Research Article

Identification of Phytochemicals from *Sargassum wightii* against *Aedes aegypti*

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ABSTRACT

Vector control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of algal origin may serve as suitable alternative, in the future. Although several plants have been reported for larvicidal activity, only few have been moved from the laboratory to field use, because they are poorly characterized, in most cases active principles are not determined and are restricted to preliminary screening. *Sargassum wightii* is a common sea weed distributed in many parts of India with rich medicinal properties. In the present study, *S.wightii* was screened for the potential bioactive natural substances against *Aedes aegypti* and human microbial pathogens. Crude extracts were made using three solvents (diethyl ether, methanol and acetone). The different extracts of *S.wightii* showed the presence of alkaloids, phenolic compounds, saponins and flavonoids with varied degree. FT-IR analysis of the extracts confirmed the presence of functional groups such as amides, phosphorous compounds, alcohols, phenols and halogen compounds. The algal extract showed larvicidal effects after 24, 48 and 72 hours of exposure. Highest mortality was recorded at 350ppm for all the instars, with methanol following acetone and diethyl ether extracts. The results of regression analysis revealed that the mortality rate (Y) is positively correlated with the period of exposure (X). Hence, LC_{50} and LC_{90} values gradually decreased with the exposure periods, having the lowest value, at 72 hours of exposure to third instar larvae, followed by second and fourth. The extracts were also subjected to antimicrobial activity against bacterial and fungal pathogens. All the three seaweed extracts have shown moderate antimicrobial activity, out of which methanol extract has shown significant activity.

Keywords: *A.aegypti,* Larvicidal activity, Phytochemicals, *S.wightii*

INTRODUCTION

Over the past several decades, seaweeds and their extracts have generated an enormous amount of interest in the drug development industry due to the fresh source of bioactive compounds with immense therapeutic potential.[1] Marine macroalgae are rich in bioactive compounds that could potentially be exploited as functional ingredients for both human and animal health applications. These include fucan, fucoidan, alkaloids, steroids, phenolic compounds, saponins and flavonoids. These natural products are known as secondary metabolites which might be a potential source of drug leads in future. Recent findings evidenced that seaweeds contained secondary antibacterial, antiviral, antifungal, cytotoxic and larvicidal potentials.

Dengue is currently one of the emerging and life-threatening disease, termed as dengue hemorrhagic fever. It is already a common knowledge that *A.aegypti* (Diptera: Culicidae) is the vector of dengue hemorrhagic fever. The overuse of chemical insecticides is not safer due to environment hazard and non-target organisms have resulted in resistant development. Hence, alternative approaches free from such problems are the need for modern time, to develop environmentally safe, biodegradable and cost effective weapons with more powerful combatable properties against such vectors. Natural products are generally preferred because of their less harmful nature and innate biodegradability. The idea of using algal extracts to kill mosquito larvae is not new. However, considering the biodiversity of macroalgae in the tropical regions, there is a need to study their larvicidal potential. The algae metabolites have also been shown in several studies to have larvicidal activities. *Sargassum,* one of the marine macroalgal genera belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the family sargassaceae and order Fucales.[2]

S. wightii is one of the important species with wide range of bioactive properties. It shows good amount of flavonoids in support of its antioxidant activity, which indicate that this genus is an ideal target for investigating the presence of bio-molecules for various medical and industrial applications. In the view of the recent increased interest in developing plant-based insecticides as an alternative to chemical insecticides, the present study focuses on extraction of bioactive compounds from *S.wightii* using different solvents and to study the phytochemical properties of different solvent extracts against mosquito vector *A.aegypti*.

MATERIALS AND METHODS

Marine brown algae were collected from the sea shores of Mandapam Island, Ramand District, Tamil Nadu, India. The different solvent extracts of *S.wightii* were tested for the identification of phytochemical constituents according to standard procedures as described previously[3].

Fourier Transform Infrared Spectroscopy (FT-IR) analysis

FT-IR analysis was carried out for methanol, acetone and diethyl ether extracts of *S.wightii*. The analysis was carried out with Shimadzu spectrophotometer system. The peak values of the FT-IR were recorded to detect the characteristic peaks and their functional groups.

