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Research Article

Ovicidal, oviposition deterrent, and larvicidal response of *Anopheles stephensi* Liston, 1901 to *Lobophora variegata* Lamouroux, 1817 from Tuticorin coast, Gulf of Mannar, India

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ABSTRACT

The objective of the present study was to investigate the mosquito ovicidal, oviposition deterrent, and larvicidal efficacy of hexane, benzene, chloroform, ethyl acetate, and methanol extracts of *Lobophora variegata* against malarial vector *Anopheles stephensi*. Among the five extract tested, the methanol extract was notable, which attained the 100% mortality at the concentration of 200.0 ppm, and the hatchability rate ranged from 71.3% to 36.3%. In laboratory oviposition deterrent test, the extract of *L. variegata* greatly reduced the number of eggs deposited by gravid *A. stephensi*. The maximum and significant diminished fecundity in *A. stephensi* was observed with methanol extract which caused 76.15-97.69% effective deterrence. Larvicidal response of *A. stephensi* was more susceptible in methanol extract. The LC_{50} value of methanol extract was 61.63 ppm and the Chi-square value were significant at $P < 0.05$ level. It is concluded that the extract of *L. variegata* could be used in control of malarial vector *A. stephensi*.

KEY WORDS: *Anopheles stephensi,* larvicidal, *Lobophora variegata,* ovicidal, oviposition deterrent

INTRODUCTION

Medical importance of mosquito plays a predominant role for the transmission of dengue, malaria, yellow fever, filariasis, and other several disease which are today among the greatest health problems in the world (Rahuman *et al.*, 2008). *Anopheles stephensi* transmits malaria in the plains of rural and urban areas of India and other parts of the world. Malaria is an entirely preventable and treatable mosquito-borne illness. In 2014, 97 countries and territories had ongoing malaria transmission. An estimated 3.3 billion people are at risk of malaria, of which 1.2 billion are at high risk. In high-risk areas, more than one malaria case occurs per 1000 population. There were an estimated 198 million cases of malaria worldwide in 2013, and an estimated 584,000 deaths. 90% of all malaria deaths occur in Africa. In 2013, an estimated 437,000 African children died before their fifth birthday due to malaria. Globally, the disease caused an estimated 453,000 under-five deaths in 2013. Between 2000 and 2013, an expansion of malaria interventions helped to reduce malaria incidence by 30% globally, and by 34% in Africa (WHO, 2014). The use of conventional pesticides in the water sources threatens the vector control program (Shelton *et al.*, 2007). Moreover, continuous application of insecticides poses serious threats to the environment against non-target species such as larval predators, bioaccumulation, hampering biodiversity, and environmental pollution (Lees *et al.*, 2014).

In this scenario, botanicals are used to control mosquito larvae, although many botanical products are obtained from terrestrial flowering plants, the number of investigations related to the use of seaweed extract against insect pests and vectors is very limited. Of some 9200 known species of seaweeds, only 250 species appear to be economically important, based on industrial, botanical, and pharmaceutical interest (Spavieri *et al.*, 2010). Since insecticides originated from seaweeds are highly effective, safe, and eco-friendly. Currently, several studies report the use of seaweed extracts as mosquito

control agent against different mosquito species (Murugan *et al.*, 2015; Kalimuthu *et al.*, 2014; Kumar *et al.*, 2012; Manilal *et al.*, 2011). Yu *et al.* (2015) gave a comprehensive review on the use of seaweed extracts and their bioactive components as mosquitocidal potential.

Screening of seaweed extracts as mosquito larvicide is going on throughout the world to find out a promising candidate for mosquito control program. Effect of methanol extract of this *Lobophora variegata* has been studied on 2nd and 3rd instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* (Manilal *et al.*, 2011). However, no work on the efficacy of *L. variegata* on *A. stephensi*. Hence, the present study was undertaken to find out the ovicidal, oviposition deterrent, and larvicidal activities of various organic solvent extracts of *L. variegata* against *A. stephensi*. This seaweed was selected on the basis of their medicinal property which has been reported earlier. Lobophora belongs to the Dictyotaceae, a family which has proven to be a particularly rich and diverse source of natural products and predominantly diterpenes (Maschek and Baker, 2008; Vallim *et al.*, 2005; Blunt *et al.*, 2015). These natural products have been particularly studied for their bioactivity for human health but also for their putative ecological role in nature. The terpenoids isolated from the Dictyotaceae exhibit various types of bioactivity such as feeding deterrence, antifungal, cytotoxic, antibiotic, anti-inflammatory, insecticidal, or antiviral activities (Vieira *et al.*, 2015).

