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LARVICIDAL POTENTIAL OF SEAGRASS EXTRACTS AGAINST DENGUE VECTOR *AEDES AEGYPTI* (INSECTA: DIPTERA: CULICIDAE)

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ABSTRACT

The present study was made an attempt to identify the larvicidal activity of the seagrass extracts. Four seagrass species and different plant extract (leaf and root) *viz., Halophila ovalis* (AUOCAS025)*, Enhalus acorodies* (AUOCAS026)*, Thalassia hemprichii* (AUOCAS027) and *Halodule pinifolia* (AUOCAS028) were tested against *A. aegypti* mosquito larvicidal activity. Batches of 25 early 4th instar larvae of *A. aegypti* were transferred to 250ml enamel bowl containing 199ml of distilled water and 1ml of plant extracts (0.01mg-0.1mg). Each experiment was conducted with three replicated and a concurrent control group. A control group consisted of 1ml of DMSO and 199ml of distilled water only. The larvae were considered dead at the end of 24 hrs and the percentage of mortality was determined with the formulae with % of test mortality -% of control mortality/100-% of control mortality X100. The extract of *H. ovalis* showed maximum larvicidal activity (LC_{50} value 0.067±0.007 µg.ml^{-1} and LC_{90} value = 0.128±0.025 µg.ml^{-1}) followed by leaf extract of *E. acorodies* (0.0852 ± 0.006 µg.ml^{-1} and LC_{90} = 0.1369±0.036). Similarly no mortality was found in extracts of *T. hemprichii* (leaf) *H. pinifolia* (leaf) showed no mortality of 4th instar larvae of *A. aegypti*. It is concluded from the present study that, the ethanolic extract of seagrass *H.ovalis* possesses and they can be developed as lead compounds for mosquito control.

KEYWORDS*: Aedes aegypti*, Dengue, Larvicidal, LC_{50}, LC_{90} Mosquito control.

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INTRODUCTION

Mosquitoes are vectors of several diseases like malaria, filariasis, dengue fever, yellow fever, etc., causing serious health problems to human beings. The present resurgence of these diseases is due to the higher number of breeding places in today's throaway society. Further, the indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance and toxic hazards to human being. Globally, there has been a conscientious effort to overcome these problems and great emphasis has been placed recently on eco-friendly and economically viable methodologies for pest control[1.] Observed that, it showed more dependency on human blood rather than on other vertebrates. Moreover, the insects in general and *A. aegypti* in particular, developed resistance to a variety of insecticides. These factors have created a search for biodegradable and target-specific insecticides for the mosquitoes. Thus, research is focused on finding newer insecticides which will be effective, safe and also easily available at low cost. Many plants produce secondary components that have insect growth inhibitory activity. Indeed, many plant extracts were studied for their analyzed to kill larvae of different species of mosquitoes[2] . So far, only a few insecticides of plant origin have reached the market. Seagrasses are the marine flowering plants. They are the only angiosperms that successfully grow in tidal and sub tidal marine environment. Seagrass belongs to the families Hydrocharitaceae and Potamogetonaceae and they are in no way related to the terrestrial grass of Poaceae[3]. There are 13 genera and 58 species available all over the world. Of these six genera (*Amphibolis, Heterzostera, Phyllospadi, Posidonia, Pseudalthenia* and *Zostera*) are mostly restricted to temperate seas and the remaining seven genera (*Cymodocea, Enhalus, Halodula, Halophila, Syringodium, Thalasia and Thalassodendeon*) are distributed in tropical seas. A variety of medicines and chemical are also prepared from seagrass and their associates[4]. Microbiologists and pharmacologists haveincreased their attention during the recent years towards seagrasses and marine algae, which constitute the potential bioactive substances[4]. There has been an exciting progress in the field of marine natural products in the last twenty five years. It has been realized that, many of these metabolites being biologically active are of biomedical importance and could be used as potential drugs[5-8] . Seagrasses produces many secondary metabolites (e.g. phenolic compounds) in order to protect itself against microorganisms and epiphytes[6,9-10] . Thus, objective of the present study is to evaluate the larvicidal effect of ethanolic extract of Seagrasses against *Aedes aegypti* mosquito larvae.

