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Phytochemical characteristics and the effect of methanolic extract of *Erythrina variegata* in the control of *Aedes*, *Anopheles* and *Culex* mosquito

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Abstract

Plant-based mosquitocidal activity has been in use control products generations in traditional practice as a personal protection against mosquitoes bite. . Commercial mosquito activities containing plant-based ingredients have gained increasing popularity and are safe when compared to synthetic larvicidal, ovicidal and repellents. The present investigation discovered the larvicidal, ovicidal and repellent activity of methanol extract of *E. variegata*. Twenty five III instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were exposed to various concentrations (50-250 ppm) in the laboratory by using the standard protocol described by WHO (2005). The ovicidal activity was determined against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* mosquito to various concentrations ranging from 70-350 ppm under the laboratory conditions. The repellent activity of *E. variegata* chemical compositions tested at concentrations of 5.0 mg/cm^2 and evaluated in a net cage (45×45× 40cm) containing 100 blood starved female mosquitoes of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* using the protocol of WHO (1996). LC_{50} and LC_{90} values of the methanol extract of *E. varegata* were found to be 121.70, 129.29, 134.64 and 217.71, 232.55, 267.40 mg/L against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, respectively. Ovicidal activity of methanol extract was assessed by assessing the egg hatchings. Highest concentrations 240 and 300 ppm of the extract exhibited 100% ovicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The repellent activity of methanol extract *E. variegata* was found to be the best and therefore the activity was ascertained at 5.0 mg/cm^2 concentration provided 100% protection up to 90, 120 and 150 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The compounds present in the leaves of *E. variegata* were analysed and determined by using GC-MS . The results clearly showed the presence of active compounds in leaves of *E. variegate*, and the bioactive compounds would pave way for the discovery of new drugs.

Keywords: *Erythrina variegata*, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, larvicidal, ovicidal and repellent activity.

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INTRODUCTION

Mosquitoes are dangerous vectors of deadly pathogens and parasites, which are responsible for the incidences of epidemics or pandemics within the increasing world population of humans and animals (Mehlhorn *et al.*, 2012; Benelli, 2015; Baranitharan *et al.*, 2017). *A. aegypti* L. is mostly called a vector for an arborvirus and answerable for infectious disease the chikungunya that is endemic to South Asia, the Pacific island space, Africa, and also the Americas. In terms of dengue, 2.5 billion folks live in danger of infection with one or a lot of the four serotypes of the virus, that cause an calculable 390 million infections a year (Bhatt *et al.*, 2013; Baranitharan and Dhanasekaran, 2014), and also the affected space has exaggerated speedily within the past 30 years (Guzman *et al.*, 2010; Baranitharan *et al.*, 2016). Chikungunya is unfolding Tiger mosquito, *A. albopictus*. Chikungunya occurrences in Europe have drawn the attention of the western world to the current disease; unfolded by the Asian tiger mosquito, *A. albopictus* (Carrieri *et al.*, 2011; Abramides *et al.*, 2013; Baranitharan *et al.*, 2014; Rogers *et al.*, 2014).

Malaria is one in all the grave scourges inflicted upon human beings. It causes human mortality and morbidity alongside giant economic loss. Roughly all tropical regions of the planet area unit experience the deadly diseases, , malaria and India is not any omission. Malaria afflicts 2020 million in 107 countries and territories placed within the tropical and semi-tropic regions in one year (Panneerselvam *et al.*, 2013; Baranitharan *et al.*, 2015). According to the recent estimates, there have been 198 million cases of malaria in 2013 and a calculable 584,000 deaths. Most deaths occur among youngsters living in the continent (WHO,

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Malaria Fact sheet, 2014). The dipteran *Cx. quinquefasciatus* is a crucial for inflicting Filariasis, West Nile Virus, Avion malaria and St. louis encephalitis (Gokulakrishnan *et al.*, 2016).

Cx. quinquefasciatus, besides known as the southern house dipteran, is extensively studied because it transmits crucial diseases (Samba Shiva Daravath and Siddaiah Reddy Naik, 2015). The estimate made in 2014 is incomplete and impure with lymphatic filariasis parasites and over 20 per cent of the planet population is at hazard of getting roundworm infection. It is reported that in Asian countries there are about 554.2 million folks are at hazard of human disease (Ghosh *et al.*, 2013). Worldwide study reported that about twenty five million men clumsy person with sex organ sickness and over 15 million folks are afflicted with lymphoedema (WHO, Lymphatic filariasis Fact sheet, 2014).