MATERIALS AND METHODS

Collection, Extraction and Preparation of Algal Specimens

Seaweed specimens were sourced from the intertidal biotope of Tuticorin coast located at southeast coast of Tamil Nadu in the Gulf of Mannar region, which is situated between India and Sri Lanka (latitude 8°48'N and longitude 78°09'E). After collection, the seaweed (*L. variegata*) samples were washed with seawater and in fresh seawater to remove the epiphytes, sand, and other calcareous matter if any and shade-dried and powdered. The dried powder was then subjected to extraction in various solvents *viz.*, hexane, benzene, chloroform, ethyl acetate, and methanol using soxhlet apparatus and solvent evaporation by vacuum evaporator. The seaweed material was reduced to a viscous dark brown residue and crude extracts were further concentrated to paste and they were covered by aluminum foil sheet and stored in a freezer until assayed. About 1 g of extracts was dissolved in acetone solvent and 1.0% stock solution was prepared. From this stock solution, different concentrations were prepared, and these solutions were used for ovicidal, oviposition deterrent, and larvicidal bioassays.

Mosquito Culture

The eggs of *A. stephensi* were collected from the Field station, Centre for Research in Medical Entomology (ICMR-Government of India), Madurai, Tamil Nadu, India. These eggs were brought to the laboratory and transferred to 18 cm × 13 cm × 4 cm enamel trays containing 500 mL of water for hatching. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. At the time of adult feeding, these mosquitoes were 3-4 days old after emergence (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. *A. stephensi* was done from 6:00 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 was maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28°C ± 2°C, 70-85% relative humidity, with a photoperiod of 12-h light and 12-h dark.

Ovicidal Bioassay

For ovicidal efficacy of *A. stephensi* eggs, slightly modified method of Su and Mulla (1998) was performed. The different leaf extracts diluted in the appropriate solvent to achieve various concentrations ranging from 50.0 to 300.0 ppm. Eggs of these mosquito species (100) were exposed to each concentration of leaf extracts. After treatment, the eggs from each concentration were count under the microscope and individually transferred to distilled water cups for hatching assessment. Each experiment was replicated 5 times along with appropriate control. The hatch rates were assessed 48 h post-treatment by following formula:

$$\% \text{ of mortality} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

Oviposition Deterrent Assay

The influence of hexane, benzene, chloroform, ethyl acetate, and methanol extracts of *L. variegata* on the oviposition activity of *A. stephensi* was studied at 30.0, 60.0,

90.0, 120.0, and 150.0 ppm concentration. Twenty gravid female mosquitoes were released into 30 × 30 × 30 cm cage and were maintained at a photoperiod of 12 h light and dark cycle. Plastic bowls used as ovitraps were kept inside the cage, and their position was changed in a cyclic manner during each trial to avoid selection bias. The ovitraps were removed after 24 h of oviposition for egg count. A total of five replicates were carried out for assessment. Trials were repeated when eggs were not laid in any one of the ovitraps placed inside the cage. Effective deterrence and oviposition active index (OAI) were calculated using the following formula:

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

Where NC is the number of eggs laid in control and NT is the number of eggs laid in treatment.

$$\text{Oviposition active index} = \frac{\text{NT} - \text{NS}}{\text{NT} + \text{NS}}$$

Where NT is the total number of eggs laid in test solution and NS is the total number of eggs laid in control solution.

OAI of +0.3 and above are considered as attractants while those with −0.3 and below are considered as deterrents (Kramer and Mulla, 1979). Positive value indicates that more eggs were deposited in treated solutions, and negative value, more eggs in control.