MATERIALS AND METHODS

(i) Plant Materials

Different plant parts (leaf and root) of seagrass plants (*Halophila ovalis* (AUOCAS025)*, Enhalus acorodies* (AUOCAS026)*, Thalassia hemprichii* (AUOCAS027) and *Halodule pinifolia* (AUOCAS028) were collected in early morning from Mandapam (Latitude 9°45′ N and Longitude 79°13′ E) of South East coast of India and were botanically authenticated by Dr. K. Eswaran, Scientist, Central Salt and Marine Chemical Research Institute (CSMCRI), Mandapam Camp, Ramanathapuram District, Tamilnadu, India. A sample voucher specimen is deposited in the herbarium facility maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, Ramanathapuram District, Tamilnadu, India. Sponsored by Indian Council of Medical Research, New Delhi.

(ii) Extract preparation

All the collected samples were washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated organism. Shade dried seagrasses were

subjected for percolation by soaking in ethanol and water mixture (3:1). After 21 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporation (>45°C) and then freeze-dried (−80°C) to obtain solid residue. The percentage of extraction was calculated by using the following formula: % of extraction = Weight of the extract / Weight of the plant material ×100. The extracts of seagrasses were further tested for the presence of phytochemical constituents following the method of Ravikumar et al. (2011a)[7].

(iii) Mosquito larval Culture

To satisfy the enormous number of mosquitoes needed for the day to day bioassays, a colony is essential. The eggs and egg rafts of *Ae. aegypti* were procured from Vector Control Research Centre (VCRC), Puducherry, India. Filter paper with attached eggs was dipped into a plastic tray containing 500 ml of dechlorinated water for 30 – 40 min, time enough to allow for eggs to hatch into larvae. They were reared indoors at 28±2°C temperature and 14:10 light and dark period cycle. The larvae were fed with powdered mixture of dog biscuits and yeast powder in 3:1 ratio. Five days after emergence, female mosquitoes were moved into a mosquito cage where the emergent adults were fed with a 10% sucrose solution and allowed to blood feed from white mice for 2-3 hrs. A few days after having a blood meal, the gravid mosquito laid their eggs.

(iv) Larvicidal activity

The larvicidal effect of ethanolic extract of 4 seagrasses species *viz., Halophila ovalis, Enhalus acorodies* (leaf and root), *Halodule pinifolia* and *Thalassia hemprichii* (leaf and root) against *Ae.aegypti* was conducted. Each seagrass extractaredissolved in DMSO to prepare a graded series of concentration. Batches of 25 early 4th instar larvae of *Ae. aegypti* were transferred to 250 ml enamel bowl containing 199 ml of distilled water and 1ml of plant extracts (0.01 mg – 0.1 mg). Each experiment was conducted with three replicates and a concurrent control group. A control group consisted of 1 ml of DMSO and 199 ml of distilled water only. After treatment, symptoms in treated larvae were observed and recorded immediately at different time intervals and no food was offered to the larva at this time. The larvae were considered dead if, at the end of 24 hrs, they showed no sign of swimming movements even after gently touching with a glass rod, as described in the World Health Organization's technical report series. Subsequently, the lower concentration of crude extract that had successfully produced more than 50% larval mortality was used in a toxicity test on a non – target organism. The percentage of mortality was calculated by with Abbott's formula: % a test mortality - % of control mortality/100 - % of control mortalityX100.

(v)Statistical analysis of data

The average larval mortality data were subjected to profit analysis to calculate LC_{50}, LC_{90} and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression equation, chi- square and analysis variation values were calculated using the Stat plus 2009 software. Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The percentage yields of extracts ranged from 2.5 to 3.14 and values are presented in Table 1. It reveals that, *H. ovalis* (3.14%) showed maximum yield followed by *E. acorodies* (2.08%). The LC_{50} and LC_{90} values of the seagrass extracts against *Ae.aegypti* listed in Table 2. The extract of *H. ovalis* showed maximum larvicidal activity (LC_{50} value 0.067±0.007 µg. ml^{-1} and LC_{90} value = 0.128±0.025 µg. ml^{-1}) followed by leaf extract of *E. acorodies* (0.0852 ± 0.006 µg. ml^{-1} and LC_{90} = 0.1369±0.036). Similarly no mortality was found in extracts of *T. hemprichii* (leaf) *H. pinifolia* (leaf) showed no mortality of 4th instar larvae of *Ae. aegypti*. The regression equation of leaf extract of *H. ovalis* and *E. acorodies* for

4th instar larvae were Y= 3.02+0.622x (R^2= 0.985) and Y=2.2+0.76x (R^2 =0.829) respectively. The chi-square and analysis of variation was significant at p<0.05 level. The preliminary phytochemical study reveals that, the extracts from seagrasses have variety of phytochemical constituents, namely, saponins, steroids, terpenoids, phenols, protein and sugars.

