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**Research Article**

# EFFICACY OF AEDES *AEGYPTI* AND *CULEX QUINQUEFASCIATUS* AGAINST *PADINA GYMNOSPORA* AND *CAULERPA RACEMOSA*

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**ABSTRACT**

This study investigated the mosquitocidal activities of marine algae collected from Gulf of Mannar. The larvae *Aedes aegypti* and *Culex quinquefaciatus* tested against *Padina gymnospora* and *Caulerpa racemosa* were extracted using different solvents like Chloroform, Methanol, Petroleum ether and Acetone. The two different seaweed extracts using four different solvents were proved statistically significant ($p<0.05$). The seaweed extract used for the LC50 assay against II and III instar larval mortality was recorded. The overall results was observed all the solvent extract against *Padina gymnospora* especially more toxic in acdtone and chloroform of *Culex quinquefaciatus* (II & III -100%) followed by chloroform in *Aedes aegypti* (II & III -83%). Likewise the similar species of mosquito larvae (*Aedes aegypti*) against *Caulerpa racemosa* extract in Chloroform, acetone and methanol showed the high mortality was noted. These findings may help in developing a prospective alternative source to control the mosquitoes.



## INTRODUCTION

Mosquitoes are responsible for the spread of more diseases. Mosquito borne diseases still remain a major health problem in both human and veterinary sectors. Mosquitoes, under the order Diptera are ravaging the humans and other animals for generations. There are nearly 2,500 mosquito species in the world but few of them transmit an array of pathogens including viruses (e.g., arboviruses), protozoan's (e.g., malaria) and nematode worms (e.g., lymphatic filariasis). According to Taubes (1997), annually more than 700 million people suffer from mosquito borne diseases. Synthetic insecticides have been widely used to control mosquito vectors of disease in various parts of the world. Plants are a source of bioactive compounds that have insecticidal properties and therefore may be suitable for mosquito control (Prophiro, 1762). the indiscriminate use of synthetic insecticides is creating multifarious problems such as environmental pollution, insecticide resistance and toxic hazardous to humanbeings (Shaalan, 2012). Larvicidal properties of marine water algae (*P. pavonica* and Zonaria sp.) were found to be most effective seaweeds (Hamlyn-Harris, 1928; Griffin, 1956; Dhillon and Mulla, 1981; Semakov and Sirenko, 1985; Pucazhendi *et al.* 1995). The frequent use of systemic insecticides to manage insect pests leads to a destabilisation of ecosystem and enhanced resistance to insecticides in pests (Kranthi *et al*., 2001; Mohan and Gujar 2003), suggesting a clear need for alternatives. 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. Although most of the algae are nutritious food for mosquito larvae, some species kill the larvae when ingested in large quantities (Marten, 1987 and 2007) while blue-green algae toxins may offer possibilities for delivery as larvicides. Certain species of green algae kill larvae primarily because they are indigestible. The algae's metabolites have also been shown in several studies which possess larvicidal activities (Poonguzhali and Nisha, 2012 & Elbanna and Hegazi, 2011). many studies on plant extracts against mosquito larvae have been conducted around the world. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Sharma *et al*., 2006; Amer and Mehlhorn 2006a, b). The Culex mosquito, better known as the common house mosquito, is one of the three major type of mosquitoes inhabiting the planet. Essential oils from plants and algal extracts may be the rich alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive

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compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. Hence in the present study was made as an attempt to find out the mosquito larvicidal efficacy of four solvent extracts of two seaweeds against *Aedes aegypti* (*Ae. aegypti*), *Culex quinquefasciatus* (*Cx. quinquefasciatus*).