Larvicidal Bioassay

Larvicidal activity of *L. variegata* was evaluated according to WHO (2005). Based on the wide range and narrow range tests, crude extract was tested at 30.0, 60.0, 90.0, 120.0, and 150.0 ppm concentrations. 20 numbers of early 3rd instar larvae were introduced into a 500 mL glass beaker containing 249 mL of dechlorinated water, and 1 mL of desired concentrations of crude extracts was added. For each concentration, five replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. Each test included a set of control groups (1 mL of acetone and 249 mL distilled water) with five replicates for each individual concentration.

Statistical Analysis

The average larval mortality data were subjected to Probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% confidence limits of upper confidence limit UCL, lower confidence limit (LCL), and chi-square values were calculated using the Statistical Package of Social Sciences 20.0 software. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS

The effect of *L. variegata* extracts on the egg hatchability of *A. stephensi* were reported in the present study (Table 1), showed that there was 100% hatchability occurred in all the control groups. The methanol extract was notable, which attained the 100% mortality at the concentration of 200.0 ppm and the hatchability rate ranged from 71.3% to 36.3% and the ethyl acetate and chloroform extract was exerted the 100% mortality at 250.0 ppm and the hatchability rate ranged from 76.4% to 28.2% and 79.2% to 34.5%. The benzene and hexane extracts exhibited the 100% mortality at a slightly higher concentration of 300.0 ppm, they indicated the hatchability rate of 83.5-24.7% and 88.3-27.2%. The rate of hatchability was higher in lower concentrations and when the concentrations of the extract increased the hatchability rate decreased. These results clearly revealed that the toxicity of extracts was dependent on its concentration and which will determine the egg hatchability.

The oviposition deterrent activity of the seaweed extracts viz., hexane, benzene, chloroform, ethyl acetate and methanol were tested against gravid *A. stephensi* at different concentrations of 30.0, 60.0, 90.0, 120.0, and 150.0 ppm and the observations are presented in Table 2. In laboratory oviposition deterrent test, the extract of *L. variegata* greatly reduced the number of eggs deposited by gravid *A. stephensi* at all the concentrations. The maximum and significant diminished fecundity in *A. stephensi* was observed with methanol extract which caused 76.15-97.69% of effective deterrence. Whereas the benzene and hexane extracts showed the least effective deterrence of 48.16–85.23% and 33.77–79.76%. The ethyl acetate and chloroform extracts also obtained their moderate oviposition deterrence with 67.35-93.74 and 59.52-92.28% and the OAI values ranged from −0.509 to −0.882 and −0.423 to −0.856. Those concentrations with an OAI of −0.3 and below are considered as oviposition deterrents and if the OAI is +0.3 and above are considered as oviposition attractant. At all concentrations, there was a significant difference between control and treated groups with respect to the number of eggs laid by gravid *A. stephensi*.

Larvicidal response of *A. stephensi* mosquito larvae to the extracts of *L. variegata* are presented in Table 3 that revealed

Table 1: Ovicidal activity of *L. variegata* extracts against *A. stephensi*

Seaweed used	Solvent used	Egg hatchability (%)						Control
		Concentration (ppm)						
		50.0	100.0	150.0	200.0	250.0	300.0	
L. variegata	Hexane	88.3±1.5	72.8±1.8	53.4±1.3	40.2±1.3	27.2±1.8	NH	100±0.0
	Benzene	83.5±1.7	69.6±1.8	48.3±1.8	37.2±1.9	24.7±1.2	NH	100±0.0
	Chloroform	79.2±1.8	65.4±1.2	45.2±1.6	34.5±1.3	NH	NH	100±0.0
	Ethyl acetate	76.4±0.6	58.4±1.7	41.2±0.9	28.2±1.6	NH	NH	100±0.0
	Methanol	71.3±0.5	52.5±0.8	36.3±0.2	NH	NH	NH	100±0.0

Each value (mean±SD) represents the mean of five replicates. NH: No hatchability (100% mortality), SD: Standard deviation, *L. variegata: Lobophora variegate, A. stephensi: Anopheles stephensi*

Table 2: Oviposition deterrent response of *A. stephensi* to *L. vaiegata* extracts