Table 1
Percentage of ethanolic leaf extracts from seagrass species

Botanical Name	Plant Parts	Weight of plant part (g)	Yield of extract	
			(g)	%
Halophila ovalis	leaf	48	1.51	3.14
Enhalus acorodies	leaf	35	0.98	2.8
	root	35	0.90	2.5
Halodule pinifolia	leaf	40	1.12	2.1
Thalassia hemprichii	leaf	38	1.06	2.7
	root	25	0.68	2.72

Table 2
Larvicidal activity of ethanolic extracts of seagrass leaves against Aedes aegypti

Name of the Species	Plant parts	LC_{50}±SE (LCL-UCL)	LC_{90}±SE	Regression Equation	R^2	X^2	P-Value
Halophila ovalis	leaf	0.067±0.007 (0.0529-0.0811)	0.128±0.025 (0.115-0.131)	Y= 3.02+0.622x	0.985*	0.483*	0.048*
Enhalus acorodies	leaf	0.0852±0.006 (0.072-0.101)	0.1369±0.036 (0.120-0.148)	Y=2.2+0.76x	0.829	0.851	0.801
	root	0.092±0.069 (0.058-0.0125)	0.115±0.068 (0.096-0.026)	Y=1.25+0.35x	0.801	0.441	0.081
Thalassia hemprichii	leaf	No Mortality					
	root	0.096±0.002 (0.054-0.123)	0.121±0.069 (0.098-0.269)	Y=1.36+036x	0.810	0.412	0.125
Halodule pinifolia	leaf	No Mortality					

**** Significant at p<0.05 level, LCL – Lower confidence level, UCL – Upper confidence level, X^2 = Chi- square***

The activity of crude plant extracts is often attributed to the complex mixture of active compounds[11] . The preliminary screening is a good mean of evaluation of the potential larvicidal activity of plants popularly used for this purpose. Bioactive marine natural products play an important role in chemotherapy. The evidence for the use of marine flora to be precise in treatment of human aliments is extensive. In Asian maritime areas, seagrass extracts were used as curative agents for various maladies such as antimalarial[9] , antibacterial[11] , antifertility[12] . The studies on larvicidal activities with seaweed extracts are too restricted; hence, the present study was investigated with three seagrass species. Among the seagrass, *H. ovalis* showed minimum LC_{50} values of 0.067±0.007 µg. ml^{-1} when compared with other seagrass species respectively; this may due to the flavonoid sulfates[13] ; the mechanism of action may due to the inhibition of *A. aegypti* which inhabits the mosquito larvae alterations in the spiracular valves of the siphon and anal papillae[10] ; The presence of phenols and reducing sugars are proved to have potential mosquito larvicidal activity[14] . Phenolic groups are highly hydroxylated which includes hydroxylcoumarins, hydroxycinnamate derivatives, flavanols, flavanones, flavones, anthocyanins, proanthocyanidins, hydroxystilbenes, aurones, etc.[14] reported that, antibacterial activity of the root extract of *Cymodocea serrulata* against the poultry pathogen might be due to the presence of major chemical classes such as alkaloid and tannins. It is evident that, all the extracts showed moderate and low larvicidal effects; however, the highest larval mortality was found

in ethanolic extract of *H. ovalis* (0.067±0.007 µg. ml^{-1}). The result reported by the present study shows the possibility of further investigations of efficacy forms the basis for further study investigation an pther marine natural products against mosquito larvicidal effect..

CONCLUSION

Present study concluded that the seagrass, which were collected from South East coast of India, showed enormous resources to find out the new marine products with mosquito larvicidal activities. Further studies on synergistic combinations and isolation of bioactive fraction / constituent may provide futuristic lead products for field application of mosquito control.

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