## MATERIAL AND METHODS

***Collection and identification of seaweeds***

The present study area Gulf of Mannar (GoM) is a transitional zone between the Arabian Sea and the Indian Ocean. Palk Strait connects GoM with the Bay of Bengal. The GoM has a chain of 21 islands located between 8° 46 N, 78° 9 E and 9° 14 N, 79° 14°E on the southeast coast of India. It has been estimated that 302 seaweed species exist in GoM in particular between pudumadam (SS-1) and Pamban (SS-2) Plate-1. Four species of seaweed samples were collected in the early morning from the intertidal zone of Rameswaram, Tamilnadu during low tide on June 2013. The seaweed *Padina gymnospora,* and *Caulerpa racemosa* were collected by hand picking. The collected macroalgae were immediately rinsed in water to remove all kinds of epiphytes and other impurities *(i.e.,* sand, mollusks, sea grasses etc). The cleaned samples were immediately kept in sterilized ziploc bags and transferred to laboratory.

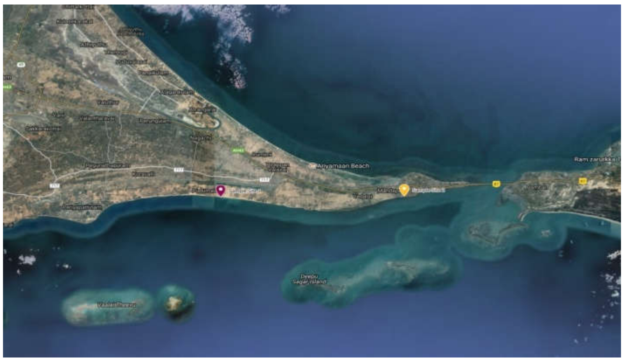

**Plate 1** Overview image of sample collection site from Google Map

***Preparation of extracts***

The cleaned macroalgae were allowed to shade dry (up to 7 days) and all individual samples were made into powder form using mixer grinder. The powdered sample of each species (10 grams) were suspended in selective solvent system (*i.e.,* Chloroform, Methanol, Petroleum ether, Acetone and Butanol) (Merck AR Grade) and kept in a borosilicate soxhlet apparatus for eight hours (WHO, 2005). After that, extracted samples were filtered using Whatman No.1 filter paper. The filtered sample was individually centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected in a separate flask. At each centrifugation, the supernatant was pooled and kept separately. Then the extract was concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland) at 40°C. The final concentrated crude extract was individually stored in sterile air tight bottles and kept in a refrigerator until further use (Celikler *et al*., 2009). The percentage of extraction was calculated by using the following formula. The extract preparation was done by following the method of Ali *et al*., (2012).

$$\textbf{Percentage of extraction} = \frac{\textbf{Weight of the extract}}{\textbf{Weight of the plant material}} \textbf{x100}$$

***Collections and Rearing of Mosquito Larva***

The eggs and egg rafts of two mosquito species *Aedes aegypti* and *Culex quinquefasciatus* were procured from CRME (Center for Research in Medical Entomology), Madurai in ICMR (Indian Council of Medical Research), Tamil nadu, India. Larvae of mosquito species *Aedes aegypti* and *Culex quinquefasciatus* were reared in enamel trays containing dechlorinated water. The larvae were fed with finely powdered mixture having 3:1 ratio of dog biscuits and dry yeast (Govindarajan *et al*., 2011). Then larvae were observed and monitored for II and III instar stages. The larvae were used for toxicological study.

***Larvicidal Bioassay***

The seaweed extracts were screened primarily for larvicidal activity. Briefly 10 numbers of II and III instar larvae were exposed with 0.5mg/ml of the chloroform, petroleum ether, acetone and methanol extract and incubated for 24 hrs at 27±2°C and 14:10 light and dark cycles (WHO, 2005). All the experiments were carried out in triplicates and control respective solvents were maintained. The selected extracts were diluted with distilled water to get desired concentrations of 12.5, 25, 50, 100, 200, 400, and 500 ppm per ml of solution. For bioassay test, 25 numbers of II and III instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were exposed to different concentrations of (12.5, 25, 50, 100, 200, 400, and 500 ppm) of extracts individually. The larvae were starved during the experiment and mortality was recorded after 24 and 48hrs of post-exposure (WHO 1981). Similarly the control was recorded from the average of three replicates. The mortality percentage was calculated using the Abbott's (1925). The $LC_{50}$ were calculated using SPSS software using linear regression. Dead larvae were counted after incubation. The larvae failed to move after probing with dropper were considered as dead. The percentage of mortality was calculated using the following formula;

$$\textbf{Percentage of mortality} = \frac{\textbf{Number of dead Larvae}}{\textbf{Number of larvae tested}} \textbf{x100}$$