Solvents used	Concentration (ppm)	Number of eggs in bowl		Effective repellence (%)	OAI
		Control	Treated		
Hexane	30.0	60.72±1.24	40.21±1.61	33.77±1.48	−0.203
	60.0	72.35±1.82	36.15±1.78	50.03±1.90	−0.333
	90.0	84.16±1.60	32.82±1.26	61.00±1.42	−0.438
	120.0	92.60±1.18	26.71±1.34	71.15±1.63	−0.552
	150.0	112.81±1.24	22.83±1.58	79.76±1.45	−0.663
Benzene	30.0	68.76±1.71	35.64±1.32	48.16±1.55	−0.317
	60.0	82.13±1.86	33.58±1.65	59.11±1.21	−0.419
	90.0	96.41±1.34	28.91±1.71	70.01±1.48	−0.538
	120.0	118.84±1.92	20.16±1.85	83.03±1.22	−0.709
	150.0	120.50±1.14	17.79±1.40	85.23±1.63	−0.742
Chloroform	30.0	75.45±1.94	30.54±1.83	59.52±1.70	−0.423
	60.0	91.83±1.85	26.72±1.74	70.90±1.16	−0.549
	90.0	117.41±1.63	21.04±1.90	82.07±1.82	−0.696
	120.0	125.64±1.58	16.53±1.66	86.84±1.92	−0.767
	150.0	136.82±1.83	10.55±1.72	92.28±1.62	−0.856
Ethyl acetate	30.0	82.62±1.20	26.82±1.90	67.53±1.54	−0.509
	60.0	98.31±1.45	20.46±1.86	79.18±1.89	−0.655
	90.0	122.36±1.78	17.23±1.74	85.91±1.55	−0.753
	120.0	130.82±1.65	13.51±1.48	89.67±1.29	−0.812
	150.0	138.70±1.32	08.66±1.22	93.74±1.38	−0.882
Methanol	30.0	94.68±1.94	22.58±1.10	76.15±1.90	−0.614
	60.0	115.45±1.86	15.71±1.59	86.39±1.22	−0.760
	90.0	134.71±1.52	12.86±1.35	90.45±1.28	−0.825
	120.0	148.50±1.30	08.73±1.14	95.46±1.96	−0.888
	150.0	152.58±1.45	02.52±1.97	97.69±1.62	−0.954

Values are mean of five replicates and standard error. OAI: Oviposition active index

the highest larvicidal activity was observed in methanol extract followed by ethyl acetate, chloroform, benzene, and hexane extracts. The LC_{50} and LC_{90} of methanol extract was 61.63 and 116.19 ppm and the chi-square values were significant at $P < 0.05$ level. The LC_{50} values of ethyl acetate, chloroform, and benzene extracts were 65.99, 71.36, and 77.05 ppm, respectively. The hexane extract showed lowest larvicidal activity on the larvae of *A. stephensi* with the LC_{50} value of 87.14 ppm. After exposure to the test concentrations, the treated larvae exhibited restless and convulsions followed by death at the bottom of the beaker. The toxicity of seaweed extracts was dependent on its concentration. The chi-square value in the bioassays indicated probably the heterogeneity of the test population. The 95% confidence limits LC_{50} (LCL-UCL) and LC_{90} (LCL-UCL) were also calculated. No mortality was recorded in the control.

DISCUSSION

The *L. variegata* is distributed worldwide in tropical to temperate waters and represents an important algal component in coral reef ecosystems. However, metabolites produced by Lobophora species have been found to exhibit a wide array of bioactivities including antibacterial, antiviral, antioxidant, antiprotozoa, antitumor, and pesticidal along with its properties, a few researchers have reported its potential role in control of mosquito vectors (Manilal *et al.*, 2011; Biano *et al.*, 2013). Keeping in view limited research work on antimosquito potential of the seaweed, the present investigations were carried out to assess the prospective use of *L. variegata* in mosquito management programs.

The ovicidal studies performed on the seaweed extracts against *A. stephensi* proved their efficacy as ovicidal agents.

