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Larvicidal Potential of *Padina gymnospora* and *Sargassum swartzii* Methanolic Extracts Against Dengue Fever Mosquito, *Aedes aegypti* (Linnaeus) (Diptera: Culicidae)

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Abstract

Dengue hemorrhagic fever causes 2 million infections, 500,000 illnesses, and 12,000 deaths. According to a recent report, more than 50 million people are at risk of contracting dengue fever around the world. Mosquito vectors transmit diseases such as dengue fever, yellow fever, zika, chikungunya, filariasis, onchocerciasis, and mansonellosis. The present study was designed to evaluate the larvicidal potential of different concentrations of methanolic extracts of *Padina gymnospora* and *Sargassum swartzii*. The larvicidal potential of the selected brown seaweed extracts were determined against 4^{th} instar larvae of *Aedes aegypti* using various concentrations *viz.,* 200, 400, 600, 800 and 1000 ppm. The mortality counts were made after 12h, 24h and 48h of incubation and LC_{50} values were calculated. The highest larvicidal activity against *A. aegypti* was [LC_{50} =621.5 and LC_{90} = 1639.0 ppm (After 12h); LC_{50} =819.8 and LC_{90} = 1448.1 ppm (After 24h) and LC_{50} =621.5 and LC_{90} =1213.1 ppm (After 48h)]. The phytochemical analysis of methanolic extracts of *P. gymnospora* and *S. swartzii* showed the presence of terpenoids, tannins, phenolic compounds, and steroids. The efficacy of the extracts of *P. gymnospora* and *S. swartzii* may be attributed to the phytochemicals present in the methanolic solvent extract. Further investigations are underway to elucidate novel compounds with mosquito repellent activity from the extracts.

Keywords

Padina gymnospora, Sargassum swartzii, Aedes aegypti, Larvicidal activity

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INTRODUCTION

Mosquito vectors transmit diseases such as dengue fever, yellow fever, zika, chikungunya, filariasis, onchocerciasis, and mansonellosis. According to a recent estimate, more than 50 million people are at risk of contracting dengue fever around the world. Dengue Virus, Chikungunya Virus, Eastern Equine Encephalitis Virus, Japanese Encephalitis Virus, La

Crosse Encephalitis, Malaria, St. Louis Encephalitis, Yellow Fever, West Nile Virus, and Zika Virus are among the new diseases spread by mosquito bites [1]. Dengue hemorrhagic fever causes 2 million infections, 500,000 illnesses, and 12,000 deaths per year [2]. Throughout the Americas, Southeast Asia, and Western Europe, it is well known that *Aedes aegypti* spreads several viruses that cause sickness. These vectors are also responsible for illnesses such as microcephaly and Guillain-Barré syndrome, which are caused indirectly by viral infections. Up to 50-100 million individuals are afflicted by diseases spread by *Aedes aegypti*, with the impact being greatest in the summer [3].

The main larvicide used worldwide to control mosquitoes are the organophosphate Temephos, but by the end of 1990's, it led to the development of resistance in *A. aegypti*. Indeed, in recent decades, indiscriminate use of synthetic insecticides (for example, domestic use of pyrethroid insecticides available on the retail market, especially during epidemic periods), combined with a lack of coordinated programmes in multi-endemic areas, has resulted in the emergence of *A. aegypti* populations resistant to various insecticides used [4]. Plant secondary metabolites are a promising source of larvicidal agents for mosquito management/control. Secondary metabolites like steroids, terpenes, alkaloids, and phenolic chemicals have been found to be larvicidal without harming human and animal health or the environment [5, 6].

Brown algae possess a broad spectrum of biological activities (anticoagulant, antithrombotic, anti-viral, anti-cancer, anti-inflammatory and antibacterial). Proteins, amino acids, polysaccharides, fatty acids, vitamins, minerals, dietary fibre, sterols, pigments, polyphenols, and other bioactive compounds are abundant and isolated from brown algae [7,8,9]. As a result, brown algae extracts have a lot of potential for treating human diseases like atherosclerosis, rheumatoid arthritis, hypertension, goitre, asthma, ulcers, menstrual disorders, syphilis, and skin illnesses. Polysaccharides isolated from algae, being one of the most prevalent and important categories of bioactive chemicals, play a vital role in the biological potential of brown algae. Different forms of polysaccharides found in brown algal cell walls, such as laminarins, alginates, and fucoidans, have sparked substantial attention in recent years, with high potential for biological uses in functional food, cosmeceutical and pharmaceutical products [8].