## RESULT

The selected two different seaweeds (*Padina gymnospora, Caulerpa racemosa,* were extracted with organic solvents like chloroform, petroleum ether, acetone and methanol. Sixteen extracts were isolated from the above mentioned marine algae and each of the extracts was weighed and calculated and the levels were statistically significant P<0.05 (Table.2).

**Table 2** Percentage (%) of different organic solvent extracts of seaweeds

| Samples | Chloroform | Petroleum ether | Acetone | Methanol |
|---|---|---|---|---|
| *Padina gymnospora* | 2.346 | 1.761 | 2.487 | 2.542 |
| *C. racemosa* | 2.482 | 1.982 | 3.197 | 2.931 |

The results showed that the highest percentage of yield for the seaweeds, *Padina gymnospora* and *Caulerpa racemosa* were observed with petroleum ether, chloroform, acetone, methanol and butanol respectively. The percentage of *P. gymnospora* crude extracts ranged from 2.542 to 1.761 % and for *C. racemosa* it was found to be 3.197 to 1.982 %. The differences of the percentage yield among the solvents were statistically significant for all the tested seaweeds at P<0.05.

Preliminary phytochemical analyse of different biomolecules such as carbohydrates, proteins, flavonoids, phenols, tannins, saponin, steroids and alkaloids were tested in sixteen different seaweed crude extracts (Table-3). Alkaloids and flavonoids did not show any positive result in *Padina gymnospora* and *Caulerpa racemosa*. The presence or absence of the phytochemicals depends upon the solvent medium used for the extraction. Carbohydrates, proteins, steroids, phenols, tannins, saponin and alkaloids flavonoids showed the maximum presence in two different extracts of *Padina gymnospora* and *Caulerpa racemosa*.

**Table 3** Analysis of phytochemical compounds from different solvent extracts against seaweeds

| Name of the Seaweeds | Phytochemicals | Chloroform | Methanol | Petroleum ether | Acetone |
|---|---|---|---|---|---|
| *Padina gymnospora* | Carbohydrates | + | + | - | - |
| | Proteins | + | - | - | + |
| | Alkaloids | - | - | - | - |
| | Steroids | - | + | - | - |
| | Tannin | + | + | + | + |
| | Phenol | + | + | + | + |
| | Saponins | + | - | + | + |
| | Flavanoids | + | - | + | - |
| *C. racemosa* | Carbohydrates | + | + | - | - |
| | Proteins | - | - | - | - |
| | Alkaloids | - | - | - | - |
| | Steroids | + | - | - | + |
| | Tannin | + | + | + | + |
| | Phenol | + | + | + | + |
| | Saponins | + | - | + | - |
| | Flavanoids | - | + | - | - |

The eight different phytochemical compound present in sixteen extracts. The results show some phytochemical compounds are more toxic. Using the two different seaweed extracts the larvicidal activities were noted. It is observed that good larval mortality rate occurred at the highest concentration of 500 mg/ml of methanol extract of *Padina gymnospora* against II instar larvae of *Aedes aegypti* (Table. 4).

