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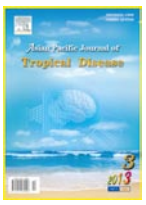

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Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*

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PEER REVIEW

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Comments

The present resurgence of these diseases is due to the higher number of breeding places in today's throwaway society. Further the indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance and toxic hazards to humans.
(Details on Page 200)

ABSTRACT

Objective: To identify the larvicidal activity of the seaweed extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* **Methods:** Seaweed extracts of *Ulva lactuca*, *Caulerpa racemosa* (*C. racemosa*), *Sargassum microystum*, *Caulerpa scalpelliformis*, *Gracilaria corticata*, *Turbinaria decurrens*, *Turbinaria conoides* and *Caulerpa toxifolia* were dissolved in DMSO to prepare a graded series of concentration. The test for the larvicidal effect of seaweeds against mosquitos larvae was conducted in accordance with the WHO standard method. Batches of 25 early 4th instar larvae of three mosquitoes were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (10-100 μg). Each experiment was conducted with triplicate with concurrent a control group. **Results:** Among the seaweeds extract, *C. racemosa* showed toxicity against 4th instar larvae of *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi* with equivalent LC_{50} value (0.055 6±0.010 3) μg/mL, (0.067 5±0.136 0) μg/mL and (0.066 1 ±0.007 6) μg/mL, respectively. **Conclusions:** The present study concluded that, the mosquito larvicidal property of *C. racemosa* might be the prospective alternative source to control the mosquitoes.

KEYWORDS

Anopheles stephensi, *Aedes aegypti*, *Culex quinquefasciatus*, *Caulerpa racemosa*, Larvicidal activity

1. Introduction

Today mosquito plays a predominant role for the transmission of dengue, malaria, yellow fever, filariasis and other several disease which are today among the greatest health problems in the world[1]. 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[2,3]. 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. Natural products are

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generally preferred because of their less harmful nature to target organisms and due to their innate biodegradability. Plant products have been used by traditionally by the human communities in different parts of the world against the vectors and species of insects. The phyto-chemicals derived from plant sources can act as larvicides and insect growth regulators and have deterrent activities observed by many researchers. Marine halophytes are the specialized group of plants adopted for high saline conditions which include seaweeds, mangrove and seagrass. The biodiversity of marine ecosystem provides important sources of chemical compounds, which have many therapeutic applications such as antiviral, antibacterial, antifungal, antifertility and anticancer activities[4-9]. They have been proven to have a rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to nematicidal and insecticidal in lower form of animals[10,11]. In this background, the present study was made as an attempt to find out the mosquito larvicidal efficacy of ethanolic extracts of seaweeds against *Aedes aegypti* (*Ae. aegypti*), *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and *Anopheles stephensi* (*An. stephensi*) vectors.

2. Materials and methods

2.1. Plant materials

Fresh sample of seaweeds *viz.*, *Ulva lactuca* (*U. lactuca*), *Caulerpa racemosa* (*C. racemosa*), *Sargassum microystum* (*S. microystum*), *Caulerpa scalpelliformis* (*C. scalpelliformis*), *Gracilaria corticata* (*G. corticata*), *Turbinaria decurrens* (*T. decurrens*), *Turbinaria conoides* (*T. conoides*) and *Caulerpa toxifolia* (*C. toxifolia*) were collected from Mandapam of south east coast of India (Latitude 9° 45′ N and longitude 79° 13′ E) in January 2011. The identified seaweeds were authenticated by Dr. K. Eswaran, Scientist, Central Salt and Marine Chemical Research Institute (CSMCRI), Mandapam Camp, Ramanathapuram District, Tamil Nadu, India. Voucher specimens were deposited in the herbarium cabinet facility (sponsored by Indian Council of Medical Research, New Delhi) maintained in the School of Marine Science, Alagappa University, Thondi campus, Thondi, Ramanathapuram Dist, 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 animals.

2.2. Extract preparation

Shade dried seaweeds samples were subjected for percolation by soaking in ethanol and water mixture (3:1). After 21 d 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,

$$\%\ \text{extraction} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100\%$$

The extracts of seaweeds were screened for the presence of phytochemical constituents by following the method of Ravikumar *et al*.

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*, *Cx. quinquefasciatus* and *An. stephensi* were procured from National Centre for Communicable Diseases (NCCD), Mettupalayam, TamilNadu, India. Filter paper attached with eggs was dipped into a plastic tray containing 500 mL of de-chlorinated 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. After five days emergence, female mosquitoes were moved into a mosquito cage where the emergent adults were fed with a 100 g/L sucrose solution and allowed for blood feed using white albino for 2-3 h. A few days after having a blood meal, the gravid mosquito laid their eggs.