Hence, in the present work we sought to screen and evaluate the larvicidal potential of *P. gymnospora* and *S. swartzii extracts* and to evaluate the efficiency of methanol extract as larvicidal agents against 4th instar larvae of *A. aegypti* in lab environment. The 4th instar larvae of *A. aegypti* was collected from Vector Control Research Centre (ICMR-Government of India), Madurai.

MATERIALS AND METHODS

Algae sample collection

Padina gymnospora and *Sargassum swartzii* (Phaeophyceae), marine brown algae were collected by hand picking from the submerged marine rocks at Manappad village, (Lat. 8°30′N; Long. 78°8′E), Tuticorin district, the Gulf of Mannar Marine biosphere, Tamil Nadu, India. Seaweed collections were made during December 2021.

Extract preparation

Algal samples were cleaned of epiphytes and extraneous matter, and necrotic parts were removed following established protocols. Plants were first cleaned in seawater before the final rinse in fresh water. The seaweeds were transferred to the laboratory in sterile polythene bags under controlled temperature and were kept in an ice box containing slush ice and transported to laboratory. Briefly, samples were rinsed with sterile distilled water, dried in the sun, chopped into small pieces, and pulverised in a mixer grinder in the lab. Fifty grams of powdered samples were packed in Soxhlet apparatus and extracted with methanol for 6 h. The extracts were pooled and the solvent were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) at 4°C and the dried extracts were stored at 4 °C in refrigerator further until further assay.

Phytochemical screening

The methanol extracts of *Padina gymnospora* and *Sargassum swartzii* were used for qualitative phytochemical studies. Screening of phytochemicals like terpenoids, tannins, cardic glycosides, steroids, alkaloids, phenolic compounds and coumarins were carried out following the standard protocols [10, 11].

Mosquito Larvicidal Bioassay

The eggs of *A. aegypti,* were received from the Field Station, Vector Control Research Centre (ICMR-Government of India), Madurai. The larvae were fed with Brewer's yeast/ dog biscuit (1:3). The larvicidal effect of methanolic extract of *P. gymnospora* and *S. swartzii* against *A. aegypti* were conducted with standard procedures recommended by the World Health Organization [12]. The methanolic extract was dissolved in 1 ml of acetone and prepared into different concentrations *viz.,* 200, 400, 600, 800 and 1000 ppm with distilled water. For the larvicidal assay, twenty larvae of the early fourth instar stage (in a 100-ml beaker) were utilized, with three replicates for each concentration. The larvae were not fed during the trial. The larval mortality was determined after 12, 24, and 48 hours of exposure.

Statistical analysis

The data are presented as a mean ± standard deviation. The statistical software SPSS (version 11.5) was used for all statistical analyses (SPSS Inc., Chicago, IL, USA). The average larval mortality data, probit analysis calculating LC_{50}, LC_{90} and other statistics, 95% confidence limits and chi-square values were calculated.

RESULTS AND DISCUSSION

Phytochemical analysis

Seaweeds extracts are considered to be a rich source of phenolic compounds [13]. In the present study, methanolic extracts of *Padina gymnospora* and *S. swartzii* showed the presence of phytochemicals such as the terpenoids, tannins, phenolic compounds and steroids and the results are presented in table 1. According to their composition and concentration, they may have an activating or inhibitory influence on microbial development [14]. Bacteria's growth and metabolism may be harmed by phenolic chemicals. Tannins have been employed as antiviral, antibacterial, antiulcer, and antioxidant agents in the medical field. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent [15]. Plant-derived steroids have been shown to have insecticidal, antibacterial, antiparasitic, and cardiotonic effects. Steroids are also used in the fields of nutrition, natural medicine, and cosmetics [16].

Table 1. Phytochemical analysis methanolic extracts of *Padina gymnospora* and *Sargassum swartzii*

S.no	Secondary metabolites	*Padina gymnospora*	*Sargassum swartzii*
1	Terpenoids	++	++
2	Tannins	++	++
3	Cardic glycosides	-	_
4	Steroids	++	++
5	Alkaloids	-	+
6	Coumarins	+	+
7	Phenolic compounds	++	++

++=strongly present; += present; - = Absent

Larvicidal activity

Mosquito control has been focused on the use of insecticides derived from plants for the past three decades. Mosquito control at the larval stages is an effective procedure because they are localized in space and time [17]. As a result, non-target creatures are less at risk, while the struggle against adults is only temporary and ineffective. The safe use of pesticides in the environment is the most important requirement for mosquito control programmes [18]. Long-term, widespread use of synthetic pesticides leads to environmental contamination. However, plant extracts have promising larvicidal efficacies because they are rich in bioactive organic chemicals, more beneficial over synthetic insecticides as they are easily bio-degradable and less toxic to the environment [19].