Organophosphates are the chemical pesticides frequently used for the control and management of mosquitoes. However, their non target effect and the harmful effects on the health and environment cannot be ignored. Besides, the mosquitoes also develop resistance against the chemical pesticides. Hence it has often been emphasized that there should be a search for an alternative eco-friendly method of mosquito management. Application of natural products including plant extracts has often been recognized as viable alternative for the use of chemical pesticides as they possess anti-larvicidal, ovicidal and repellent properties (Govindaraju *et al.*, 2015). It has been reported that there are several plants contain chemicals that are useful for the management of insects and are helpful for field applications in mosquito management programmes (Patil *et al.*, 2014). Extracts of several plants have been found effective against larvae of mosquitoes (Dhanasekaran *et al.*, 2013; Elumalai *et al.*, 2013; Baranitharan *et al.*, 2016; Murugan *et al.*, 2015).

Erythrina variegata is a species of *Erythrina* native to the tropical and subtropical regions of eastern Africa, the Indian Subcontinent, northern Australia and the islands of the Indian Ocean and the western Pacific Ocean east to Fiji. It is a thorny deciduous tree growing to 27 m (89 ft) tall. The leaves are pinnate with a 20 cm petiole and three leaflets, each leaflet up to 20 cm long and broad. It has dense clusters of scarlet or crimson flowers and black seeds. It is valued as an ornamental tree. Several cultivars have been selected, including 'Alba' with white flowers. In Vietnam, the leaves are used to wrap fermented meat (Vietnamese: nem). In Siddha medicine it is used especially for menstrual disorders and fissures at penis tip (Rahman *et al.*, 2010). The leaves, bark and root are used in India for the treatment of various diseases, and *E. variegata* species shows anti-osteoporotic (Augustine *et al.*, 2001), cytotoxic (Palanivelu and Jesupillai, 2009), anthelmintic (Sakat Sachin and Juvekar, 2009), antiulcer (Jesupillai *et al.*, 2008), diuretic (Runia and Mohammad, 2006), analgesic (Chatterjee and Gurman, 1981), cardiovascular effect, respiratory effect (Saraswathy *et al.*, 2008) and antioxidant activity (Irfan and Atiya, 2005).

MATERIALS AND METHODS

Sample collection

Young leaves of *E. variegata* were collected from Velankanni (10°40'49.09"N and 79°50'58.91"E), Nagapattinam District, Tamil Nadu in India. Plant is legitimately validated in the Department of Botany, Poompuhar college.

Preparation of plant extracts

The dried leaves (100g) were powdered by using electrical stainless-steel liquidizer, and extracted consecutively with methanol, ethanol, chloroform and acetone using Soxhlet apparatus. The extract was exposed to reduced pressure 22–26 mmHg at 45°C by 'Rotavapour' and therefore the residue obtained was held at 4°C.The condensed crude leaves extract was used for the investigation of larvicidal, ovicidal and repellent activities.

Test mosquitoes

All tests were applied against laboratory reared vector mosquitoes viz., *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* freed from exposure to pesticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 25-29 °C and 80-90 buddy wetness within the insectoriums. Larvae were exhausted larval food (powdered dog biscuit and yeast within the quantitative relation of 3:1) and adult mosquitoes on 10 blood sugar resolution. Female person mosquitoes were sporadically blood-fed on restrained unusual person mice for egg production.

Larvicidal activity

Larvicidal activity of the crude *E. variegata* concentrates were assessed according to the convention of WHO (2005). In view of the wide range and thin range tests, all concentrates from 50 to 250 ppm were readied and they were tried against the newly shed (0-6 shrs) third instar hatchlings of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The plant extracts were disintegrated in 1 ml DMSO (Dimethyl sulfoxide) and afterward diluted in 249 ml of dechlorinated faucet water. The control was readied utilizing 1ml of DMSO as a part of 249 ml of dechlorinated water. The hatchlings of test species (25) were collected in 250 ml plastic glass containing 250 ml of fluid medium (249 ml of dechlorinated water + 1ml of Dimethyl Sulfoxide) and the required measure of compound syntheses was included. The larval mortality was observed and

recorded after 24 h of post treatment. For every examination, five recreates were kept up at once. Per cent mortality was determined using control (Abbott, 1925).