Table 3: Larvicidal efficacy of *L. variegata* against *A. stephensi*

Solvents used	Concentration (ppm)	% of mortality±SD	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2 (df=4)
Hexane	Control	0.0±0.0			
	30.0	15.28±1.36			
	60.0	32.50±1.72	87.140	149.521	5.807
	90.0	53.15±1.82	(81.276-93.158)	(139.773-161.830)	
	120.0	72.82±1.96			
	150.0	89.43±1.58			
Benzene	Control	0.0±0.0			
	30.0	21.53±1.66			
	60.0	41.74±1.80	77.053	138.637	9.509
	90.0	59.18±1.44	(63.823-90.255)	(120.626-168.430)	
	120.0	80.56±1.34			
	150.0	92.17±1.62			
Chloroform	Control	0.0±0.0			
	30.0	25.46±1.85			
	60.0	44.20±1.26	71.363	129.705	11.274
	90.0	62.83±1.90	(57.136-85.141)	(111.706-160.332)	
	120.0	86.12±1.36			
	150.0	94.55±1.42			
Ethyl acetate	Control	0.0±0.0			
	30.0	29.34±1.59			
	60.0	48.82±1.65	65.993	122.249	13.159
	90.0	67.63±1.72	(50.531-80.507)	(103.928-154.631)	
	120.0	88.16±1.45			
	150.0	96.70±1.38			
Methanol	Control	0.0±0.0			
	30.0	33.75±2.42			
	60.0	52.96±1.64	61.638	116.199	17.265
	90.0	69.18±1.28	(43.481-78.123)	(96.377-154.681)	
	120.0	90.54±1.22			
	150.0	98.32±1.80			

LCL: Lower confidence limits, UCL: Upper confidence limits, χ^2: Chi-square values are significant at $P<0.05$, df: Degrees of freedom, SD: Standard deviation

The maximum efficacy was exhibited by the methanol extract of *L. variegata* which resulted in 100% mortality at 200.0 ppm. Our results are comparable with earlier reports of Prathibha *et al*. (2014). They determined that the ovicidal activity of petroleum ether and ethyl acetate extracts of the leaves of *Eugenia jambolana*, *Solidago canadensis*, *Euodia ridleyi*, and *Spilanthes mauritiana* against the three vector mosquito species, namely *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. Among the four plant extracts tested for ovicidal activity against *A. stephensi, A. aegypti*, and *C. quinquefasciatus*, the ethyl acetate extracts of *S. mauritiana* exerted 100% mortality (zero hatchability) at 100 ppm against all the three mosquito species, followed by *S. canadensis, E. ridleyi*, and *E. jambolana*. Govindarajan *et al*. (2008a) have also observed that the leaf extract of *Azadirachta indica* with different solvents, viz., benzene, chloroform, ethyl acetate, and methanol, had larvicidal activity, ovicidal activity, and oviposition attractancy against *A. stephensi*. Mullai *et al*. (2008) studied that the leaf extract of *Citrullus vulgaris* with different solvents viz. benzene, petroleum ether, ethyl acetate, and methanol for larvicidal, ovicidal, repellent and insect growth regulatory activities against *A. stephensi*. The larvicidal and ovicidal activity of *Ervamatia alba, Cardiospermum halicacabum,* and *Andrographis paniculata*, was tested against the early 3rd instar larvae of *A. stephensi* (Govindarajan *et al*., 2011). The leaf methanol extract of *Cassia fistula* was tested for larvicidal and ovicidal activity of *Cassia fistula* against *C. quinquefasciatus* and *A. stephensi*, with the LC_{50} values of 17.97 and 20.57 mg/l, respectively (Govindarajan *et al*., 2008b).

In the present investigation, extracts of *L. variegata* proved to be oviposition deterrent activity against gravid female *A. stephensi*. The mean number of eggs in control group was greater than the treated groups at all the concentration of seaweed extracts tested. The OAI also determined that the gravid and oviposited females were deterred by *L. variegata*. These results indicate that adults of *A. stephensi* were acutely sensitive to the changes induced in the physiology and behavior of the adult mosquito species reflected by their egg-laying capacity. Some phytochemicals act as general toxicants against both adult and larval stages of mosquitoes, while others interfere with growth and development (growth inhibitors) or with reproduction (chemosterilent) or produce olfactory stimuli acting as repellent or attractant. In this regard, Rajkumar and Jebanesan (2009) have reported the oviposition deterrence