2.4. Larvicidal activity

The test for the larvicidal effect of ethanolic extract derived from seaweeds against mosquitoes larvae (*Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*) was conducted in accordance with the WHO standard method[12]. Each seaweed extract was dissolved in DMSO to prepare a graded series of concentration. Batches of 25 early 4th instar larvae of three mosquitoes (*Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*) were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (10-100 μg). 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 with at time intervals and no food was offered to the larvae. At the end of 24 h, the larvae were considered dead, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the WHO's technical report series. 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 by with Abbott's formula[13],

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

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 and analysis variation values were calculated using the Stat plus 2009 software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The percentage yields of extracts ranged from 1.64% to 8.69% and are represented in Table 1. It revealed that, the *C. racemosa* (8.69%) showed maximum yield and followed by *C. scalpelliformis* (7.26%) (Table 1).

Table 1
Percentage extraction of ethanolic extracts from seaweed species

Species name	Family	Weight of plant part (g)	Yield of extract	
			Weight (g)	Percent (%)
Ulva lactuca	Ulvaceae	31	1.65±0.92	5.32
C. racemosa	Caulerpaceae	84	7.30±2.21	8.69
S. myriocystum	Sargassaceae	43	0.89±0.25	2.66
C. scalpelliformis	Caulerpaceae	79	10.81±1.10	7.26
G. corticata	Gracilariaceae	80	1.31±0.62	1.64
T. decurrens	Sargassaceae	90	4.18±1.69	4.64
C. toxifolia	Caulerpaceae	62	0.41±0.12	0.66
T. conoides	Sargassaceae	51	2.88±1.90	5.65

The LC_{50} and LC_{90} values of the seaweeds extracts against mosquito larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* are listed in Table 2. The seaweed extract of *C. racemosa* showed minimum level of LC_{50} value (0.055 6± 0.010 3) μg/mL, LCL-UCL=0.031 7-0.105 0, LC_{90}=0.130 6 and the extract of *C. scalpelliformis* showed minimum level of LC_{50} value of (0.0708±0.007) μg/mL, LCL-UCL=0.066 8-0.104 9, LC_{90}=0.140 2. The regression equation of *C. racemosa* and *C. scalpelliformis* for 4th instar larvae were $Y=1.133+0.787x$ (R^2= 0.995) and $Y=0.462x$ (R^2=0.921 2) respectively. The *Chi*-square value was significant at $P<0.05$ level (Table 2). The extract of *T. conoides* and *C. toxifolia* observed no mortality of *Ae. aegypti* and no mortality was observed in the control (Table 2). The tested plant extract observed to 4th instar mosquito larvae of *Cx. quinquefasciatus* was examined (Table 3). *C. racemosa* extract showed minimum level of LC_{50} value of (0.056 9±0.021 3) μg/mL, LCL-UCL=0.017-0.405, LC_{90}=0.258 and the extract of *C. scalpelliformis* showed minimum level of LC_{50} value of (0.067 5±0.136 0) μg/mL, LCL-UCL=0.057-0.119, LC_{90}=0.622. The regression equation of *C. racemosa* and *C. scalpelliformis* for 4th instar larvae were $Y=1.102+0.797x$, (R^2= 0.981) and $Y=0.831+0.619x$ (R^2=0.917) respectively. The *Chi*-square value was significant at $P<0.05$ level (Table 3). The extract of *T. conoides*, *Turbinaria decurrens* and *C. toxifolia* observed no mortality of *Cx. quinquefasciatus* and no mortality was observed in the control (Table 3).

Table 4 shows the toxic effects of tested plant extract on early 4th instar larva of *An. stephensi*. *C. racemosa* extract showed minimum level of LC_{50} value of (0.066 1±0.007 6) μg/mL, LCL-UCL=0.040 2-0.091 0, LC_{90}=0.150 4 and followed by extract of *C. scalpelliformis* showed minimum level of LC_{50} value of (0.066 8±0.117 0) μg/mL, LCL-UCL=0.056 8-0.104 0, LC_{90}=0.140 2. The regression equation of *C. racemosa* and *C. scalpelliformis* for 4th instar larvae were $Y=0.222+0.817x$, (R^2=0.985) and $Y=0302+0.941x$, (R^2=0.899) respectively. The *Chi*-square value was significant at $P<0.05$ level (Table 4). The extract of *S. microystum*, *C. toxifolia* and *T. conoides* observed no mortality of *An. stephensi* and no mortality was observed in the control. The preliminary phytochemical study revealed that, the extracts from seaweeds had variety of phytochemical constituents, namely, carbohydrate, saponin, steroids, terpenoid, phenols, protein and sugars (Table 5).

