

## Original Research

Larvicidal activity of two seaweeds, *Chaetomorpha antennina* (Bory de Saint-Vincent) Kützing and *Sargassum wightii* Greville against mosquito vector, *Anopheles stephensi*

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**ABSTRACT:**

Tropical and subtropical regions of the world are the most prevalent area of malaria and are mainly caused by infection with species of Plasmodium, through *Anopheles* mosquitoes to human. It is critical to manage the spread of disease causing agents by the use of conventional synthetic chemical insecticides to control the mosquitoes. When mosquitoes develop resistance to the insecticides the effectiveness of these chemicals for vector control is diminished. In addition to that, the use of synthetic chemical insecticides leads to environmental pollution and some evidence suggests that these materials act as immune suppressants. The approaches for control of malarial transmission are by interrupting mosquito life cycle at larval stage. The present study is to determine the larvicidal potential of two macroalgae collected from eastern coast of India against malarial parasite *Anopheles stephensi*.

**Keywords:**

Larvicidal potential, *Anopheles stephensi*, *Chaetomorpha antennina* and *Sargassum wightii*.

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## INTRODUCTION

The most dangerous and spreading major public health problem in more than 100 countries, inhabited by a total of some 2.4 billion people, or close to half of the world's population is malaria (Persidis, 2000). Tropical and subtropical regions of the world are the most prevalent area of malaria and are mainly caused by infection with *Plasmodium falciparum*, *P. vivax*, *P. ovale*, or *P. malariae*, which are transmitted to humans only by female *Anopheles* mosquitoes.

It is critical to manage the spread of disease causing agents by use of conventional synthetic chemical insecticides to control the mosquitoes. When mosquitoes develop resistance to the insecticides the effectiveness of these chemicals for vector control is diminished (Tabashnik *et al.*, 1991). In addition to that, the use of synthetic chemical insecticides lead to the cause of environmental pollution. Apart from this, these materials also act as immuno suppressants (Sengottayan, 2007). Chemical control methods have been practiced against the immature or the adult of malaria vectors. Using chemical larvicides has been performed during the fight against malaria in Iran and still considered as an important part of vector mosquito control. But chemical larvicides are now found to be toxic to fish and other non-target organisms as well as to the environment. This also leads to insecticide resistance in arthropods. Extract of whole leaf and essential oil of certain plants have been investigated, and found to be toxic effect against some public health pests (Hadjiakhoondi *et al.*, 2006 and Hadjiakhoondi *et al.*, 2003).

Most of the researches deal with ovicidal, larvicidal and adulticidal activities using plant derived products for controlling the malarial vector (Gbolade, 2001). The larval stage is the most vulnerable and best stage to attack mosquitoes as they are concentrated in smaller areas during their developmental stage. Thus, the approaches for control of malarial transmission are by interrupting mosquito life cycle at

larval stage.

Several species of marine algae from coastlines of India have been reported for this purpose (Sohrabipour and Rabii, 1999). Different secondary metabolites are produced by marine algae with a wide range of bio activities (Mayer *et al.*, 2003-04). Many studies have been achieved on the screening of biological effects of marine organisms and many active compounds were isolated and characterized (Blunden, 2001). Larvicidal property of *Ulva fasciata* and *Grateloupia lithophila* against *Culex quinquefasciatus* has already been carried out by (Poonguzhali and Nisha, 2012). This study was aimed to determine the larvicidal activity of different extracts of *Chaetomorpha antennina* (Bory de Saint-Vincent) Kützing and *Sargassum wightii* Greville, collected from peninsular coast of India, against malarial vector *Anopheles stephensi*.

## MATERIALS AND METHODS

### Collection of algae and extract preparation

Two seaweed samples, *Chaetomorpha antennina* and *Sargassum wightii* were collected from the peninsular coast of India. The healthy algal material were harvested manually and washed thoroughly in running water to remove epizoon, 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 using methanol, acetone and benzene were prepared by submerging the powder in three different flasks 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 three solvents alone) and negative controls (larvae exposed to dechlorinated water without solvent).

**Test mosquito larvae**

Larvae of *Anopheles stephensi* 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 (WHO, 1981). Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides, WHO/VBC, 81:807, with slight modifications. Bioassay were conducted with larvae 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 was 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. The LC<sub>50</sub> and LC<sub>90</sub> value was calculated by using Probit analysis (Finney, 1971).

