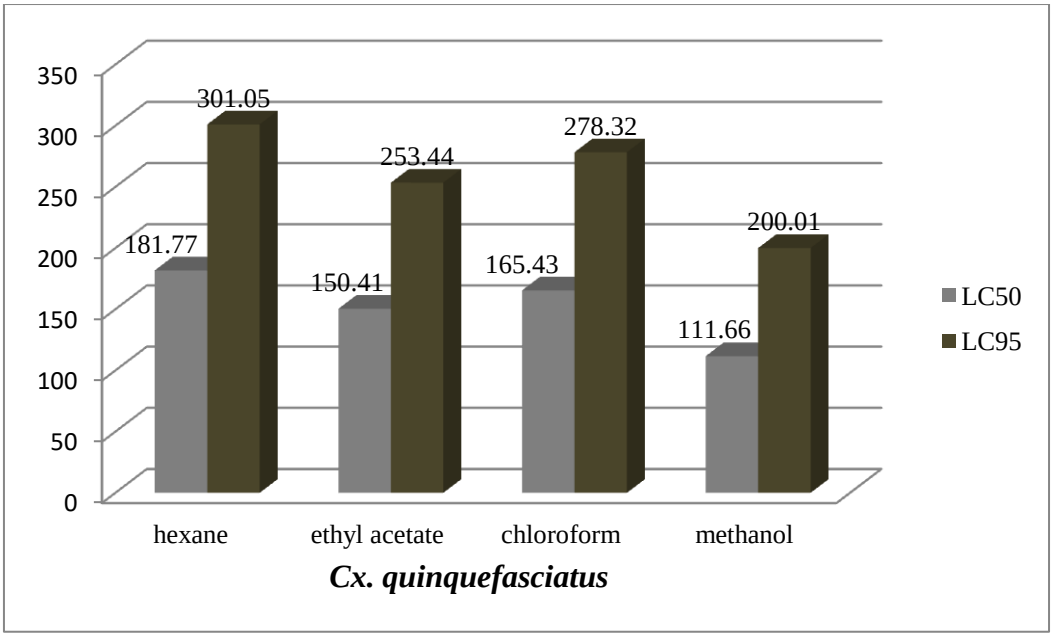

Figure 2. Graph showing the LC_{50} and LC_{95} values of *C. quinquefasciatus.*

Table 1. Percentage mortality of mosquito larvae of *Ae. aegypti* and *Cx. quinquefasciatus* exposed to different concentrations of different solvent leaf extracts of *P. murex.*

Extracts	Concentration (ppm)	*Ae. aegypti*	*Cx. quinquefasciatus*
		% of mortality±SD[a]	% of mortality±SD[a]
Hexane	Control	0.00±0.0	0.00±0.0
	50	5.2 ±2.1	8.2±2.1
	100	11.6±2.5	20.2±2.8
	150	19.8±1.7	36.2±2.4
	200	34.6±2.0	52.8±3.5
	250	58.4±3.3	80.2±2.4
Ethyl acetate	Control	0.00±0.0	0.00±0.0
	50	8.8±2.7	12.6±1.5
	100	21.6±3.2	25.8±2.1
	150	38.6±2.0	45.2±1.3
	200	57.8±2.2	74.8±1.9
	250	83.2±2.7	90.2±2.8
Chloroform	Control	0.00±0.0	0.00±0.0
	50	7.4±2.7	10.6±1.9
	100	15.8±1.7	23.2±3.4
	150	22.8±2.7	42.4±2.6
	200	38.4±2.6	60.2±1.7
	250	64.6±1.5	86.8±2.3
Methanol	Control	0.00±0.0	0.00±0.0
	50	18.4±2.1	22.2±2.4
	100	35.8±2.6	41.8±3.0
	150	59.2±2.5	65.6±1.8
	200	78.4±2.8	89.8±2.0
	250	98.2±1.4	100.0±0.0

SD = Standard deviation, [a] Values are mean ± SD of four replicates.