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

Name of the species	Parts	LC_{50} (LCL-UCL)	LC_{90}	Regression equation	R^2	χ^2	*P*-value
U. lactuca	Whole	0.082±0.007 (0.072-0.102)	0.141	$Y=0.6+0.781x$	0.812	48.213	0.723
C. racemosa	Whole	0.055 0±0.010 3 (0.031-0.105)	0.130	$Y=1.133+0.787x$	0.995	7.982**	0.038*
S. microystum	Whole	0.086±0.006 (0.074-0.099)	0.139	$Y=1.266+0.703x$	0.961	11.056	0.198
C. scalpelliformis	Whole	0.070±0.007 (0.066-0.104)	0.140	$Y=0.462x$	0.921	4.351	0.824*
G. corticata	Whole	0.087±0.007 (0.071-0.102)	0.148	$Y=0.8+0.654x$	0.941	2.365	0.967
T. decurrens	Whole	0.079±0.008 (0.062-0.096)	0.143	$Y=0.8+0.727x$	0.879	4.387	0.820
C. toxifolia	Whole			NM			
T. conoides	Whole			NM			

*Significant at $P<0.05$ level, LCL: Lower confidence level, UCL: Upper confidence level, χ^2=*Chi*-square, NM: No mortality.

Table 3
Larvicidal activity of ethanolic extracts of seaweed plant against *Cx. quinquefasciatus*.

Name of the species	Parts	LC_{50} (LCL-UCL)	LC_{90}	Regression equation	R^2	χ^2	*P*-value
U. lactuca	Whole	0.082±0.007 (0.072-0.102)	1.140	$Y=2.8+3.77x$	0.812	8.2133	0.723
C. racemosa	Whole	0.056±0.021 (0.017-0.405)	0.258	$Y=1.102+0.797x$	0.981	8.422**	0.048*
S. microystum	Whole	0.098±0.013 (0.074-0.099)	0.368	$Y=2.169+0.503x$	0.831	10.051	0.182
C. scalpelliformis	Whole	0.067±0.136 (0.057-0.119)	0.622	$Y=0.831+0.698x$	0.917	8.326	0.044*
G. corticata	Whole	0.091±0.070 (0.080-0.101)	0.148	$Y=0.8+0.654x$	0.941	3.256	0.854
T. decurrens	Whole			NM			
C. toxifolia	Whole			NM			
T. conoides	Whole			NM			

*Significant at $P<0.05$ level, LCL: Lower confidence level, UCL: Upper confidence level, χ^2=*Chi*-square, NM: No mortality.

Table 4
Larvicidal activity of ethanolic extracts of seaweed plant against *Anopheles stephensi*

Name of the species	Parts	LC_{50} (LCL-UCL)	LC_{90}	Regression equation	R^2	χ^2	*P*-value
U. lactuca	Whole	0.091±0.017 (0.072- 0.102)	1.012	$Y=0.6+0.781x$	0.812	48.213	0.723
C. racemosa	Whole	0.066±0.007 (0.040-0.091)	0.150	$Y=0.222+0.817x$	0.985	8.150**	0.041*
S. microystum	Whole			NM			
C. scalpelliformis	Whole	0.066±0.117 (0.056-0.104)	0.140	$Y=0.302+0.914x$	0.899	6.352	2.270*
G. corticata	Whole	0.0910±0.1126 (0.071-0.102)	0.135	$Y=0.7+0.567x$	0.826	3.255	1.867
T. decurrens	Whole	0.0990±0.0884 (0.062-0.096)	0.138	$Y=0.9+0.317x$	0.779	4.387	0.916
C. toxifolia	Whole			NM			
T. conoides	Whole			NM			

*Significant at $P<0.05$ level, LCL: Lower confidence level, UCL: Upper confidence level, χ^2=*Chi*-square, NM: No mortality.

Table 5
Phytochemical constituents of seaweeds extracts.