Ovicidal activity

Evaluation of the plant extract for ovicidal activity was made following the method of Su and Mulla (1998). Eggs were exposed to different concentrations starting from 70 to 350 ppm. The required concentrations were achieved by adding 1.0 cubic centimeter of an acceptable stock to 99 cubic centimeter of water. Every egg raft containing 100 eggs of *Cx. quinquefasciatus* and hundred eggs of *Ae. Aegypti* and *An. Stephensi* each were exposed to every dose of extract for 48hr. Count of eggs was done beneath a magnifier. DSMO served as management. Four replicates for every concentration were maintained. After twenty four hours of incubation, the egg rafts or eggs exposed to every concentration were transferred to H_2O cups. The hatch rates were calculated by using 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 *E. variegata* plant crude extract against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* was evaluated by victimization and the minutes of protection in respect to dose technique was made as proposed by World Health Organization (2009). Three-day-old blood- starved feminine *An. stephensi* and *Cx. quinquefasciatus* mosquitoes (100) were unbroken in a web 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, solely 25 cm^2 dorsal facet of the skin on every arms was exposed and therefore the remaining space lined by rubber gloves. The crude extracts were applied at 3.5 mg/cm^2 on an individual basis within the exposed space of the fore arm. The time of observation was not obsessed with whether or not the target mosquitoes were day or night biters. *An. stephensi* and *Cx. quinquefasciatus* in an unit area were tested throughout the dark from 20:00 to 4:00, while *A. aegypti* was tested throughout the day time, 8:00 to 16:00. The management and treated arm were introduced at the same time in to the experimental cages, and the mosquitoes were activated. Every observation at each concentration was for five times. The observation was made at every concentration by inserting the treated and management arm in to an equivalent cage for one full minute at an interval of 5 minutes. The mosquitoes that landed on the hand were recorded and so jolted off before uptake any blood; creating out a five minutes protection. The proportion of repellency was calculated by the following formula.

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

Where T_a is the quantity of mosquitoes in the control gathering and T_b is the quantity of mosquitoes in the treated gathering.

Gas chromatography analysis

Examination was carried on GC Clarus 500 Perkin Elmer equipment with a mass detector Turbo mass gold-Perkin Elmer and Turbomass 5.2 software. Helium at a stream rate of 1.0 ml min^{-1} and 8 psi channel weight was utilized as a bearer gas. Oven temperature modified up to 200 °C at the rate of 5 °C/min^{-9} min hold. The injector temperature 250 °C and indicator temperatures were kept up at 200 °C and 280 °C, respectively. The sample (2 il) was infused with 10:1 split proportion.

Gas chromatography – mass spectrometry analysis

Gas Chromatography – Mass Spectroscopic (GC-MS) test was performed on a mass detector Turbo mass gold-Perkin Elmer particular identifier and an Elite-5MS (5% Diphenyl/95% Dimethyl poly siloxane) slender segment. The stove temperature was customized from 50 to 280 °C at the rate of 5 °C min^{-9} and commend at this temperature for 36 min. The delta and interface temperatures were 200 °C and 280 °C, individually. The transporter gas was helium at a stream rate of 1.0 ml min^{-1} (consistent stream). The sample (2µl) was infused with a split of 10:1. Electron sway mass spectrometry was conveyed at 70 eV. Particle source and four fold temperature were kept up at 250 °C and 150 °C separately (Kumaravel *et al.*, 2010).

Identification of components

Interpretation of mass spectrum of GC-MS was conducted using the databases of IIT, Chennai version (NIST08s) WILEY8, FAME. The spectrum of the unknown components was compared with the known components stored in the NIST08, WILEY8, FAME library. The name, molecular weight and structure of the components of the test materials were ascertained.

Statistical analysis

The examination program probit (Finney, 1971) was utilized for the determination of LC_{50}, LC_{90} and different insights at mean, standard deviation, chi-square qualities were figured utilizing the SPSS 16.0 programming.

RESULTS

The larvicidal activity of crude methanol, chloroform, ethyl acetate and hexane solvent extracts of *E. variegata* against *Ae. Aegypti, An. stephensi* and *Cx. Quinquefasciatus* was recorded. The methanol extract of *E. variegata* reported in the present study showed the mosquitocidal properties of the plant suggest that