Table 2. LC_{50}, LC_{95}, slope and regression of *P. murex* different extracts tested against selected mosquitoes.

Extracts	Species	LC_{50} (mg/L)	LCL-UCL	LC_{95} (mg/L)	LCL-UCL	Slope	χ^2 (*df*)
Hexane	*Ae. aegypti*	235.52	218.13 – 259.31	377.72	338.55 – 437.72	2.379714	1.336 (3)
	Cx. quinquefasciatus	181.77	170.21 – 194.67	301.05	277.47 – 333.72	2.974411	1.773 (3)
Ethyl acetate	*Ae. aegypti*	173.58	162.54 – 185.50	288.11	266.63 – 317.41	3.098786	1.266 (3)
	Cx. quinquefasciatus	150.41	140.23 – 160.62	253.44	236.39 – 275.84	3.39369	1.542 (3)
Chroloform	*Ae. aegypti*	221.07	205.03 – 242.15	364.86	328.02 – 420.44	2.369891	2.602 (3)
	Cx. quinquefasciatus	165.43	154.59 – 176.85	278.32	257.96 – 305.86	3.136601	2.245 (3)
Methanol	*Ae. aegypti*	127.08	116.71 – 136.91	226.00	210.70 – 245.95	3.841205	4.765 (3)
	Cx. quinquefasciatus	111.66	101.67 – 120.90	200.01	186.65 – 217.23	3.191929	4.732 (3)

Values represent mean of five replications. Mortality of the after 24 h of exposure period LC_{50}= Lethal Concentration brings out 50% mortality and LC_{90}= Lethal Concentration brings out 90% mortality. LCL= Lower Confidence Limit, UCL= Upper Confidence Limit, χ^2 = Chi-squire, Significant at $p<0.05$.

Table 3. Ovicidal activity of *P. murex* different extracts tested against selected mosquitoes.

Species	Solvents	Ovicidal activity					
		Concentrations tested (ppm)					
		60	120	180	240	300	360
Ae. aegypti	Hexane	70.8±2.2	65.6±1.9	57.8±3.0	53.2±2.5	48.6±2.1	42.4±2.9
Cx. quinquefasciatus		58.3±1.5	46.5±1.4	37.8±1.8	32.1±1.2	23.6±1.7	18.6±1.6
Ae. aegypti	Ethyl acetate	57.8±1.9	48.2±2.5	36.2±1.4	30.2±2.1	24.6±2.3	17.8±1.9
Cx. quinquefasciatus		46.1±1.3	35.4±1.8	24.9±1.6	19.3±1.7	12.8±1.6	NH
Ae. aegypti	Chloroform	59.4±1.8	46.2±1.0	37.4±1.8	31.2±2.1	26.4±1.8	20.2±2.1
Cx. quinquefasciatus		50.4±2.3	36.2±1.7	27.4±1.5	24.2±1.6	13.4±1.1	9.6±0.5
Ae. aegypti	Methanol	51.8±2.1	43.4±2.0	31.2±2.1	22.6±0.8	16.2±1.0	12.2±2.1
Cx. quinquefasciatus		41.9±1.2	31.7±1.6	21.4±1.4	12.2±2.7	NH	NH

Control- Nil mortality, Values represent mean ± S.D. of five replications. Significant at $p<0.05$.

Table 4. Repellent activity of the *P. murex* extracts against *Ae. aegypti* and *Cx. quinquefasciatus* at 3.5 mg/cm^2.

Species	Extracts	% of repellency					
		Time post application of repellent (min)					
		40	80	120	160	200	240
A. aegypti	Hexane	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.6±1.6
	Ethyl acetate	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
	Chloroform	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
	Methanol	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
Cx.quinquefasciatus	Hexane	100±0.0	100±0.0	100±0.0	100±0.0	84.6±2.7	76.6±2.6
	Ethyl acetate	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.4±1.9
	Chloroform	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93.2±1.4
	Methanol	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	98.7±2.6

Value represents mean ±S.D. of five replications.