**Table 4** Mortality percentage of different solvent extracts of the seaweed *Padina gymnospora* against II and III instar larvae of *Aedes aegypti* (n= 100)

| Concentrations (mg/ml) | II instar larval mortality (%) | | | |
|---|---|---|---|---|
| | Chloroform | Petroleum ether | Acetone | Methanol |
| 100 | $26^{c}$ | $2^{cd}$ | $25^{c}$ | $25^{c}$ |
| 200 | $45^{bc}$ | $9^{cd}$ | $54^{bc}$ | $45^{bc}$ |
| 300 | $66^{b}$ | $13^{cd}$ | $71^{b}$ | $59^{bc}$ |
| 400 | $86^{ab}$ | $21^{c}$ | $88^{a}$ | $79^{ab}$ |
| 500 | $97^{a}$ | $28^{c}$ | $98^{a}$ | $100^{a}$ |
| | III instar larval mortality (%) | | | |
| 100 | $20^{cd}$ | $0^{d}$ | $12^{cd}$ | $10^{cd}$ |
| 200 | $34^{c}$ | $10^{cd}$ | $37^{c}$ | $35^{c}$ |
| 300 | $45^{bc}$ | $15^{cd}$ | $68^{b}$ | $60^{b}$ |
| 400 | $77^{ab}$ | $19^{cd}$ | $78^{ab}$ | $70^{b}$ |
| 500 | $87^{a}$ | $21^{cd}$ | $92^{a}$ | $83^{ab}$ |
| Control | $0^{d}$ | $0^{d}$ | $0^{d}$ | $0^{d}$ |

The columns, mean followed by the same letter do not significantly using Duncan's test
p≤0.05. d-Reference control

The other concentrations of 100, 200, 300 and 400 mg/ml showed moderate larval mortality. In comparison with the control, all concentrations of *Padina gymnospora* contributed high larvicidal activity against *Ae. aegypti*. The lowest mortality was observed in petroleum ether extract at 28 % level in 500 mg/ml concentrations. In III instar, larval mortality was observed in acetone extract 92 % at 500 mg/ml concentration. For the *S. wightii* against II and III instar larvae of *Culex quinquefasciatus* 100 percent larval mortality were detected in chloroform and acetone extracts at 500 mg/ml concentration level. Low mortality rate was found in petroleum ether extract (Table. 5). Dead larvae were not observed in the entire control group.

**Table 5** Mortality percentage of different solvent extracts of the seaweed *Padina gymnospora* against II and III instar larvae of *Culex quinquefasciatus* (n= 100)

| Concentrations (mg/ml) | II instar larval mortality (%) | | | |
|---|---|---|---|---|
| | Chloroform | Petroleum ether | Acetone | Methanol |
| 100 | $22^{cd}$ | $10^{cd}$ | $31^{c}$ | $23^{cd}$ |
| 200 | $68^{b}$ | $25^{c}$ | $63^{bc}$ | $44^{bc}$ |
| 300 | $90^{a}$ | $35^{c}$ | $96^{a}$ | $56^{bc}$ |
| 400 | $98^{a}$ | $38^{c}$ | $99^{a}$ | $77^{ab}$ |
| 500 | $100^{a}$ | $45^{bc}$ | $100^{a}$ | $99^{a}$ |
| | III instar larval mortality (%) | | | |
| 100 | $23^{cd}$ | $2^{cd}$ | $23^{cd}$ | $10^{cd}$ |
| 200 | $45^{bc}$ | $6^{cd}$ | $38^{c}$ | $17^{cd}$ |
| 300 | $76^{b}$ | $16^{cd}$ | $78^{ab}$ | $34^{c}$ |
| 400 | $90^{a}$ | $25^{c}$ | $87^{a}$ | $60^{bc}$ |
| 500 | $100^{a}$ | $37^{c}$ | $100^{a}$ | $92^{a}$ |
| Control | $0^{d}$ | $0^{d}$ | $0^{d}$ | $0^{d}$ |

The columns, mean followed by the same letter do not significantly using Duncan's test
p≤0.05. D-Reference control

The data obtained from 400 and 500 mg/ml of acetone and 500 mg/ml of chloroform and methanol extract of *Caulerpa racemosa* gave 100 % mortality and were recorded in II instar larvae of *Ae. aegypti* (Table. 6).