it could be used in mosquito population control. The methanol extracts of *E. variegata* showed larval mortality. *Cx. quinquefasciatus* was more vulnerable followed by *An. stephensi* and *Ae. aegypti*. The methanol extract of *E. variegata* exhibited the maximum larvicidal activity with LC_{50} and LC_{90} values of 121.70 and 217.71 mg/L against the larvae of *Cx. quinquefasciatus*, followed by, the ethanol, chloroform and acetone extract of *E. variegata* with LC_{50} and LC_{90} values of 132.56, 142.05, 170.97 and 236.13, 242.89, 272.59 mg/L respectively. The methanol, ethyl acetate, chloroform and hexane extracts of *E. variegata* against *An. stephensi* showed LC_{50} and LC_{90} values as 129.29, 138.40, 151.06 and 180.84, and 232.55, 238.93, 256.75 and 278.31 mg/L respectively, but against *Ae. aegypti* they were 134.64, 156.12, 172.50 and 194.87, and 267.40, 273.94, 284.53 and 292.24 mg/L, respectively (Table 1 and Fig. 1). Among the extracts tested for ovicidal activity against *An. stephensi* and *Cx. quinquefasciatus*, the methanol extract of *E. variegata* exerted 100% mortality (*i.e.*, no hatchability was recorded) at 240 and 300 ppm, respectively (Table 2). The repellent action of the *E. variegata* extract showed important repellent activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. It showed that repellency depends on the potency of the 5.0 mg/cm² provided 100% protection up to 120 and 150 min against *An. stephensi* and *Cx. quinquefasciatus*, respectively (Table 3). Hence, it was fractioned using TLC with varying solvent systems. Ethyl acetate: ethanol (2:8) gave 2 and 4 fraction, two fractions have been obtained in ethyl acetate: ethanol (1.5:8.5), three fractions were obtained in ethyl acetate: ethanol (1:9) and the maximum of five fractions were obtained in ethyl acetate: ethanol (0.5:9.5). Further, the peaks of compounds and retention times of methanol extract of *E. variegata* identified by GC-MS analysis have been reported in chromatogram (Fig. 2). The compositions of the leaves of *E. variegata*, with their retention time (RT), and area (%) are presented in Table 4. Qualitative analysis of the leaf extracts of *E. variegata*, showed 6 compounds and identified. Among all, 12-Octadecenoic acid, methyl ester, Methyl hexadecanoate, 4,8-Decadienal, 5,9-dimethyl-, 6-Methyl-á-ionone, 3,10b-Ethanonaphtho [1,2-c] pyran,1,3,4,6,6a,7,8,9,10,1a, 10b- undecahydro-8á-[dimethoxymethyl] -3-methoxy-7-cyanomethyl-4,4,8-trimethyl-, and Nonadecanoic acid, 18-oxo-, methyl ester compounds showed prominent occurrence.

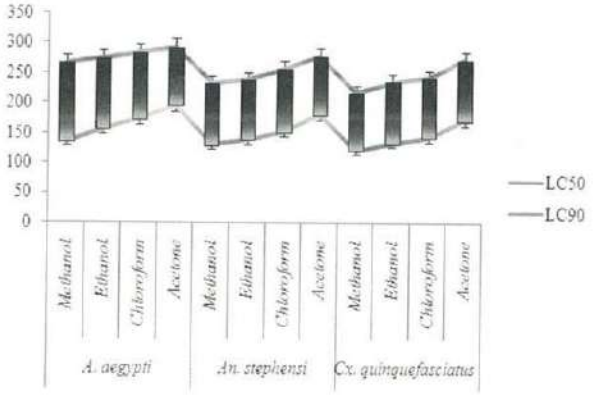

Fig. 1. LC_{50} and LC_{90} values of *E. variegata* extract against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*

DISCUSSION

Plants are well known sources of bioactive compounds that can be utilized to develop environmentally safe