DISCUSSION

Phyto-chemicals may dish up as appropriate alternatives to man-made insecticides in the upcoming as these are comparatively safe, low-cost and are willingly available in a lot of parts of the world. Dissimilar parts of plants include a complex of chemicals with only one of its kind biological activity which is idea to be due to toxins and secondary metabolites, which perform as Mosquitocidal agents. In recent studies on the anti-bacterial, anti-microbial, anti-oxidant, aphrodisiac, anti-hyperlipidemic, nephroprotector activities from the *P. murex* fruits extract (Elumalai *et al.*, 2011). Antimicrobial activity was practical in *P. murex* root methanolic extract against gram positive bacteria, Streptococcus pyogenes and Enterococcus faecalis than the negative bacteria (Muruganantham, 2011). Anandanayaki and Uma (2014) the reported that the alcoholic extract possess good antimicrobial activity against selected test bacteria and fungi. Murugesan Sakthivadivel *et al.,* (2015) results show the LC_{50} and LC_{90} values are crude aerial extracts (petroleum ether, chloroform and acetone) of *Hyptis suaveolens* against *Cx. quinquefasciatus* were 493.44, 625.97, 485.61 and 875.75, 1032.88, 840.79 mg/L, respectively. Deepa *et al.,* (2014) the investigated that the mosquitocidal (larvicidal, ovicidal and repellent) activity of *Polygala arvensis* benzene and methanol extracts tested against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* with maximum LC_{50} and LC_{90} values of methanol extract of *Polygala arvensis* were 58.21, 46.37 and 42.68 ppm; 208.45, 189.82 and 130.44 ppm, respectively. The maximum ovicidal activity of methanol extracts against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* at 200 ppm concentration. The highest repellent activity of methanol extracts provided 100% protection against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* for 280 minutes. Kamakshi *et al.,* (2015) investigated that the highest ocividal action was *Cereus hildmannianus* petroleum ether extract with 52.8% at 1000 mg/L. The uppermost repellent activity of petroleum ether extract demonstrated protection time of 137 minutes at 5.0 mg/cm^2 against *Ae. aegypti*. Appadurai Daniel *et al.,* (2013) reported the ovicidal and oviposition deterrent activity of five plants, *Aegle marmelos*, *Limonia acidissima*, *Sphaeranthus indicus*, *Sphaeranthus amaranthaides* and *Chromolaena odorata* extracts (hexane, ethyl acetate and methanol) against *Ae. aegypti* and *Cx. quinquefasciatus*. Among the various extracts of the plants show the hexane extract of *Limonia acidissima* evidenced the highest ovicidal test of 79.2% and 60% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*. Among the *Aegle marmelos*, *Limonia acidissima*, *Sphaeranthus indicus*, *Sphaeranthus amaranthaides* and *Chromolaena odorata* extract showed, the hexane extract of *Limonia acidissima* noted the 100% oviposition deterrent activity at experimented concentrations against *Cx. quinquefasciatus* and *Ae. aegypti* adult females. Raveen *et al.,* (2014) statement that the larvicidal activity of *Nerium oleander* hexane and aqueous extracts against *Cx. quinquefasciatus*. LC_{50} values are hexane extract of 102.54 ppm at 24h and 61.11 ppm at 48h; aqueous extract of 2758.87 ppm at 24h and 168.84 ppm at 48h. LC_{90} values are hexane extract of 7731.80 ppm at 24h and 4916.44 ppm at 48h; aqueous extract of 11011.93 ppm at 24h and 7882.93 ppm at 48h, respectively.

CONCLUSION

The conclusion, the methanol extract of *P. murex* was the most used as a treatment of larvicidal, ovicidal and repellent activity against *Ae. aegypti* and *Cx. quinquefasciatus* mosquito vectors. Supported on these results, the methanol extract of *P. murex* can be used in vector mosquito control and possibly further searched to isolate the active component responsible for the bioactivities.

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