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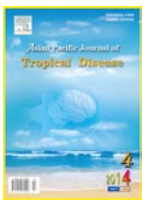

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Bioactivity of sea grass against the malarial fever mosquito *Culex quinquefasciatus*

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PEER REVIEW

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Comments

The present resurgence of vector-borne diseases is due to the higher number of breeding places in today's society. Further the indiscriminate use of synthetic insecticides is creating multifarious problems, insecticides resistance and toxic hazards to human. The marine plants are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential.
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ABSTRACT

Objective: To identify the larvicidal activity of the seagrass extracts against *Culex quinquefasciatus* (*Cx. quinquefasciatus*)
Methods: Seagrass extracts, *Halodule pinifolia* (*H. pinifolia*), *Cymodocea serrulata* (*C. serrulata*) and *Thalasia testudinum* (*T. testudinum*) were dissolved in dimethylsulfoxide to prepare a graded series of concentration. Batches of 25 early 4th instars larvae of *Cx. quinquefasciatus* 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 dimethylsulfoxide and 199 mL of distilled water only.
Results: The root extract of *H. pinifolia* showed maximum larvicidal activity with minimum concentration of extract of LC_{50} value of (0.614±0.006) μg/mL with lower confidence limit-upper confidence limit value of (0.052-0.072) and LC_{90} value of 0.9120 μg/mL followed by leaf extract of *C. serrulata* LC_{50} value of (0.074±0.008) μg/mL and LC_{90} value of 0.1487 μg/mL. *T. testudinum* leaf extract showed LC_{50} value of (0.082±0.006) μg/mL. The regression equation of root and leaf extract of *H. pinifolia* for 4 th instar larvae of *Cx. quinquefasciatus* were Y=5.229+1.36x (R^2=0.993) and Y=2.369+1.21x (R^2=0.878) respectively and analysis of variation was significant at P<0.05 level. The result of the preliminary phytochemical constituents showed the presence of saponin, steroids, terpenoid, phenols, protein and sugars.
Conclusions: From the present study the ethanolic extracts of seagrass of *H. pinifolia* possess lead compound for development of larvicidal activity.

KEYWORDS

Malarial fever, Larvicides, *Halodule pinifolia*, *Cymodocea serrulata*, *Thalasia testudinum*, *Culex quinquefasciatus*

1. Introduction

Mosquito born diseases are major health problems in tropical regions. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) a vector of malaria is widely distributed in Asia, Africa, Central and South America. However, no part of the world is free from vector-borne diseases[1]. Mosquitoes are the most important single group of insects, which transmit a number of diseases such as malaria filariasis, dengue, Japanese encephalitis *etc*., causing millions of death every year. *Cx. quinquefasciatus* is the major malaria vectors in India. With an annual incidence

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of 300-500 million clinically manifest cases and a death toll of 1.1-2.7 million. Malaria is still one of the most important communicable diseases. Currently about 40% of the world's population lives in areas where malaria is endemic[2,3]. One can speculate that people controlled and killed mosquitoes and other domestic insect pests by physically removing them or by using plant parts and plant derivatives before the advent of synthetic chemicals.

The present resurgence of these diseases is due to higher number of breeding places in today's throwaway society. Further, the indiscriminate use of synthetic insecticides is creating multifarious problems such as environmental pollution, insecticide resistance and toxic hazardous to humanbeings. Synthetic insecticides such as organochlorine, organophosphourous, carbamates, pyrethrins and pyrethroids are commonly used for controlling the ever increasing population of vectors. The overuse of these chemical insecticides is not safer due to environment hazard and non target organisms has resulted in resistant development[4,5]. Hence, alternative approaches free from such problems are the need for modern time to development of environmentally safe, biodegradable, cost effective indigenous methods consisting of legal scientific and technological weapons with more powerful combatable properties against such vectors.

Marine organisms are 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 being developed as new pharmaceuticals[6-12]. Seagrass are marine flowering plants that successfully grow in tidal marine environment. A variety of medicines and chemicals are prepared from seagrass and their associates[9,13]. In this background, the present study was made as an attempt to find out the mosquito larvicidal efficacy of ethanolic extracts of seagrass against the 4th instar larva of *Cx. quinquefasciatus* mosquito.

