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Larvicidal Activity of Two Seaweeds *Enteromorpha flexuosa* and *Gracilara corticata* Against Mosquito Vector *Culex quinquefasciatus*

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The use of plant products is one of the best alternatives for mosquito control. Hence it remains a top research issue for scientists associated with alternative vector control (Redwane *et al.,* 2002). *C.quinquefasciatus* is the most widely distributed mosquito in India, mainly found in urban and suburbanareas. The most efficient approach to control the vector is totarget the immature stages of the life cycle. *C.quinquefasciatus*, a vector of lymphatic filariasis, is a widely distributed tropical diseasewith around 120 million people infected worldwide and 44 million people having common chronic manifestation(Bernhard *et al.,* 2003). It can transmit a number of illnesses that can present serious health problems to human beings. It is known to spread West Nile Virus and also diseases such as filariasis, and encephalitis. Dry algal powder in methanol, acetone and benzene in varying concentration were used as biolarvicide against *C.quinquefasciatus* results from biostatistics showed that *Gracilara corticata* in acetone extract served as efficient larvicide when compared to *Enteromorpha flexuosa*.

Keywords: *Gracilara corticata, Enteromorpha flexuosa*., Biolarvicide, *C.quinquefasciatus* and biostatistics.

The attractive targets for larvicides are at the larval stages of Mosquitoes, because they breed in water and, thus, are easy to deal with them in this habitat. The conventional methods that have been used early for mosquito control were chemical pesticides, which has resulted in the development of resistance (Severini *et al.,* 1993 and WHO 1970), undesirable effects on non-target organisms and fostered environmental and human health concerns (Forget, 1989). The search for herbal preparations is mainly due to the fact that they do not produce any adverse effects in the non-target organisms and are easily biodegradable. The use of plant products is one of the best alternatives for mosquito control. Hence it remains a top research issue for scientists associated with alternative vector control (Redwane *et al.,* 2002).

C. quinquefasciatus is the most widely distributed mosquito in India, mainly found in urban and suburban areas. The most efficient approach to control the vector is to target the immature stages of the life cycle. *C.quinquefasciatus*, a vector of lymphatic filariasis, is a widely distributed tropical disease with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard *et al.,* 2003). It can transmit a number of

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illnesses that can present serious health problems to human beings. It is known to spread West Nile Virus and also diseases such as filariasis, and encephalitis.

Petroleum ether (60–80°C) extracts of the leaves of *Vitex negundo* were evaluated for larvicidal activity against larval stages of the mosquito *C.tritaeniorhynchus* in the laboratory (Karunamoorthi *et al,*. 2008); the acetone leaf extract of *S. trilobatum* was evaluated against the ovicidal activity of *C.quinquefasciatus* and *C.tritaeniorhynchus* (Rajkumar & Jebanesan 2005); the crude acetone, hexane, ethyl acetate, methanol and petroleum ether extracts of the leaf of *Centella asiatica, Datura metal*, *Mukia scabrella* and *Toddalia asiatic*a, extracts of whole plant of *Citrullu scolocynthis* and *Sphaeranthus indicus* were assayed for their toxicity against the early fourth instar larvae of *C.quinquefasciatus* (Rahuman *et al.,* 2008d).

The hexane extract of *Apium graveolens* showed larvicidal and adulticidal potency, but remarkable repellency and four fractions from *A. graveolens* seeds showed strong repellent activity (Tuetun *et al.,* 2005, 2004); the ethanolic extract of Curcuma aromatica showed repellent activity (Pitasawat *et. al.,* 2003); the hexane fraction from *Kaempferia galangal* possessed repellency (Choochote *et al.,* 1999) against *C.tritaeniorhynchus.*

The ethanolic extracts of seaweed of *Enteromorpha intestinalis, Dictyota dichotoma* possess active compounds for development of larvicidal activity. (Margaret Beula *et al.*, 2011). Larvicidal property of *Ulva fasciata* and *Grateloupia lithophila* against *Culex quinquefasciatus* has already been carried out by Poonguzhali & Nisha (2012). Keeping this background into account, the present study was initiated to explore the potential of two major seaweeds *Enteromorpha flexuosa* and *Gracilara corticata* infested along southwest coast of India as a potential source of marine biolarvicide.