effects of the extracts of *Cassia obtusifolia* with repellency at higher concentration (400 mg/L). The present study results are favourably supported by the oviposition deterrent activity of *Bryopsis pinnata* investigated by Yu *et al*. (2015). He determined the oviposition deterrent activity viz., hexane, chloroform, methanol and aqueous extract of *B. pinnata*. Among the four plant extracts tested for oviposition deterrent activity against *A. aegypti* and *Aedes albopictus* the methanol extract of *B. pinnata* exhibited the maximum oviposition deterrent at 44.36 and 51.60 µg/mL. Oviposition deterrence of *B. pinnata* increased with the higher concentration. The ovicidal, oviposition deterrent and larvicidal activity of the *L. variegata* might be due to the variety of compounds in this seaweed including phenolics, terpenoids, alkaloids, and fatty acids (Manilal *et al*., 2012; Thennarasan *et al*., 2015). These compounds may jointly or independently contribute to cause these biological activities.

Table 3 observations found that the organic solvent extracts from *L. variegata* proved the larvicidal potential of methanol extract with lowest LC_{50} of 61.63 ppm against *A. stephensi* followed by ethyl acetate, chloroform, benzene and hexane extracts, respectively. Similarly, Manilal *et al*. (2011) reported the efficacy of methanol extract of *L. variegata* against the second and third instars of *A. aegypti* with LD_{50} of 70.38, 79.43 µg/mL and *C. quinquefasciatus* had the following LD_{50} values of 95.52, 96.52 µg/mL. The ethyl acetate fraction of *Sargassum swartzii* has shown active larvicidal effect against mosquito larvae of *A. stephensi* (Khanavi *et al*., 2011). Earlier reports showed that the mosquito larvicidal activity of marine plants with LC_{50} Value of 17 mg/L in *Rhizophora apiculata* Blume stilt root against *A. stephensi* (Thangam *et al*., 1988). The use of synthetic pesticides is undesirable because of their persistence in the environment and collateral effects in humans and other non-target organisms. Less toxic and environmentally acceptable substitutes are therefore needed, which has created a significant market opportunity for alternative products such as botanical pesticides (Ntalli *et al*., 2012). Since bioactive compounds from marine source are often active against specific target insects, less expensive, easily biodegradable to non-toxic products and potentially suitable for use in mosquito control program.

CONCLUSION

It is concluded from the present study that the seaweed (*L. variegata*) which were collected from Tuticorin coast, Gulf of Mannar, India, showed significant ovicidal, oviposition deterrent, and larvicidal activities against *A. stephensi* and could serve as alternative to synthetic insecticides. Therefore, we suggest the extension of studies to establish the potential of these extracts for use in natural environments, considering the potential low impact on non-target organisms.

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REFERENCES

Biano EM, Luana P, Geanne KN, Kamilla AD, Thiago NV, Edson RT, *et al*. Larvicidal activity of seaweeds from North Eastern Brazil and of a halogenated sesquiterpene against the dengue mosquito (*Aedes aegypti*). Ind Crops Prod 2013;43:270-5.

Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. Marine natural products. Nat Prod Rep 2015;32:116-211.

Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K. Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res 2008;103:691-5.

Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitol Res 2008;102:289-92.

Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activity of botanical extracts against *Culex quinquefasiatus*, *Aedes aegypti* and *Anopheles stephensi,* (Diptera: Culicidae). Asian Pac J Trop Med 2011;1:43-8.

Kalimuthu K, Lin SM, Tseng LC, Murugan K, Hwang JS. Bioefficacy potential of seaweed *Gracilaria firma* with copepod, *Megacyclops formosanus* for the control larvae of dengue vector *Aedes aegypti*. Hydrobiologia 2014;741:113-23.

Khanavi M, Toulabi PB, Abai MR, Sadati N, Hadjiakhoondi F, Hadjiakhoondi A, *et al*. Larvicidal activity of marine algae, *Sargassum swartzii* and *Chondria dasyphylla*, against malaria vector *Anopheles stephensi*. J Vector Borne Dis 2011;48:241-4.

Kramer WL, Mulla S. Oviposition attractants and repellents of mosquitoes: Oviposition responses of *Culex* mosquitoes to organic infusions. Environ Entomol 1979;8:1111-7.