**Table 6** Mortality percentage of different solvent extracts of the seaweed *Caulerpa racemosa* against II and III instar larvae of *Aedes aegypti* (n= 100)

| Concentrations (mg/ml) | II instar larval mortality (%) | | | |
|---|---|---|---|---|
| | Chloroform | Petroleum ether | Acetone | Methanol |
| 100 | $36^{c}$ | $14^{cd}$ | $29^{c}$ | $23^{cd}$ |
| 200 | $62^{bc}$ | $25^{c}$ | $62^{bc}$ | $50^{bc}$ |
| 300 | $93^{a}$ | $33^{c}$ | $93^{a}$ | $75^{b}$ |
| 400 | $99^{a}$ | $45^{bc}$ | $100^{a}$ | $91^{a}$ |
| 500 | $100^{a}$ | $66^{b}$ | $100^{a}$ | $100^{a}$ |
| | III instar larval mortality (%) | | | |
| 100 | $41^{c}$ | $12^{cd}$ | $28^{c}$ | $21^{cd}$ |
| 200 | $76^{b}$ | $16^{cd}$ | $65^{bc}$ | $50^{bc}$ |
| 300 | $95^{a}$ | $33^{c}$ | $90^{a}$ | $73^{b}$ |
| 400 | $100^{a}$ | $56^{bc}$ | $100^{a}$ | $91^{a}$ |
| 500 | $100^{a}$ | $81^{ab}$ | $100^{a}$ | $100^{a}$ |
| Control | $0^{d}$ | $0^{d}$ | $0^{d}$ | $0^{d}$ |

The columns, mean followed by the same letter do not significantly using Duncan's test
p≤0.05. D-Reference control

The III instar larvae also exhibited 100 % mortality at 400 and 500 mg/ml of chloroform and acetone extracts and 500 mg/ml methanol extract. All the extracts showed concentration dependent mortality. Similar results were obtained in *Cx. quinquefasciatus* II and III instar (Table. 7).

**Table 7** Mortality percentage of different solvent extracts of the seaweed *Caulerpa racemosa* against II and III instar larvae of *Culex quinquefasciatus* (n= 100)

| Concentrations (mg/ml) | II instar larval mortality (%) | | | |
|---|---|---|---|---|
| | Chloroform | Petroleum ether | Acetone | Methanol |
| 100 | 33[c] | 20[cd] | 32[c] | 27[c] |
| 200 | 65[bc] | 33[c] | 62[bc] | 48[bc] |
| 300 | 90[a] | 40[c] | 88[a] | 69[b] |
| 400 | 100[a] | 55[bc] | 99[a] | 91[a] |
| 500 | 100[a] | 71[b] | 100[a] | 100[a] |
| | **III instar larval mortality (%)** | | | |
| 100 | 23[cd] | 9[cd] | 20[cd] | 21[cd] |
| 200 | 47[bc] | 21[cd] | 38[c] | 30[c] |
| 300 | 75[b] | 24[cd] | 70[b] | 52[bc] |
| 400 | 93[a] | 38[c] | 88[ab] | 68[b] |
| 500 | 100[a] | 56[bc] | 92[a] | 90[a] |
| Control | 0[d] | 0[d] | 0[d] | 0[d] |

The columns, mean followed by the same letter do not significantly using Duncan's test
p≤0.05. d-Reference control

## DISCUSSION

Algae synthesize a number of chemically diversified secondary metabolites. Among them, some of the compounds are recognized as insecticides. The control of adult mosquito is an unsuccessful strategy as the adult stage occurs beside human inhabitation and they can easily overcome remedial measures (Service, 1983 and 1992).