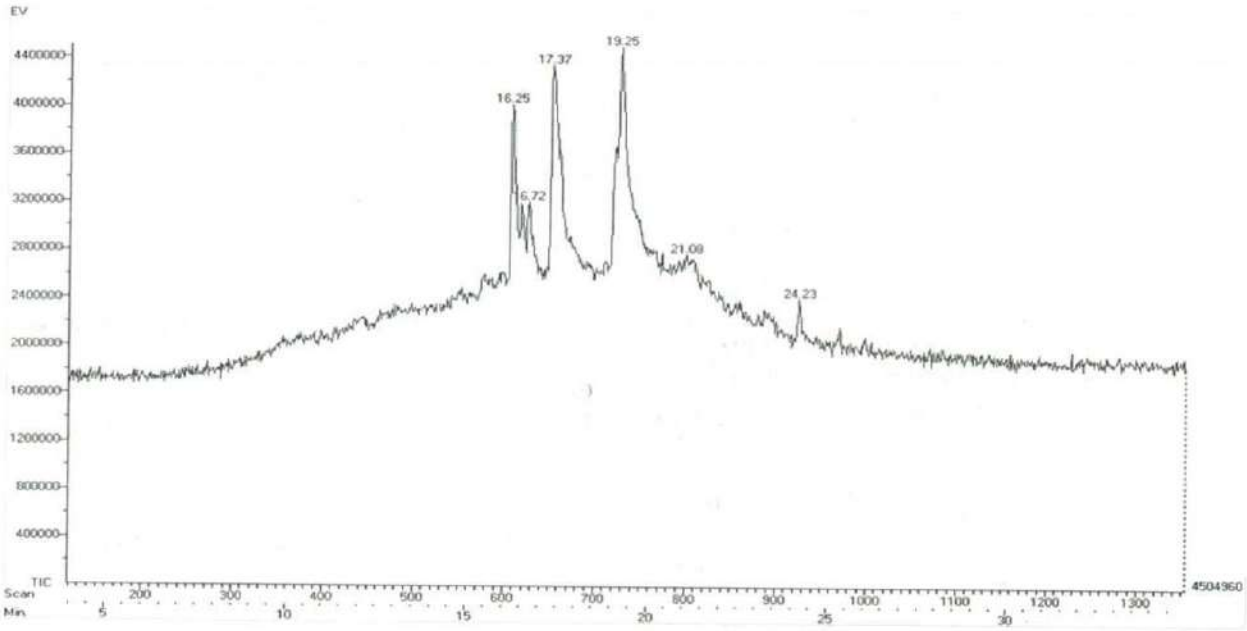

Fig. 2. GC-MS Chromatogram of methanol leaf extract of *E. variegata*

vector and pest managing agents. Medicinal plant extracts are rising as possible mosquito control agents, with low cost, easy to administer and hazard free properties. Simple medicinal plant extracts have been used as insecticides in many countries. The results of the present study revealed the different larvicidal, ovicidal and repellent activity of hexane, ethyl acetate, chloroform and methanol extracts against of *E. variegate, An. stephensi* and *Cx. quinquefasciatus*. The highest larvicidal, ovicidal and repellent activities were recorded with methanol extract of *E. variegata* tested against *Cx. quinquefasciatus*. The results of the present study are comparable with earlier reports with the ethanol fractions of *Eichhornia crassipes* which

