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Larvicidal activity of two seaweeds, *Enteromorpha flexuosa* J. Agardh and *Gracilara corticata* J. Agardh Against Mosquito Vector, *Anopheles stephensi*

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The *Anopheles* mosquito is also capable of transmitting filarial worms, various arboviruses, onyong-nyong, tataguine, equine encephalitis, as well as other viruses, but malaria is unquestionably the most threatening disease. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. The bioinsecticides are generally pest-specific, readily biodegradable and usually lack toxicity to higher animals. This study was undertaken to investigate the larvicidal potential of the two different seaweeds *Enteromorpha flexuosa* J. Agardh and *Gracilara corticata* J. Agardh against the medically important species of malaria vector *Anopheles.* Of the two algae screened *G. corticata* was found to effective against the larva *A.stephensi*. It is concluded that the seaweeds such as *E.flexuosa* and *G. Corticata* serves as an excellent biopotent, which can be exploited for larvicidal property and can be cultivated in the coastal areas of the South East Coast of India.

Key words: Larvicidal activity, Seaweeds, *Anopheles stephensi,* Malaria.

Plants are the rich source of bioactive potentials (Wink, 1993) their products are greatly preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability. There are a number of factors, which have economic impact, including loss in commercial and labour outputs. In countries, particularly with tropical and subtropical climates, mosquito borne diseases is one among them the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year. The major disease burden in India is malaria and other vector-borne disease.

With approximately 460 different species living on the planet, the mosquito known as the *Anopheles* has been evolving since it came into existence over 150 million years ago. Despite their very short live span, certain species of the *Anopheles* mosquito are highly devastating to the human population.

Out of the 460 species of *Anopheles*, about 60 have been documented as having transmitted malaria to humans. In certain areas of the world, specific species of *Anopheles* are prevalent. Some dangerous ones include the *A. freeborni* in North America, *A.gambiae* in Africa, and approximately 45 different species have been reported in India.

The *Anopheles* is well known for spreading illnesses to humans, the most dangerous one being malaria which has killed hundreds of

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million people worldwide, and continues to kill over one million individuals each year. Malaria is transmitted to humans by the female mosquito which requires a blood meal to provide nourishment to her eggs after mating. Once the female bites an infected human, she will then transmit the malarial parasites to the next person she feeds on.

The *Anopheles* mosquito is also capable of transmitting filarial worms, various arboviruses, onyong-nyong, tataguine, equine encephalitis, as well as other viruses, but malaria is unquestionably the most threatening disease.

To prevent mosquito-borne diseases and improve public health, it is necessary to control them. However, in recent years, mosquito control programmes have failed because of the ever increasing insecticide resistance (WHO, 1992). It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern (Brown, 1986), which initiated a search for alternative control measures. The bio insecticides are generally pest-specific, readily biodegradable and usually lack toxicity to higher animals (Bowers, 1992).

Certain species of green algae in the order Chlorococcales kill larvae primarily because they are indigestible (Gerald, 2007). *Gracilaria crassa* and *Hypnea valentia* in methanolic extract have shown good larvicidal activity with a LC50 of about 52.2 and 53.4 respectively (Anandhan and Sorna kumara, 2011). Larvicidal property of *Ulva fasciata* and *Grateloupia lithophila* against *Culex quinquefasciatus* has already been carried out by Poonguzhali and Nisha (2012). This study was undertaken to investigate the larvicidal potential of the two different seaweeds *Enteromorpha flexuosa* J. Agardh and *Gracilara corticata* J. Agardh against the medically important species of malaria vector *Anopheles stephensi.*

MATERIALS AND METHODS

Collection of algae and extract preparation

Two seaweed samples, *Enteromorpha flexuosa* J. Agardh and *Gracilara corticata* J. Agardh. were collected from the Kovalam, near Chennai. Healthy algal material were harvested manually and washed thoroughly in running water to remove epizoones, epiphytes, animal castings, sand, calcareous and other adhering detritus matters. Cleaned algal materials were shade dried under room temperature for 4 -5 days. The completely dried material was powdered using electric blender.

Three different extracts (methanol, acetone and benzene) were prepared by submerging the powder in three different flask of each containing 1000 mg/L and placed at 35°C in a shaker at 120 rpm for 7 days for the extraction of active ingredients. From this stock solution dilutions were made to prepare different concentrations Such as 100, 200, 300, 400 and 500 mg/L, respectively, including positive (with 2% methanol, acetone and benzene) and negative controls (larvae exposed to dechlorinated water without methanol, acetone and benzene).

Test mosquito larvae

Larvae of *Anopheles stephensi* were collected from rice field and stagnant water areas of Chennai. It was maintained at 27 ± 2 °C, 75–85% relative humidity and 14L: 10D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio.

Bioassay

The larvicidal bioassay followed the World Health Organization (WHO) standard protocols (World Health Organization, 1981). Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC, 81:807.) with slight modifications. Bioassay were conducted with larvae collected with a Pasteur pipette, placed on filter paper for removal of excess water and transferred (25 per test) with a tiny brush into beakers containing different concentrations of algal extracts (100, 200, 300, 400 and 500 mg/L) with 1000 ml of tap water each. Larvae were exposed to the samples at room temperature for 48 hours and the mortality/survival was registered after the first 24 hrs. **Each test was run in triplicate**

The persistence of larvicidal activity of the algal extract was tested by running bioassays with the same samples after 15, 30 and 60 days.