The larval stages of mosquitoes are exclusively aquatic hence, systematic treatment of algal based larvicides in their breeding habitat is a successful and safer way to interrupt larval stages of vectors rather than at the adult stage [20]. Periodic larviciding [21] is very helpful in favourable conditions. So far, a number of plants derived compounds has been tested for mosquito control [22, 23].

In the present investigation, methanolic extracts of *S. swartzii* had remarkable larvicidal activity against *A. aegypti* [LC_{50} =621.5 and LC_{90} = 1639.0 ppm (After 12h); LC_{50} =819.8 ppm and LC_{90} = 1448.1 ppm (After 24h)] and the methanolic extracts of *P. gymnospora* had larvicidal activity against *A. aegypti* [LC_{50} =621.5 ppm and LC_{90} =1213.1 ppm (After 48h) and LC_{50} =621.5 and LC_{90} = 1639.0 ppm (After 12h); LC_{50} =819.8 ppm and LC_{90} = 1448.1 ppm (After 24h) and LC_{50} =621.5 ppm and LC_{90} =1213.1 ppm (After 48h)] and the results are represented in Table 2. Mahnaz Khanavi [24] reported that the fractions tested against *A. aegypti* larvae, EtOAc fraction of *S. swartzii* and C. *dasyphylla*, showed mortality rate of 96 and 95%, respectively. The LC_{50} and LC_{90} values for *S. swartzii* were 11.75 and 53.47 ppm, respectively, and 10.62 and 56.39 ppm for *C. dasyphylla*, respectively, according to Probit analysis of logarithmic concentration from regression line.

The acetone extracts of the marine seaweeds *Caulerpa scapelliformis, D. dichotoma, Enteromorpha clathrata, E. intestinalis* and *Ulva lactuca* were active against the fourth instar larvae of *A. aegypti* with LC_{50} values of 53.70, 61.65, 85.11, 67.70 and 91.20 ppm, respectively [20]. *Padina minor* showed significantly increased larvicidal activity compared to *D. linearis* at LC_{50}. Higher larvicidal activity of *Padina* than *Dictyota* was also observed in *P. tetrastromatica* and *D. dichotoma* from southwest Coast of India[25]. The LD_{50} of *P. tetrastromatica* on the II instar larvae of *C.*

quinquefasciatus is 0.96 mg/ml (960 µg/ml) while *D. dichotoma* is 0.25 mg/ml (256 µg/ml). Both *P. minor* and *D. linearis* showed greater potency

Table 2. Larvicidal properties methanol extracts of Padina gymnospora and Sargassum swartzii against the larvae of A. aegypti after 12, 24 and 48 h of exposure period

Name of the seaweeds	Time	Concentration (ppm)	% mortality ± SE	LC_{50} (LCL- UCL) [a]	LC_{90}(LCL- UCL) [a]	X^2(d=4) [b]
P. gymnospora	After 12h	200	6.4 ± 0.40			
		400	14.5 ± 0.50			
		600	24.0 ± 1.0	953.6 (874.5 – 1066.5)	1062.2 (1414.4 – 1902.8)	0.08
		800	38.0 ± 0.50			
		1000	53.6 ± 1.50			
	After 24h	200	10.5 ± 0.50			
		400	19.1 ± 0.28			
		600	28.5 ± 0.50	894.1 (817.1 – 1001.5)	1593 (1400.2 – 14069)	0.44
		800	41.0 ± 1.00			
		1000	59.6 ± 1.52			
	After 48h	200	14.5 ± 0.50			
		400	25.0 ±1.50			
		600	48.0 ± 0.50	698.6 (6407 – 763.7)	1331.8 (1196.3 – 1535.5)	2.147
		800	58.6 ± 1.50			
		1000	70 ± 1 .25			
S. swartzii	After 12h	200	8.1 ± 0.36			
		400	14.2 ± 1.10			
		600	25.0 ± 1.00	951.9 (869.2 – 1071.6)	1639.0 (1437.4 – 1967.4)	0.81
		800	43.0 ± 1.00			
		1000	61.0 ± 1.50			
	After 24h	200	11.5 ± 0.50			
		400	18.7 ± 0.68			
		600	30.0 ± 0.10	819.8 (756.5 – 900.4)	1448.1 (1294.8 – 1682.1)	0.81
		800	51.0 ± 1.00			
		1000	71.1 ± 0.36			
	After 48h	200	16.5 ± 0.50			
		400	30.5 ± 0.50			
		600	53.0 ± 0.10	621.5 (566.7 – 677.5)	1213.4 (1100.3 – 1377.8)	1.52
		800	65.6 ± 0.68			
		1000	77.0 ± 1.00			