Kumar KP, Murugan K, Kovendan K, Kumar AN, Hwang JS, Barnard DR. Combined effect of seaweed (*Sargassum wightii*) and *Bacillus thuringiensis* var. *Israelensis* on the coastal

mosquito *Anopheles sundaicus* in Tamil Nadu, India. Sci Asia 2012;38:141-6.

Lees RS, Knols B, Bellini R, Benedict MQ, Bheecarry A, Bossin HC, *et al.* Review: Improving our knowledge of male mosquito biology in relation to genetic control programmes. Acta Trop 2014;132:S2-11.

Manilal A, Selvin J, Thajuddin N, Sujith S, Panikkar M, Idhayadhulla A, *et al.* Biopotentials of marine alga, *Lobophora variegata* collected from the South Indian littoral. Thalassas 2012;28:47-54.

Manilal A, Thajuddin N, Selvin J, Idhayadhulla A, Kumar RS, Sujith S. *In vitro* mosquito larvicidal activity of marine algae against the human vectors, *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). Int J Zool Res 2011;7:272-8.

Maschek JA, Baker BJ. The chemistry of algal secondary metabolism. In: Amsler CD, editor. Algal Chemical Ecology. Berlin: Springer; 2008.

Mullai K, Jebanesan A, Pushpanathan T. Effect of bioactive fractions of *Citrullus vulgaris* Schrad. Leaf extract against *Anopheles stephensi* and *Aedes aegypti*. Parasitol Res 2008;102:951-5.

Murugan K, Benelli G, Ayyappan S, Dinesh D, Panneerselvam C, Nicoletti M, *et al.* Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*. Parasitol Res 2015;114:2243-53.

Ntalli NG, Caboni P. Botanical nematicides: A review. J Agric Food Chem 2012;60:9929-40.

Prathibha KP, Raghavendra BS, Vijayan VA. Larvicidal, ovicidal and oviposition-deterrent activities of four plant extracts against three mosquito species. Environ Sci Pollut Res 2014;21:6736-43.

Rahuman AA, Venkatesan P, Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. Parasitol Res 2008;103:1383-90.

Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res 2009;104:337-40.

Shelton AM, Wang P, Zhao JZ, Roush RT. Resistance to insect pathogens and strategies to manage resistance: An update. In: Laceyand LA, Kaya HK, editors. Field Manual of Techniques in Invertebrate Pathology. New York: Springer; 2007.

Spavieri J, Allmendinger A, Kaiser M, Casey R, Hingley Wilson S, Lalvani A, *et al.* Antimycobacterial, antiprotozoal and cytotoxic potential of twenty-one brown algae (*Phaeophyceae*) from British and Irish waters. Phytother Res 2010;24:1724-9.

SuT, Mulla MS. Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Am Mosq Control Assoc 1998;14:204-9.

Thangam TS, Kathiresan K. Toxic effect of mangrove plant extracts on mosquito larvae *Anopheles stephensi* L. Curr Sci 1988;57:914-5.

Thennarasan S. Biochecmial composition of marine brown alga *Lobophora variegata* from Mandapam in the South East Coast of Tamil Nadu. Int J Pharm Pharm Sci 2015;5:25-9.

Vallim MA, De Paula JC, Pereira RC, Teixeira VL. The diterpenes from Dictyotacean marine brown algae in the tropical Atlantic American region. Biochem Syst Ecol 2005;33:1-16.

Vieira C, Gaubert J, Clerck OD, Payri C, Culioli G, Thomas OP. Biological activities associated to the chemodiversity of the brown algae belonging to genus *Lobophora* (Dictyotales, Phaeophyceae). Phytochem Rev 2015;63:152-3.

WHO. Fact Sheet on the World Malaria Report; 2014. Available from: http://www.who.int/malaria/media/world_malaria_report_2014/en/. [Last accessed on 2014 Dec].

World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides [WHO/CDS/WHOPES/GCDPP/2005.13.2005]. Geneva: WHO; 2005.

Yu KX, Wong CL, Ahmad R, Jantan I. Mosquitocidal and oviposition repellent activities of the extracts of seaweed *Bryopsis pennata* on *Aedes aegypti* and *Aedes albopictus*. Molecules 2015;20:14082-102.