Data analysis

The larval mortality in each concentration and control was recorded after 24 hours of exposure. Percentage mortalities were corrected for the natural mortality observed in the negative controls using Abbots (1925) formula; P = PI – C /

1 – C, where PI denotes the observed mortality rate and C means the natural mortality. The median lethal concentration or dose (LC50 and LD90) was calculated using 'Probit' analysis (Finney, 1971) that has been recommended by OECD guideline as appropriate statistical method for toxicity data analysis. After linearization of response curve by logarithmic transformation of concentrations, 95% confidence limits and slope function were calculated to provide a consistent presentation of the toxicity data.

RESULTS

Results of the larvicidal activity of three different extracts (methanol, acetone, and benzene) of *E.flexuosa* and *G.corticata* against the larvae of *A. stephensi* was performed under laboratory evaluation. It illustrates that the larval mortality rate of *A. stephensi* after the treatment of the three different extracts of *E.flexuosa* and *G. corticata* at different concentrations (100 - 500 mg/L). In terms of lethal concentration for 50% and 90% mortality

Table 1. Effect of methanolic, acetone and benzene extracts of *E.flexuosa* against mosquito larvae *A. stephensi*

Extract	LC50	95% Confidence Limits		LC90	95% Confidence Limits	
	(mg/L)	LCL	UCL	(mg/L)	LCL	UCL
Methanol	498.91	462.49	543.49	763.78	698.50	852.22
Acetone	537.79	479.50	621.46	875.58	761.77	1058.18
Benzene	491.58	443.80	554.77	866.98	765.55	1019.12

LC_{50} = lethal concentration to cause 50% mortality in population.;
LC_{90} = lethal concentration to cause 90% mortality in population.
LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit.

Table 2. Effect of methanolic, acetone and benzene extracts of *G. corticata* against mosquito larvae *A. stephensi*

Extract	LC50	95% Confidence Limits		LC90	95% Confidence Limits	
	(mg/L)	LCL	UCL	(mg/L)	LCL	UCL
Methanol	189.69	142.55	225.42	497.55 `	444.03	580.29
Acetone	145.38	112.01	171.21	330.70	300.31	373.28
Benzene	297.40	261.83	333.04	633.79	569.49	725.52

LC_{50} = lethal concentration to cause 50% mortality in population.;
LC_{90} = lethal concentration to cause 90% mortality in population.
LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit.

(LC_{50} and LC_{90}) values were represented as follows: LC_{50} value of the methanol extract of *E.flexuosa* was 498.91, acetone extract was 537.79 and benzene extract was 491.58 while for *G. corticata* LC_{50} value of the methanol extract was 189.69 followed by acetone extract 145.38 benzene extract 297.40, LC_{90} value of the acetone extract of *E.flexuosa* was 875.58, followed by benzene extract and methanol extract; while for *G. corticata* LC_{90} value of the benzene extract was 633.79 followed by methanol extract and acetone extract was. (Table 1&2). The LC_{50} values of *E.flexuosa* revealed that the larvae *A. stephensi Culex* was more susceptible to acetone followed by methanol and benzene extract (Acetone > Methanol > Benzene) whereas the LC_{90} values shows that there is a mild variation in the lethality of extracts. LC_{90} values revealed that *A. stephensi* was more susceptible to both seaweeds extracted using benzene followed by acetone and methanol extracts (Benzene > Acetone > Methanol).

DISCUSSION

Mosquitoes are important blood sucking insects. They transmit disease agents that cause malaria, dengue, yellow fever, encephalitis, and filariasis. Many studies have been achieved on

the screening of biological effects of marine organisms and many active compounds were isolated and characterized (Blunden, 2001). Red algae from genus *Chondria* are known as a producer of cyclic polysulfides, terpenoids, amino acids and amines. Domoic acid derivatives with larvicidal and lowering blood pressure activity have been identified in *Chondria armata* (Mangala and Solimabi, 2000). Secondary metabolites with cytotoxic and antitumor activity have been extracted and identified in *Sargassum* species (Numata *et al.*,1991; Tang *et al.,* 2002).

The seaweeds (*U.fasciata* and *H. musciformis*) produced 100% larval mortality at 10 mg/mL (Selvin and Lipton, 2004). There is no previous report on the mosquito larvicidal activity of *E.flexuosa* and *G. corticata* from the Kovalam coast (Chennai) of Tamil Nadu. Of the two algae screened, *G. lithophila* was found to be effective against *A. stephensi* larva in all the 3 extracts. Among the two seaweeds *G. Corticata* showed an LC_{50} value with minimum concentration when compared with *E. flexuosa.* This may be due to the presence of polysaccharides (Andrews *et al.,* 2005). The post coital contraceptive activity from a crude extract in marine algae *Gelidiella acerosa* is due to the presence of various phytochemical components such as alkaloids, flavonoids, phenols, amino acid, steroids, tannins and carbohydrates was demonstrated by Osman *et al.* (2010). Chapagain *et al.* (2008) reported that, saponins serves as natural larvicidal compounds. Previous report of seaweeds showed that red algae had high potency than green algae (Manilal *et al.,* 2011). The phytochemical component saponins serve as natural larvicidal compound as reported by Chapagain *et al.* (2008) Extracts of *Gracilaria crassa* and *Hypnea valentia* have shown good larvicidal activity with a LC_{50} of about 52.2 and 53.4 mg/L respectively against *Aedes* sp.(Anandhan and Sorna, 2011).

CONCLUSION

From the present study it is concluded that the seaweeds such as *E.flexuosa* and *G. Corticata* serves as an excellent biopotent, which can be exploited for larvicidal property and can be cultivated in the coastal areas of the South East Coast of India. These algal extracts showed the ability they have an effective mosquito control properties and also can act as a low cost eco-friendly, bio-pesticide for further vector control programs.

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