	Phytochemical constituents												
Seaweeds plants	Alkaloids	Carboxylic acid	Coumarins	Flavanoids	Quinones	Phenols	Saponins	Xanthoproteins	Protein	Resins	Steroids	Tannins	Sugars
U. lactuca	+	-	-	+	-	-	+	-	-	-	-	-	+
C. racemosa	++	-	-	+	-	-	++	-	++	-	-	-	++
S. microystum	-	-	-	-	-	+	-	-	+	-	-	-	++
C. scalpelliformis	-	-	-	-	-	+	-	-	+	-	-	-	++
G. corticata	+	-	-	+	-	-	-	-	+	-	-	-	+
T. decurrens	-	-	-	-	-	-	-	-	++	-	-	-	+
C. toxifolia	+	-	-	-	-	-	++	+	-	-	-	-	-
T. conoides	-	-	-	-	-	-	-	-	-	-	-	-	-

(-)- Absent, (+) - Medium, (++) - High

4. Discussion

Plant extracts and phytochemical have potential as mosquito control properties because many of them are selective, may biodegrade to nontoxic products and may be applied to mosquito breeding places in the same way as conventional insecticides[14]. The marine environment is incomparable reservoirs of bioactive natural products, many of which exhibit structural features that have not been found in terrestrial natural products[15]. 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. Marine algae are found to be vital source of useful bioactive substance since two decades. Several studies have demonstrated with biological activity such as antibacterial[6,8], antiviral[16,17], larvicidal, antifeedant and anticoagulant[18,19]. The studies on mosquito larvicidal activities with seaweeds extracts are too restricted; hence, the present study was investigated with several seaweeds extracts. Among the seaweeds, *C. racemosa* showed minimum LC_{50} values of *Ae. aegypti*, *Cx. quinquefasciatus*

and *An. stephensi* larvae [LC_{50}=(0.0556±0.0103) μg/mL, (0.0569±0.0213) μg/mL, (0.0660±0.0076) μg/mL] when compared with control respectively; this might due the presence saponin and triterpenoids[20]. Since the commercial product is a mixture of various substances highly enriched with saponin, 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. A study along these lines is currently under way by us. Another promising approach to the problem of elucidating the larvicidal action of saponin consists of studying the glycosidic and/or the lipophilic portion of the saponin molecule. Based on the above-cited reports on the effect of saponin on permeabilization and the integrity of the plasma membrane[21]. The ethanol extracts from *Dictyota dichotoma*, *Enteromorpha intestinalis* and *Acanthopora spicifera* were evaluated for mosquito larvicidal activity against *Ae. aegypti* by Ravikumar *et al*. Thangam and Kathiresan reported that, *Caulerpa scalpelliformis* and *Dictyota dichotoma* were found most effective with LC_{50} values of 53.70 and 61.65 mg/L, respectively[22]. Similarly, LC_{50} value of permethrin 10 EC has been documented as 0.36-105 (% v/v) against larvae of *Cx. quinquefasciatus*[23,24], whereas, LC_{50} values for *Pimpinella anisum* oil ranged from 63.3-64.5 g/mL against three mosquito species. Result of present investigation indicate that, *C. racemosa* was minimum larvicidal activity against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. Stephensi*. However, Tawatsin *et al*. have reported the repellent and larvicidal activity against *Ae. aegypti*, *Anopheles dirus* and *Cx. quinquefasciatus* which is due to 5% vanillin which has been added to the essential oil of *Curcuma longa*[25]. Sosan *et al*. reported that larvicidal activities of essential oils of *Ocimum gratissium*, *Cymbopogon citrus* and *Ageratum conyzoides* against *Ae. aegypti* and achieved 100% mortality at 120, 200 and 300 mg/L concentrations respectively[26]. 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 both species of mosquitoes[7].

It is concluded from the present study that the seaweeds, 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

Synthetic insecticides such as organochlorine, organophosphourous, carbamates, pyrethrins and pyrethroids are commonly used for controlling the ever increasing population of vectors. The over use of these chemical insecticides is not safer due to environment hazard and non target organisms has resulted in development of resistance. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to nematicidal and insecticidal in lower form of animals.

Research frontiers

The studies are carried out to investigate the mosquito larvicidal activity of the seaweeds against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* vectors Among different extracts, present investigation indicate that, *C. racemosa* was minimum larvicidal activity against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*. showed minimum concentration of maximum activity mosquito larvicidal.

Related reports

Compared with all the seaweeds extract of *C. racemosa* showed minimum concentration of maximum activity. Previously Syed Ali found ethanol extracts from *Dictyota dichotoma, Enteromorpha intestinalis* and *Acanthopora spicifera* were evaluated for mosquito larvicidal activity against *Ae. aegypti*.

Innovations & breakthroughs

The study showed that the seaweeds plant have some phytochemicals, which are responsible for the activity against the mosquito larvicidal. showed enormous resources to find out the new marine product with mosquito larvicidal activities.

Applications

Seaweeds, 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.

Peer review

The present resurgence of these diseases is due to the higher number of breeding places in today's throwaway society. Further the indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance and toxic hazards to humans. They are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to nematicidal and insecticidal in lower form of animals.

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