2. Materials and methods

2.1. Sample collection and extract preparation

Live and healthy samples of the seagrasses like *Halodule pinifolia* (*H. pinifolia*), *Cymodocea serullata* (*C. serullata*) and *Thalasia testudinum* (*T. testudinum*) (leaves and root) were collected from Tuticorin coast (Lat. 8048′ N and Long. 78011′ E) of Gulf of Mannar, India. These samples were thoroughly washed with seawater to remove all epiphytes, shells etc., and again washed with fresh water to remove the surface salts, sand particles if any and allowed to dry in the shady place for 3 to 4 d. The collected samples were identified by using standard books and manuals. 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 *et a l*[12].

2.2. Mosquito larval culture

To satisfy the enormous number of mosquitoes need for the day to day bioassays, a colony is essential. The eggs of *Cx. quinquefasciatus* were procured from National Centre for Communicable Diseases (NCCD), Mettupalayam, Tamil Nadu, 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.

2.3. Larvicidal activity

The larvicidal effect of ethanolic crude extract of three seagrasses *viz.*, *H. pinifolia*, *C. serrulata* and *T. testudinum* against *Cu. quinquefasciatus* was conducted in accordance with the WHO standard method[14]. Seagrass extracts were dissolved in dimethylsulfoxide to prepare a graded series of concentration. Batches of 25 early 4th instar larvae of *Cx. quinquefasciatus* were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL 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 dimethylsulfoxide 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[15].

$$\% \text{ mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100\%$$

2.4. 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 variations values were calcuted using Stat Plus 2009 software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The percentage yield of extracts ranged from 2.49% to 6.27% and values are presented in Table 1. It revealed that *H. pinifolia* root extract (6.27%) showed maximum yield followed by *C. serrulata* root extract (3.54%). The LC_{50} and LC_{90} values of the seagrass against *Cx. quinquefasciatus* are listed in Table 2. The root extract of *H. pinifolia* showed maximum larvicidal activity with minimum concentration of the extract of LC_{50} at dose of (0.614 0±0.005 0) μg/mL and LC_{90} of 0.912 0 μg/mL followed by leaf extract of *C. serrulata* LC_{50} (0.074 0±0.008 0) μg/mL and *T. testudimum* LC_{50} (0.0820±0.0080) μg/mL. The *Chi*-square and analysis of variation was siginificant at $P<0.05$ level. The preliminary phytochemical study reveals that the extracts from seagrass have variety of phytochemical constituents such as saponin, steroids, terpenoid, phenols, proteins and sugars (Table 3).

Table 1

Extractive value of chosen seagrass species.

Seagrass species	Plant parts	Weight of plant part (g)	Yield [g (%)]
H. pinifolia	leaf	52.00±2.01	1.29 (2.49)
	root	100.00±1.59	6.00 (6.27)
C. serrulata	leaf	68.00±2.45	1.98 (3.24)
	root	86.00±2.01	2.61 (3.54)
T. testudinum	leaf	74.00±2.80	1.85 (2.95)

Table 2

Larvicidal activity of ethnolic extracts of seagrass against *Cx. quinquefasciatus*.

Name of the seagrass	Plant parts	LC_{50} (LCL-UCL) (μg/mL)	LC_{90} (μg/mL)	Regrassion equation	R^2	X^2	P-value
H. pinifolia	leaf	0.064 0±0.006 0 (0.056 9-0.073 1)	0.8990	Y=2.369±1.21x	0.878	21.067 0	0.763
	Root	0.061 4±0.005 0 (0.052 0-0.072 0)	0.9120	Y=5.229±1.36x	0.993	25.843 0**	0.048*
C. serrulata	leaf	0.074 0±0.008 0 (0.054 0-0.080 0)	0.1487	Y=3.446±0.82x	0.612	1.443 0	0.682
	Root			No mortality			
T. testudimum	leaf	0.082 0±0.006 0 (0.620 0-0.090 0)	0.1686	Y=3.645±0.94x	0.680	1.623 4	0.743

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

Table 3

Phytochemical constituents in chosen seagrass species.