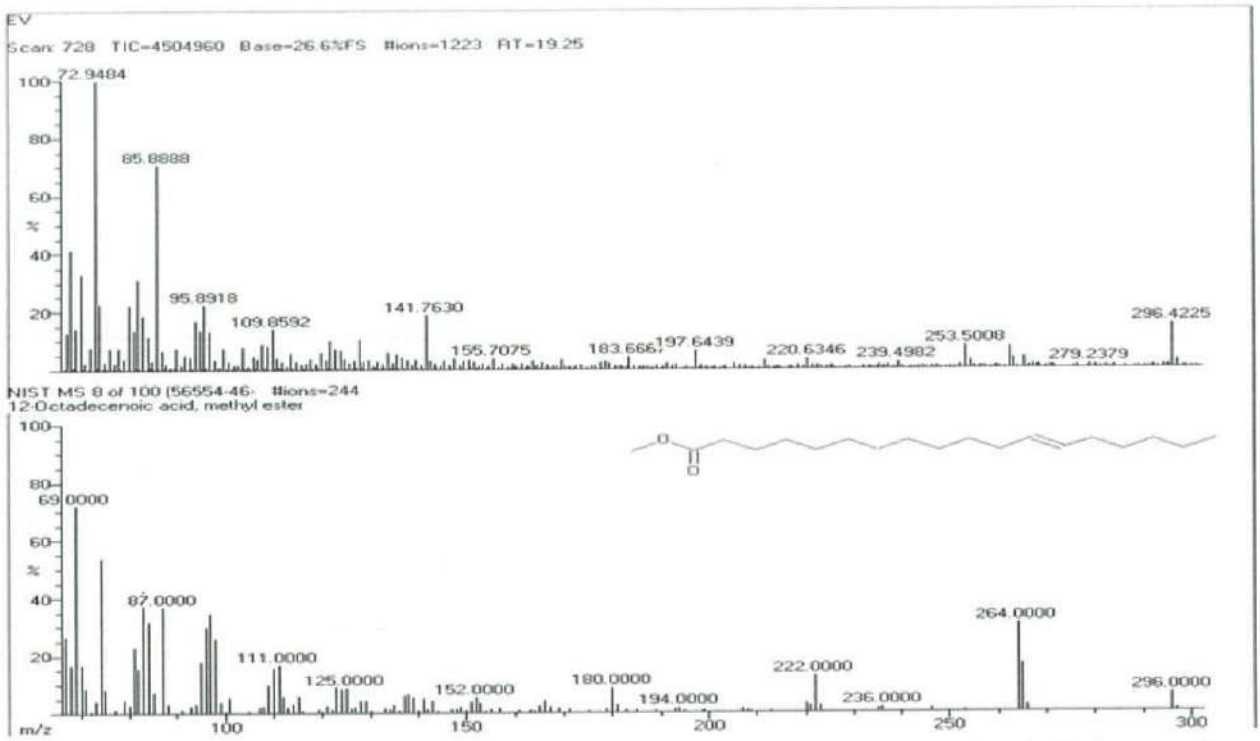

Fig. 3. Mass spectra of 12-Octadecenoic acid, methyl ester compound in the methanolic leaf extract *of E. variegata*

Table 1. Larvicidal activity of three medicinal plants against third instars larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*

Species name	Extracts	LC_{50} (mg/L)	95% Confidence limits		LC_{90} (mg/L)	95% Confidence limits		χ^2
			LCL	UCL		LCL	UCL	
Ae. aegypti	Methanol	134.64	122.4	146.06	267.4	246.65	295.72	3.673
	Ethanol	156.12	144.88	167.65	273.94	253.16	302.19	2.906
	Chloroform	172.5	161.66	184.16	284.53	263.75	312.68	2.678
	Acetone	194.87	184.67	206.35	292.24	272.85	318.36	2.352
An. stephensi	Methanol	129.29	118.63	139.43	232.55	216.4	253.79	5.396
	Ethanol	138.4	128.23	148.33	238.93	222.88	259.93	4.517
	Chloroform	151.06	140.7	161.49	256.75	239.09	280.1	1.13
	Acetone	180.84	170.99	191.5	278.31	260.28	302.25	1.174
Cx. quinquefasciatus	Methanol	121.7	90.6	147.77	217.71	184.5	287.01	7.851
	Ethanol	132.56	121.96	142.7	236.13	219.81	257.59	3.76
	Chloroform	142.05	131.93	152.02	242.89	226.64	264.14	2.786
	Acetone	170.97	160.94	181.59	272.59	254.42	296.71	1.109

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

Table 2. Ovicidal activity of *P. granatum* extracts against *Ae. aegypti, An. stephensi and Cx. quinquefasciatus.*

Species	Extracts	Percentage of egg hatch ability					
		Concentration (ppm)					
		60	120	180	240	300	Control
Ae. aegypti	Methanol	20.4±2.30^{ab}	40.2±1.92^{c}	59.6±2.30^{d}	79.2±2.16^{e}	98.8±1.09^{f}	100±0.00^{f}
	Ethanol	15.4±1.81^{a}	31.6±2.50^{bc}	49.4±2.50^{c}	71.2±2.16^{de}	89.6±1.94^{ef}	100±0.00^{f}
	Chloroform	12.6±1.94^{a}	25.8±2.16^{b}	39.4±2.30^{c}	55.6±1.94^{cd}	70.6±1.81^{de}	100±0.00^{f}
	Acetone	4.6±1.94^{a}	10.2±1.48^{a}	21.2±2.28^{ab}	34.2±2.16^{bc}	42.4±2.30^{c}	100±0.00^{f}
An. stephensi	Methanol	24.6±1.14^{ab}	49.4±2.30^{c}	72.2±2.28^{de}	95.8±1.48^{f}	NH	100±0.00^{f}
	Ethanol	17.8±2.28^{a}	38.6±1.94^{c}	59.8±2.16^{d}	79.4±2.30^{e}	96.6±1.81^{f}	100±0.00^{f}
	Chloroform	14.4±2.30^{a}	30.4±1.81^{ab}	45.6±2.30^{c}	69.8±2.16^{de}	87.6±1.94^{ef}	100±0.00^{f}
	Acetone	7.8±1.64^{a}	16.4±1.14^{a}	26.6±1.51^{ab}	37.4±1.81^{c}	48.8±2.28^{cd}	100±0.00^{f}
Cx. quinquefaciatus	Methanol	26.8±2.16^{ab}	54.2±1.48^{cd}	82.6±2.50^{e}	NH	NH	100±0.00^{f}
	Ethanol	20.4±1.81^{ab}	45.8±2.16^{c}	67.2±1.92^{de}	93.6±1.94^{f}	NH	100±0.00^{f}
	Chloroform	18.4±2.30^{a}	39.2±1.92^{c}	58.8±1.78^{d}	78.6±2.19^{e}	98.2±1.48^{f}	100±0.00^{f}
	Acetone	8.6±1.94^{a}	18.6±1.81^{a}	36.2±2.16^{bc}	55.6±1.94^{cd}	76.8±2.77^{e}	100±0.00^{f}

