Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

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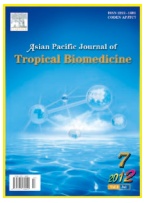

Document heading doi:10.1016/S2221-1691(12)60099-9

Bioactivity of seagrass against the dengue fever mosquito *Aedes aegypti* larvae

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ARTICLE INFO

Article history:
Received 15 August 2011
Received in revised form 3 October 2011
Accepted 3 December 2011
Available online 28 July 2012

Keywords:
Aedes aegypti
Bioinsecticides
Dengue fever
Larvicides
Seagrass
Syringodium isoetifolium
Cymodocea serrulata
Halophila beccarii
Mosquito
Extract

ABSTRACT

Objective: To identify the larvicidal activity of the seagrass extracts. **Methods:** Seagrass extracts, *Syringodium isoetifolium* (*S. isoetifolium*), *Cymodocea serrulata* and *Halophila beccarii*, were dissolved in DMSO to prepare a graded series of concentration. Batches of 25 early 4th instars larvae of *Aedes aegypti* (*Ae. aegypti*) were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (0.01 mg - 0.1 mg). After 24 h the mortality rate was identified with the formulae [(% of test mortality - % of control mortality)/(100 - % of control mortality)] × 100. 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. **Results:** The root extract of *S. isoetifolium* showed maximum larvicidal activity with minimum concentration of extract of LC_{50}= 0.0604 ± 0.0040) μg/mL with lower confidence limit (LCL) - upper confidence limit (UCL) = (0.051-0.071) and LC_{90}=0.0972 μg/mL followed by leaf extract of *S. isoetifolium* showed LC_{50}= (0.062 ± 0.005) μg/mL. The regression equation of root and leaf extract of *S. isoetifolium* for 4th instar larvae were Y= 4.909 + 1.32x (R^2= 0.909) and Y= 2.066 + 1.21x (R^2 =0.897) respectively. The results of the preliminary phytochemical constituents shows the presence of saponin, steroids, terpenoid, phenols, protein and sugars. **Conclusions:** From the present study the ethanolic extracts of seagrass of *S. isoetifolium* possesses lead compound for development of larvicidal activity.

1. Introduction

Insect- transmitted diseases are major health problems in tropical regions. *Aedes aegypti* (*Ae. aegypti*) (Culicidae) occurs in Asia, Africa and Central and South America. It transmits virus of *Flavivirus* genus, etiologic agents of human diseases like dengue and yellow fever[1]. Various synthetic products and devices have been designed to combat resistance developed by various mosquito species. Most of the mosquito control programmes target the larval stage in their breeding sites, while adulticides may only reduce the adult population temporarily[2,3]. The chemicals derived from plants have been projected as weapons in future mosquito control programme as they are shown to function as general toxicant, growth and reproductive inhibitors, repllents and oviposition-deterrent[4]. Pyrethrin based products have been widely used to protect people from mosquito bites through their repellent and killing effects. Many other products of botanical origin especially, essential oils hold significant promise in insect vector management[5]. Marine organisms are a rich source of structurally novel and biologically active metabolites. Many chemically unique compounds of marine origin with different biologically activity have been isolated and a number of them are under investigation and /or are being developed as new pharmaceuticals[6-12]. Seagrass are marine flowering plants that successfully grow in tidal marine environment. Seagrasses consist of about 60 species marine flowering plants, which form the most widespread and productive coastal systems in the world[13]. A variety of medicines and chemicals are prepared from seagrass and their associates[9,14]. Several species of seagrass produce antimicrobial compounds that may act to reduce or control

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Foundation Project: Supported by Ministry of Environment and Forest [Grant No. D.O. NO. 3/2/2004 CS(M)-11.12.2006].

microbial growth[15-17]. New trends in drug discovery from natural source emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities for the treatment of many diseases such as cancer, inflammatory condition, arthritis, malaria and large variety of viral bacterial, fungal disease[16-20]. In folklore medicine, seagrasses have been used for a variety of remedial purposes, *e.g.* for the treatment of fever and skin diseases, muscle pains, wounds and stomach problems, remedy against stings of different kinds of rays, tranquillizer for babies[21]. The objective of the present study was to evaluate larvicidal effect of ethanolic extract of seagrasses against the 4th instar larva of *Ae. aegypti* mosquito.