Phytochemical constituents	*H. pinifolia*		*C. serrulata*		T.testudinum
	Leaf	Root	Leaf	Root	Leaf
Alkaloids	+	+	-	+	+
Carboxylic acid	+	+	-	-	-
Coumarins	-	-	-	-	-
Flavanoids	++	+	-	-	+
Quinones	+	-	-	+	-
Phenols	-	+	-	+	-
Saponins	-	+	-	-	-
Xanthoproteins	+	-	-	-	-
Proteins	+	-	-	-	-
Resins	-	-	-	-	-
Steriods	-	+	-	-	-
Tannins	-	+	-	-	-
Sugars	-	+	++	-	-

-: Absent; +: Medium; ++: High.

4. Discussion

The bioactivity of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts, age of plant parts, solvent used in extraction and mosquito species[16]. Plants and plant parts have been provided as a good source of novel drug compounds, as plant derived drugs have made large contribution to human health. The use of plant extracts, as well as other alternative forms of medical treatment, is enjoying great popularity in the late 1990s. Mangroves are widespread in tropical and subtropical regions and grow in the saline intertidal zones of sheltered coast lines[17]. 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], antibacterial[18,19], antihelmintic, cough, antipyretic, wound healing, treatment of gallstone and goiter[20-28].

Each year, an increasing number of novel marine metabolites are reported in the literature, indicating that the marine environments is likely to continue to be a prolific sources of more natural products for many years to come. The studies on mosquito larvicidal activities with seagrass extracts are too restricted; hence, the present study was investigated with seagrass root extracts of *H. pinifolia* showed maximum larvicidal extract with minimum concentration of the extract LC_{50} value of (0.614 0 ±0.005 0) μg/mL when compared with other seagrass species respectively. This might be due to the presence of saponin and triterpenoids which inhibit the mosquito larvae alterations in the spiracular values of the siphon and papillae[29,30]. Since the commercial product is a mixture of

various substances highly enriched with saponin, it would be of basic interest to isolate its major components and test the larvicidal activity of each of these on mosquito larvae.

The biological activity of this marine plant extracts might be due to various compounds, including phenolic, terpenoides, flavonoids, saponins and alkaloids existing in plant. These compounds may jointly or independently contribute to produce larvicidal activity against mosquitoes[11]. The presence of phenols and reducing sugars are proved to have potential mosquito larvicidal activity[31]. Phenolic groups are highly hydroxylated which includes flavonols, hydroxycoumarins, hydroxycinnamate derivatives, flavanols, flavanones, anthocyanins, proanthocyanidins, hydroxystilbene, aurones *etc*. Ravikumar *et al*.[10] 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 *H. pinifolia* [(0.614±0.0050) μg/mL]. It is concluded from present findings that, the root extract of *H. pinifolia* can be used as potential larvicidal agent against *Cx. quinquefasciatus* mosquito larvae.

It is concluded from the present study that the seagrass, which were collected from south east coast of India, showed enormous resources to find out the new marine product 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.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

According to WHO, The Africa reported 207 million cases of malaria occur in children under fever from 2010-2012. In 2012 malaria killed an estimated 483 000 children in less than five years. Malaria is caused by mosquito born disease that is prevalent in Africa; this parasite victimizes children mostly below the age of fifteen. This study attempts to develop a way to prevent malaria parasite carrying mosquitoes from harming children.

Research frontiers

The studies are carried out to investigate the seagrass extracts against the larvicidal activity of *Cx. quinquefasciatus*.

Related reports

Compared with all seagrass extract *H. pinifolia* had minimum concentration of maximum activity. Previously Mohamed Yacoob evaluated ethanol extracts from seaweeds and mangrove plants extracts for mosquito larvicidal activity against *Ae. aegypti*.

Innovations & breakthroughs

This study showed that the seagrasses have some phytochemicals, which are responsible for the activity against mosquito larvicidal.

Applications

H. pinifolia which were collected from Gulf of Manner, India, showed great potential of mosquito larvicidal activities. Further studies on synergistic combinations and isolation of bioactive fractions are needed.

Peer review

The present resurgence of vector-borne diseases is due to the higher number of breeding places in today's society. Further the indiscriminate use of synthetic insecticides is creating multifarious problems, insecticides resistance and toxic hazards to human. The marine plants are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential.

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