MATERIALS AND METHODS

Collection of algae and extract preparation

Two seaweed samples, *Enteromorpha flexuosa* and *Gracilara corticata* were collected from the Kovalam coast, 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

Culex larvae used for the study 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 was carried out with larvae, which were 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 h and the mortality/ survival were registered after the first 24 h. 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 h 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

The larvicidal activity of three different extracts (methanol, acetone, and benzene) of *E.flexuosa* and *G.corticata* against the larvae of *C.quinquefasciatus* was performed under laboratory evaluation showed that ($LC_{50\ and}$ LC_{90}) values were calculated in the case of *E.flexuosa* LC_{50} value of the methanol extract was *550.12825* acetone extract was *545.98847* and benzene extract was *575.75454* and for *G.lithophila* LC_{50} value of the methanol extract was *184.31237*, acetone extract was *159.72002* and benzene extract was *444.76765*. *E.flexuosa* showed a value of *898.63373* for methanol extract *887.90287* for acetone and *959.40607 for* benzene extract. For *G.corticata* LC_{90} value showed a maximum of *901.88268* for benzene extract.

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

Extract	LC50	95% Confidence Limits		LC90	95% Confidence Limits	
	(mg/L)	LCL	UCL	(mg/L)	LCL	UCL
Methanol	550.12825	498.35041	619.85503	898.63373	797.47265	1047.61986
Acetone	545.98847	495.46893	613.53647	887.90287	789.76092	1031.47776
Benzene	575.75454	516.05649	659.76322	959.40607	839.99754	1142.05693

LC50 and LC90 = Concentration required to kill 50 and 90% of the test populations respectively; 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 *Culex* mosquito larvae

Extract	LC50	95% Confidence Limits		LC90	95% Confidence Limits	
	(mg/L)	LCL	UCL	(mg/L)	LCL	UCL
Methanol	184.31237	160.54831	205.04537	343.93769	316.09194	381.87838
Acetone	159.72002	130.23956	183.53942	336.93372	307.28370	378.09259
Benzene	444.76765	377.46698	546.69159	901.88268	742.48255	1215.06889

LC50 and LC90 = Concentration required to kill 50 and 90% of the test populations respectively; LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit.

Table 1 illustrates the larval mortality rate of *C.quinquefasciatus* after the treatment for a period of 24 hours of the three different extracts of *E.flexuosa* at different concentrations ranging from 100 - 500 mg/L. Both the LC_{50} and LC_{90} values revealed that the larvae *Culex* was more susceptible to benzene extract followed by acetone and methanol extract. The order of hierarchy of larvicidal activity was Acetone > Methanol > Benzene.

The larval mortality rate of *C.quinquefasciatus* after the treatment of the three different extracts of *G. Corticata* for a period of 24 hours different concentrations (100 - 500 mg/L). Both the LC_{50} and LC_{90} values revealed that the larvae *Culex* was more susceptible to acetone extract followed by benzene and methanol extract. The order of hierarchy of larvicidal activity Acetone > Methanol > Benzene (Table 2).

DISCUSSION

Seaweeds are an excellent source of components with biological activity such as antibacterial (Ravi Kumar *et al.,* 2002), antifungal (Ravi kumar *et al.,2009*), antiviral (Wang *et al., 2009*), anti-inflammatory (Tan *et al.,* 2000), cytotoxic (Jimenez *et al., 2010*), nematicidal (Manilal *et al., 2009*) larvicidal ((Manilal *et al., 2009*) and also they contain many useful medicinal properties. The studies on larvicidal activities with seaweed extracts are too restricted (Subhash *et al., 2010*); hence, the present study was to investigate the larvicidal activity of the two seaweed extracts. Among the two seaweeds *G. Corticata s*howed an LC_{50} value with minimum concentration when compared with *E.flexuosa* 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 a*l., (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 was reported by Chapagain *et al.,* 2008) extracts *Gracilaria crassa* and *Hypnea valentia* have shown good larvicidal activity with a LC50 of about 52.2 and 53.4 mg/L respectively against *Aedes* sp. (Anandhan & Sorna 2011).

Our also showed that, the seaweed *G. corticata* is highly efficient in causing mortality of mosquito larvae with the minimum lethal concentration than *E.flexuosa.*

CONCLUSION

From the present study it is concluded that the seaweeds are 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 a effective mosquito control properties and also can act as an eco-friendly, bio-pesticide for further vector control programs.

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