LCL lower confidence level, UCL upper confidence level
a 95 % Confidence interval
b Degrees of freedom

compared to *Ulva fasciata* and *Grateloupia lithophila* collected from Kovalam, near Chennai [26]. Rajashekar *et al.*[27] showed ethanol extracts of *Cassia fistula, Curcuma amada, Manilkara zapota, Momordica charantia, Sansevieria trifasciata* and *Solanum indicum* at dosages of 25, 50, 75 and 100% for their larvicidal effects against the third instar larva of *A. aegypti*. It was reported that the maximum larval mortality was evident at minimum LC_{50}, LC_{90} and LC_{99} in *C. amada* (LC_{50} =0.14, LC_{90}=9.28 and LC_{99}=277.97). In the present study, varied larvicidal effects with methanol extracts were observed. Here we confirm that the methanolic extracts of *P. gymnospora* and *S. swartzii* had significant larvicidal activity against *A. aegypti*. The larvicidal activity of three different extracts (methanol, acetone, and benzene) of *C. antenina* and *S. wightii* against the larvae of *A. aegypti* was performed under laboratory conditions. In terms of lethal concentration for 50% and 90% mortality (LC_{50} and LC_{90}) values were represented as follows: For *C. antenina* LC_{50} value of the benzene extract was 448.03ppm, followed by acetone and methanol extracts while for *S. wightii* LC_{50} value of the methanol extract 415.10 ppm was followed by acetone and benzene extract, LC_{90} value of the benzene extract of *C. antenina* was 800.72 ppm, followed by acetone extract and methanol extract; while for *S. wightii* LC_{90} value of the acetone extract was 686.37 ppm, followed by methanol and benzene extract[28]. Rahuman and Venkatesan [29] have reported that the petroleum ether extract of *Citrullus colocynthis (C. colocythis),* methanol extracts of *Cannabis indica*, *C. sativus*, *Momordica charantia* and acetone extract of *Trichosanthes anguina* against the larvae of *A. aegypti* (LC_{50}=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *C. quinquefasciatus* (LC_{50}=88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively.

Kumari *et al.* [30] have demonstrated that the brown alga, *Turbinaria conoides*, exhibited the effective mosquito larvicidal and bactericidal effect against the *C. quinquefasciatus* larvae and *S. aureus* bacterium respectively. They have also found that diethyl ether and acetone extracts of *T. conoides* were superior in mosquito-larvicidal effect (LC_{50} 104 mg/L, 469 mg/L) and antibacterial activity (4 mm ZoI at 0.1mg and 1.2mg extract), respectively. Such recorded toxicity was related to the presence of major algal - compounds viz; 1,2, dicarboxylic derivatives of benzene, Phthalic acid, Pentane 2,2,4-trimethyl, and titanium 1,3,5,7 cyclooctatetraene 2,4 cyclopentadien 1-Yl in the acetone extract of the *T. conoides*. Trivedi *et al.* [31] reported the industrial applications like anti-mosquito & antimicrobial agents by the AgNPs synthesized from the brown seaweed, *Sargassum muticum* of Red sea. Amutha *et al*. (2019) have documented the effective mosquitocidal property of the nanoparticles that they synthesized through the seagrass, *Cymodocea serrulate* species. The varying biological activity of plant extracts might be due to various compounds, including phenolics, terpenoids, and alkaloids present in plants [32].

CONCLUSION

Finally, it can be concluded that the methanol extract of *S. swartzii* and *P. gymnospora* may find use as broad-spectrum larvicidal agent in the near future. More work would be undertaken to evaluate the utility of these plant extracts for field applications considering the promising leads given by the present study. Our further investigations will elucidate the novel antirepellent compounds.

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