Phytochemicals are naturally present in the seaweeds. phytochemicals in marine algae may reduce the risk of human diseases, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects (Boonchum *et al*., 2011; Oumaskour *et al*., 2012; Abirami *et al*., 2012). The preliminary phytochemical screening is a part of chemical evaluation of the seaweeds. Seenivasan *et al*. (2012) found that the results with the highest total phenol and flavanoid was in the brown seaweed *Padina gymnospora.* The green seaweed like *Ulva* (Elmegeed *et al.,* 2014) contain saponin, alkaloids, steroids and terpenoids. Tannin and phenolic content shows positive result to all solvent extract from both seaweeds. ). The phytochemical component saponins serve as biocontrol agent against vector mosquitoes as reported by Chapagain *et al*. (2008). Phenol compounds are also responsible for the antimicrobial, anti inflammatory, antifeedant, antiviral, anticancer and vasodilatory actions (Aliyu *et al*., 2009; Valentina *et al*., 2015 and Gupta, 2011). The tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent (Kolodziej *et al*., 2005). In addition to similar evidence of polyphenols it may be associated with various carbohydrates and organic acids (Manach *et al.*, 2004).

Larval stages of mosquitoes are exclusively aquatic, systematic exposure of algal based larvicides in their breeding habitat is a successful and safer way to interrupt larval stages of vectors rather than the adult stage (Thangam and Kathiresan, 1991). Periodic larvicide is very helpful in favorable conditions (WHO, 1975 and Becker *et al*., 2003). The marine algae, *C. racemosa* showed minimum $LC_{50}$ values of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* larvae [$LC_{50}$=(0.055 6±0.010 3) µg/mL, (0.056 9±0.021 3) µg/mL, (0.066 0±0.007 6) µg/mL] when compared with control respectively; this might due the presence saponin and triterpenoids by Ali *et al*., 2012, (Syed ali *et al*., 2013). The extract of *D. dichotoma* showed minimum level of $LC_{50}$value (0.0683±0.0084 µg/mL) and $LC_{90}$value was 0.140 1. The regression equations of *D. dichotoma* and *E. intestinalis* for 4th instar larvae were Y=0.333 + 0.684x ($R^2$=0.946) and Y=0.600 + 0.781x ($R^2$=0.812), respectively ( Beula,M *et al*., 2011).

The present study reveals that different solvent extracts of the selected seaweeds were tested for their larvicidal effect on the two mosquito larvae on their two life stages. Based on the results it is evident that all the extracts of the four algal samples were effective against both larvae. The $LC_{50}$ values showed that ethyl acetate extract of *Padina gymnospora* was found to be more effective against both II and III instar larvae of *Ae. Aegypti* ($LC_{50}$ 162.23 and 253.78 mg/ml) and *Cx. quinquefasciatus* ($LC_{50}$142.87 and 180.37 mg/ml). Ethyl acetate extract of *C. racemosa* was found to be more effective to II instar (138.32 mg/ml) and Methanol (197.21 mg/ml) was found to be highly active in III instar of *Ae. aegypti* and II and III of *Cx. quinquefasciatus* (113.32, 200.47 mg/ml). A broad spectrum of algae species like *Caulerpa prolifera*, *Caulerpa serrulata*, *U. lactuca, Lobophora variegata*, *Spatoglossum asperum*, *Dictyota dichotoma*, *U. fasciata* and *Grateloupia lithophila* were screened for their high effects on mosquito larvae (Elbanna and Hegazi, 2011; Ravikumar *et al*., 2011). The solvent extracts of *U. lactuca* caused significant larvicidal effect against *Cx. pipiens* larvae. Acetone extract was the most potent larvicidal extract with $LC_{50}$ value of 5.46 mg/ml. On the other hand, marine algae *C. racemosa* and *U. lactuca* have been reported to possess nymphicidal, anti-ovipositional activity, reduced fecundity, hatchability and adult longevity (Abbassy *et al*., 2014).

## CONCLUSIONS

It can be concluded from the present study that, the chloroform and methanol extracts of seaweed of *C. racemosa* possess more active compounds present in the above mentioned solvent against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

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