**RESULTS**

Results of the larvicidal activity of three different extracts (methanol, acetone, and benzene) of *C. antennina* and *S. wightii* against the larvae of *A. stephensi* was performed under laboratory evaluation. The results exhibited larval mortality rate of *A. stephensi* after the treatment of the three different extracts of *C. antennina* and *S. wightii* at different concentrations (100-500 mg/L). In terms of lethal concentration for 50% and 90% mortality (LC<sub>50</sub> and LC<sub>90</sub>) values were represented as follows: LC<sub>50</sub> value of *A. stephensi* extract of *C. antennina* was 432.26, followed by other extracts while LC<sub>50</sub> value of the benzene extract of *S. wightii* was 435.24 followed by methanolic and acetone extract, for *A. stephensi* LC<sub>90</sub> value of the methanol extract of *C. antennina* was 838.87, followed by benzene extract and acetone extract; while LC<sub>90</sub> value of the methanol extract of *S. wightii* was 864.63, followed by benzene and acetone extract (Table 1 and 2). The LC<sub>50</sub> values of *S. wightii* revealed that the larvae *A. stephensi* was more susceptible to benzene extract *C. antennina* followed by methanol and acetone (Benzene> Methanol> Acetone). The larval mortality rate of *A. stephensi* after the treatment of the three different extracts at different concentrations (100-500 mg/L) showed that methanol extract was more efficient than the other extracts and the lethal hierarchy is (Methanol> Benzene> Acetone) (Table 2).

**Table 1 Effect of methanolic, acetone and benzene extracts of *Cheatomorpha antennina* against mosquito larvae *A. stephensi***

Extract	LC <sub>50</sub> (mg/L)	95% Confidence Limits		LC <sub>90</sub> (mg/L)	95% Confidence Limits	
		LCL	UCL		LCL	UCL
Methanol	432.26	386.68687	491.41	838.87	732.30711	1006.40095
Acetone	359.71	304.97613	425.66	728.45	617.45753	930.00502
Benzene	426.74	391.23655	524.24	795.80	681.07149	994.52389

LC<sub>50</sub> = lethal concentration to cause 50% mortality in population.

LC<sub>90</sub> = lethal concentration to cause 90% mortality in population.

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit.

**Table 2 Effect of methanolic, acetone and benzene extracts of *Sargassum wightii* against mosquito larvae *A. stephensi***

Extract	LC <sub>50</sub> (mg/L)	95% Confidence Limits		LC <sub>90</sub> (mg/L)	95% Confidence Limits	
		LCL	UCL		LCL	UCL
Methanol	424.57	362.34390	512.20613	864.63	721.26315	1132.02774
Acetone	363.14	311.81629	424.19872	757.54	648.34725	944.78106
Benzene	435.24	379.38059	511.75093	814.36	694.89253	1020.66012

LC<sub>50</sub> = lethal concentration to cause 50% mortality in population.

LC<sub>90</sub> = lethal concentration to cause 90% mortality in population.

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit.

## DISCUSSION

Mosquito control and the management of the spread of disease causing agents is critical and is based primarily on the use of conventional synthetic chemical insecticides. A number of research from terrestrial plant extracts have been studied previously for toxicity to larvae of *Anopheles* mosquitoes, including those from *Calophyllum inophyllum* (Clusiaceae), *Rhinacanthus nasutus* (Acanthaceae), *Solanum surattense* (Solanaceae), *Samadera indica* (Simaroubaceae), and *Myriophyllum spicatum* (Haloragaceae) (Pushpanathan *et al.*, 2006 and Thangam and Kathiresan, 1991). As India is a peninsular country having wide coastal area with rich reserves of seaweeds they can be easily exploited for various potentials. Our investigations were focused on larvicidal potential of seaweeds.

The acetone extract of seaweeds *Caulerpa scalpelliformis* and *Dictyota dichotoma* exhibited mosquito larvicidal activity against *Aedes aegypti* (Thangam and Kathiresan, 1991). Albeit the larvicidal potential of seaweeds was well established, no evidence was available on the field trials of seaweed based mosquito repellents. (Mullai and Jebanesan, 2006) reported the larvicidal efficacy of the leaf extract of *Cucumis pubescens* with four different solvents against late third instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus*, and *A. aegypti*. The seaweeds (*U. fasciata* and *H. musciformis*) produced 100% larva mortality at 10 mg/mL (Selvin and Lipton, 2004). There is no previous report on the mosquito larvicidal activity

of *C. antennina* and *S. wightii* from the coasts of Tamil Nadu. Of the two algae screened, *S. wightii* was found to be more effective against *A. stephensi* larva in all the three extracts.

*Sargassum wightii* Greville (Sargassaceae) is an abundant marine brown alga commonly found in the shorelines of India. It is a macroscopic, multicellular, photosynthetic, non vascular, pelagic marine species (Sumich and Morrissey, 2004) rich in sulphated polysaccharides that manifest potent free radical scavenging (Park *et al.*, 2005) and antioxidant effects. These properties justify that *S. wightii* from our present study suggest that its biologically active compounds that may be useful in mosquito control and as alternatives to conventional synthetic larvicide. Similarly in the present study, *S. wightii* frond extract treatment resulted in higher larval mortality which might be due to the multiple actions of dioctyl phthalate and other bioactive compounds present in the seaweed.

## CONCLUSION

This study poses the following suggestions: *S. wightii* which is promising locally available seaweed can be cultivated in coastal areas. As the use of seaweeds for larvicidal activity was potent and safe to non target cohabitants, their bioactives which is efficient in larvicidal activity can be explored. The extracts from these algae may be useful for the improvement of new natural insecticides, however, further investigations are needed to identify and purify the effective components and their mechanisms of action of these algae.

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