2.Materials and methods

2.1. Plant materials

Fresh seagrasses of *Syringodium isoetifolium* (*S. isoetifolium*), *Cymodocea serrulata* (*C. serrulata*) and *Halophila beccarii* (*H. beccariii*) (leaves and root) were collected from Thondi (Latitude 9°44′ N, Longitude 79°18′ E) coast and authenticated by Dr. K Eswaran, Scientist, Central Salt and Marine Chemical Research Institute, Mandapam Camp, Ramanathapuram District, Tamil Nadu, India. A voucher specimen is deposited in the herbarium cabinet facility, maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, Ramanathapuram District, Tamil Nadu, India.

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

2.2. Extract preparation

Shade dried seagrasses were subjected to 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 ℃) and then freeze-dried at -80 ℃ 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 by following the methods of Ravikumar S *et al*[12].

2.3. Mosquito larval culture

To satisfy the enormous number of mosquitoes need 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, 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) ℃ 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 h. A few days after having a blood meal, the gravid mosquito laid their eggs.

2.4. Larvicidal activity

The larvicidal effect of ethanolic crude extract of three seagrasses *viz.*, *S. isoetifolium*, *C. serrulata* and *H. beccarii* against *Ae. aegypti* was conducted in accordance with the WHO standard method[22]. Seagrass extracts were dissolved 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 different concentration of plant extracts (0.01 mg - 0.1 mg). After treatment, symptoms in treated larvae were observed and recorded immediately at different time intervals and no food was offered to the larvae at this time. The larvae were considered dead if, at the end of 24 h, showed no sign of swimming movements even after gentle touching with a glass rod, as described in the World Health Organization′s technical report series. 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. Subsequently, the lower concentration of crude extract that had successfully produced more than 50% larval mortality rate was used in a toxicity test on a non-target organism. The percentage of mortality was calculated with Abbott′s formula: [(% of test mortality- % of control mortality- % of control mortality)/(100 - % of control mortality)] × 100.

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis to calculate LC_{50}, LC_{90} and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression equation, *Chi*- sequre. Analysis variation were assessed using StatPlus 2009 software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

Table 1

Extractive values of chosen seagrass species.

Seagrass species	Plant parts	Weight of plant part (g)	Yield [g (%)]
S. isoetifolium	leaf	55.00 ± 2.01	1.59 (2.89)
	root	110.00 ± 1.69	8.00 (7.27)
C. serrulata	leaf	63.00 ± 2.45	1.98 (3.14)
	root	81.00 ± 2.01	2.71 (3.34)
H. beccariii	leaf	60.00 ± 2.60	1.65 (2.75)

The percentage yields of extracts ranged from 2.75 to 7.27 and the values are presented in Table 1. It reveals that *S. isoetifolium* root extract (7.27%) showed maximum yield followed by *C. serrulata* root extract (3.34%). The LC_{50} and LC_{90} values of the seagrass extracts against *Ae. aegypti* are listed in Table 2. The root extract of *S. isoetifolium* showed

Table 2
Larvicidal activity of ethanolic extracts of seagrass against *Ae. aegypti*.

Name of the seagrass species	Plant parts	LC_{50} (LCL-UCL) (μg/mL)	LC_{90} (μg/mL)	Regression equation	R^2	X^2	*P*-value
S. isoetifolium	Leaf	0.0620 ± 0.0050 (0.0529-0.0711)	0.8970	Y= 2.066 + 1.21x	0.859	20.0757	0.733
	Root	0.0604 ± 0.0040 (0.0510-0.0710)	0.9090	Y= 4.909 + 1.32x	0.995	25.7650**	0.049*
C. serrulata	Leaf	0.0780 ± 0.0090 (0.0580-0.0900)	0.1675	Y= 3.667 + 0.84x	0.625	1.3334	0.762
	Root			No mortality			
H. beccarii	Leaf			No mortality			