NH- No hatchability; values are mean of five replicates ±SD. Within each row, different letters indicate significant differences (ANOVA, Duncan's new multiple range method test).

Table 3. Repellent activity of the *S. indicum* extract against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* at 5.0 mg/cm^2

Species	Extracts	% of repellency					
		Time post application of repellent (min)					
		30	60	90	120	150	180
Ae. aegypti	Methanol	100±0.00	100±0.00	100±0.00	95.6±2.88^{d}	82.4±2.30^{c}	68.8±2.28^{b}
	Ethanol	100±0.00	100±0.00	92.8±1.78^{d}	78.2±2.48^{c}	65.4±1.51^{b}	53.8±1.64^{a}
	Chloroform	100±0.00	94.4±1.51^{d}	80.8±2.28^{c}	66.2±1.78^{b}	53.8±2.28^{a}	40.6±2.19^{a}
	Acetone	91.4±2.30^{d}	78.6±2.60^{c}	64.2±2.16^{b}	53.4±1.51^{a}	39.2±1.48^{a}	26.6±1.94^{a}
An. stephensi	Methanol	100±0.00	100±0.00	100±0.00	100±0.00	96.2±2.16^{d}	85.6±2.19^{c}
	Ethanol	100±0.00	100±0.00	100±0.00	95.4±1.81^{d}	80.8±2.16^{c}	73.4±2.50^{b}
	Chloroform	100±0.00	100±0.00	94.2±2.48^{d}	81.2±2.04^{c}	70.6±1.94^{b}	58.6±2.19^{b}
	Acetone	100±0.00	96.2±2.48^{d}	85.4±2.30^{c}	73.4±1.51^{b}	61.2±2.16^{b}	49.6±2.19^{a}
Cx . quinquefasciatus	Methanol	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	95.4±2.19^{d}
	Ethanol	100±0.00	100±0.00	100±0.00	100±0.00	97.8±1.09^{d}	89.2±2.38^{c}
	Chloroform	100±0.00	100±0.00	100±0.00	94.2±1.78^{d}	81.6±1.94^{c}	69.2±2.16^{b}
	Acetone	100±0.00	100±0.00	94.4±2.30^{d}	81.2±1.48^{c}	69.4±2.30^{b}	58.2±2.77^{b}

Mean ± SD value of the five replications. Within each row, different letters indicate significant differences (ANOVA, Duncan's new multiple range method test)

displayed the larvicidal and pupicidal activity against *Cx. quinquefasciatu,* The analysis of the solvent extracts and fractionates showed that the LC_{50} values were 71.43, 94.68, 120.42, 152.15 and 173.35 ppm for first, second, third, fourth and pupae respectively. The fractions were tested for larvicidal, ovicidal and repellent against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus.* The larvicidal activity was observed more with 11-octadecenoic acid and methyl ester compound against *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* with LC_{50} values of 20.51, 22.32 and 23.90 ppm than the ovicidal activity in the present investigation, 100% mortality was exerted by 11-octadecenoic acid, methyl ester compound tested at 40 ppm. Furthermore, high repellence of 11-octadecenoic acid, methyl ester compound tested at 2.5 mg/cm^2 was observed in "arm in cage" tests for at least 320 min (Baranitharan *et al.*, 2017). Presence of metabolites like flavonoides, alkaloids, anthroquinones and anthocyanins in the proved extracts might be the reason for the larvicidal and pupicidal action of the plant extracts and fractions of water hyacinth.