*- P <0.05; X^2 = *Chi*- square**. LCL- Lower confidence level; UCL- Upper confidence level.

maximum larvicidal activity with minimum concentration of the extract of LC_{50} at does of (0.0604 ± 0.0040) μg/mL and LC_{90} (0.0972 μg/mL) followed by leaf extract of *S. isoetifolium* LC_{50} (0.062 ± 0.005) μg/mL and LC_{90} (0.0992 μg/mL) and leaf extract of *C. serrulata* LC_{50} (0.078 ± 0.009) μg/mL. 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, such as saponin, steroids, terpenoid, phenols, protein and sugars (Table 3).

Table 3
Phytochemical constituents in chosen seagrass species.

Phytochemical constituents	*S. isoetifolium*		*C. serrulata*		*H. beccarii*
	Root	Leaf	Root	Leaf	Leaf
Alkaloids	+	+	+	-	+
Carboxylic acid	+	+	-	-	-
Coumarins	-	-	-	-	-
Flavanoids	++	+	-	-	-
Quinones	-	-	+	-	-
Phenols	+	-	+	-	-
Saponins	+	-	-	-	-
Xanthoproteins	-	-	-	-	-
Protein	-	-	-	-	-
Resins	-	-	-	-	-
Steroids	+	-	-	-	-
Tannins	+	-	+	-	-
Sugars	++	+	-	-	+

-: Absent, +: Medium, ++: High.

4. Discussion

Large number of terrestrial plants were screened for mosquito larvicidal activity to avoid the environment pollution caused by using synthetic chemicals in mosquito control practices. Natural products of plant origin with insecticidal properties have been tried in the past for the control of variety of insect pests and vectors. They are generally preferred because of their less harmful nature to non-target organisms due to their innate biodegradability. Plants are considered as a rich source of bioactive chemicals[23] and they may be an alternative source of mosquito control agents. 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 are used as curative agents for various maladies such as anti malarial[11,12,20], antibacterial[16,17], antihelmintic, cough, antipyretic, wound healing, treatment of gallstone and goiter[24-32]. The studies on seaweed extracts with larvicidal activities are too restricted. Hence, the present study was carried out to find out the mosquito larvicidal effect of seagrass extract. The seagrass root extract of *S. isoetifolium* showed maximum larvicidal extract with minimum concentration of the extract of LC_{50} values of (0.0604 ± 0.0040) μg/mL when compared with other seagrass species involved in this study. This might be due to the flavonoid sulfates which inhibits the mosquito larvae alterations in the spiracular valves of the siphon and anal papillae[33, 34]. The presence of phenols and reducing sugars are proved to have potential mosquito larvicidal activity[35]. Phenolic groups are highly hydroxylated which includes flavonols, hydroxycoumarins, hydroxycinnamate derivatives, flavanols, flavanones, anthocyanins, proanthocyanidins, hydroxystilbene, aurones *etc*. Ravikumar *et al* reported that, the antibacterial activity of root extracts of *C. serrulata* against the poultry pathogen might be due to the presence of major chemical classes such as alkaloid and tannins. It was evident that, all the extracts showed moderate and low larvicidal effects; however, the highest larval mortality was found in ethanolic root extract of *S. isoetifolium* (0.0604 ± 0.0040) μg/mL. It is concluded from present findings that, the root extract of *S. isoetifolium* can be used as potential larvicidal agent against *Ae. aegypti* mosquito larvae.

This report demonstrating the mosquito larvicidal activity of the *S. isoetifolium* is an encouraging trend unraveling the potential of the Indian coastline as a source of marine organisms worthy of further investigation. These organisms are currently being investigated in detail with the objective of isolating biologically active molecules which could be lead chemicals for bio-insecticides.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to the authorities of the Alagappa University for providing the required facilities and also to the Ministry of Environment and Forest, Delhi for providing financial assistance to carry out Ph.D degree for M Syed Ali, Junior Research Fellow.

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