Table 4. List of identified phytocompounds in the methanol leaf extract *of Erythrina variegata*

Peak	Compounds	Scan code	RT(min) *	Concentration (%)
1	4,8-Decadienal, 5,9-dimethyl-	609	16.27	24.55%
2	6-Methyl-á-ionone	627	16.72	9.08%
3	Methyl hexadecanoate	653	17.37	27.66%
4	12-Octadecenoic acid, methyl ester	728	19.25	37.31%
5	Nonadecanoic acid, 18-oxo-, methyl ester	801	21.08	2.27%
6	3,10b-Ethanonaphtho[1,2-c]pyran, 1,3,4,6,6a,7,8,9,10,1a,10b-undecahydro-8á-[dimethoxymethyl]-3-methoxy-7-cyanomethyl-4,4,8-trimethyl-	926	24.23	6.08%

*RT- Retention Time

Repellent action was not exhibited by these extracts at the tested concentrations. The potential of the aquatic *Eichhornia crassipes* in the successful control of the filarial vector, *Cx. quinquefasciatus* has been reported (Jayanthi *et al.*, 2012). N-hexane (LC_{50}=298.8 ppm), chloroform (LC_{50}=418.3 ppm) fractions were more effective on the larvicidal activity than other fraction on *An. gambiae* larvae. The highest mortality per cent of the pupae was also recorded with N-hexane and chloroform fractions on *An. gambiae* at 2500 ppm (Younoussa Lame *et al.*, 2015). The larvicidal activity of essential oil from *Mentha* and *Pulegium* against *Cx. quinquefasciatu*, and. *Mentha longifolia* and *M. suaveolens* essential oil, which contained a majority share of piperitenone oxide, showed the highest effects. The LD_{50} value was estimated at 17 mg/l for both essential oil and LD_{90} value was estimated at 28 mg/l (Pavela *et al.*, 2014). Among the tested compounds, eucalyptol (1,8-cineole) and α-terpinyl acetate were considered to be inactive as the LC_{50}was50.0 mg L^{-1} (Cheng *et al.*, 2009).

The LC_{50} and LC_{90} values of citronellal component from *Melissa officinalis* against *An. stephensi* were 85.44 and 159.73 mg/L, respectively. The ovicidal activity of citronellal component exerted 45 mg/L, and repellent activity was observed at 0.75 and 1.50 mg/cm^2 concentrations which gave 100% protection up to 210 min against *An. stephensi* (Baranitharan *et al.*, 2016). Larvicidal leaf extract of *Gymnema sylvestre* showed the highest mortality in the concentration of 1000 ppm against *An. subpictus* (LC_{50}=166.28 ppm) and the maximum efficacy was observed in gymnemagenol compound isolated from petroleum ether extract of *Gymnema sylvestre* with LC_{50} values against *An. subpictus* at 22.99 ppm and *Cx. quinquefasciatus* at 15.92 ppm, respectively (Khanna *et al.*, 2011). The phytochemical confirmed the presence of various photochemical compounds including glycosidase, saponin, fixed oil and fats, protein, carbohydrates and tannin. The most effective larvicidal activity with concentrations of 0.4% *Cassia tora* extracts gave 80% mortality of the larvae of *An. stephensi* (Swati Supare and Mansi Patil, 2015). The larvicidal activity of essential oils against mosquito species, the monoterpenes â-asatone, ñ-cymene, (+)-limonene, linaly acetate, myrcene, á-phellandrene, (+)-â-pinene, (-)-â-pinene, ã-terpinene and terpinolene, á-terpinene, phenylpropenes safrole and eugenol, and the sulfur containing compound daillyl disulfide has been reported on one or more species of mosquitoes (Pohilt *et al.*, 2011). A new tetranortriterpenoid, meliatralenone [24,25,26,27-tetranorapotircalla-(apoeupha)-6alpha-O-methyl, 7alpha-senecioyl (7-deacetyl)-11alpha, 12alpha, 21,23-tetrahydrocxy-21,23epoxy-2,14,20(22)-trien-1, 16-dione] (1), was isolated from the methanolic extract of crisp leaf of *Azadirachta indica* along with the known compound odoratone (3) which showed mortality on IV instar larvae of *An. stephensi* with LC_{50} values of 16 and 154 ppm (Siddiqui *et al.*, 2004). Overall, this research provides useful information for the development of newer and safer mosquito control tools. Concerning the composition of the *E. variegata* methanol extract, it was mainly composed of 12-Octadecenoic acid, methyl ester. Mosquitocidal activities of *E. variegata* methanol extract against *Cx. quinquefasciatus* larvae, even at low dosages, have been clearly demonstrated. Further studies are needed to validate and develop efficient mosquito larvae, egg and repellent with least impact on human health and environment.

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