Collection of eggs and larvicidal activity

The eggs and egg rafts of *A. aegypti* were procured from Indian Council for Medical Research (ICMR), Madurai. Filter paper attached with eggs was dipped into plastic tray containing 500mL of de-chlorinated water and allowed to hatch into larvae. They were reared indoors at (28±2)˚C temperature. The larvae were fed with powdered mixture of dog biscuits and yeast powder in 3:1 ratio. The test for the larvicidal effect of different extracts derived from *S. wightii* against mosquito larvae *(A.aegypti)* was conducted in accordance with the World Health Organization (WHO) standard method (2005). The potential toxicity of chemical substances often is presented as their LC_{50}. LC_{50} is the concentration of a substance that is lethal to 50% of the organisms in a toxicity test.[4]

Antimicrobial activity

Antimicrobial activity was performed against the following pathogenic bacterial and fungal strains, *Bacillus subtilis*, *Pseudomonas aeruginosa, Staphylococcus auerus, Salmonella typhi, Klebsiella pneumoniae, Candida albicans, Aspergillus niger* and *Aspergillus flavus.* The test was carried out in Bose clinical Laboratory, Madurai, Tamilnadu. Antibacterial activity was measured using a well diffusion method [5]. Standard antibiotic Ampicillin was used as control for bacterial strains and Amphotericin was used as control for fungal strains.

RESULTS AND DISCUSSION

Extraction of Bioactive Compounds:

Extraction of crude bioactive compounds from the alga was carried out with four different solvents (Hexane, Diethyl ether, Acetone, Methanol) of increasing polarity. The percentage yield of extracts ranging from 0.22(w/w) to 0.96 (w/w), is represented in Figure 1. It revealed that, maximum yield was obtained with acetone (0.969%) and minimum yield with hexane (0.225%). The solid residue thus, obtained was subjected to phytochemical screening.

Phytochemical Screening

Preliminary phytochemical screening of different bioactive compounds (steroids, terpenoids, alkaloids, glycosides, flavonoids, saponins, tannins, proteins, free amino acids and carbohydrates) were tested in four different extracts of *S.wightii*. The analysis of crude bioactive compounds was carried out with standard procedure and the results are tabulated in Table 1. The studies indicated the presence of steroids, terpenoids and flavonoids in all four extracts. Alkaloids were present in methanol and acetone extracts. The presence of glycosides was noted with diethyl ether and acetone extracts of *S.wightii*. Saponin is present in all the three extracts except hexane. Tannins were present in acetone and methanol extract. Among the ten different compounds tested, acetone and methanol extracts showed the presence of maximum number of compounds.

The presence of flavonoids and alkaloids were noted in ethyl acetate, chloroform and ethanolic extracts of *S.wightii* was reported.[1]

The presence of steroids, terpenoids, tannins, glycosides, alkaloids and flavonoids were identified in acetone, methanol and petroleum ether extracts of *S.wightii.*[6] Phenolic compounds are reported to have several biological activities including antioxidant properties. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects.[7] Therefore; from the results it is clear that, the solvent extracts of *S.wightii* may possess various bio potential characteristics.

FTIR analysis of the solvent extracts of *S.wightii*

The FT-IR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. Analysis of crude methanolic and acetone extracts of *S.wightii* displayed the presence of functional groups such as Iodine- halogen by recording its peak signal at 370.31, 397.31 and 426.24cm^{-1}, bromine (659.61cm^{-1}), fluorine (1012.56 and 1026.06 cm^{-1}), and carbon – nitrogen covalent bonding (1112.85 cm^{-1}), carbon – carbon covalent bonding (1400.22 and 2073.33cm^{-1}**),** nitro group (1456.30, 1622.02and 21cm^{-1}), hydroxyl group (2580.09 and 2923.88cm^{-1}) carbon – hydrogen covalent bonding (2837.09 and 3303.83 cm^{-1}) on the vibration stretches. FTIR analysis of diethyl ether extract showed different peaks which confirmed the presence of functional groups such as amides, phosphorus compounds, alcohols, phenols and halogen compounds (Figure 2) Alcohols, phenols and alkanes have resulted in breaking the cell wall and inhibiting protein synthesis of gram positive and gram negative bacterial strains, there by exhibiting higher anti-microbial properties. Aromatic elements from the plant materials exhibits antibacterial efficacy.[8] The amine groups present in the ethanolic extract of *L.inermis* were also contributing for its antimicrobial activity.[9]

Mosquito larvicidal activity

Algae synthesize a number of chemically diversified secondary metabolites. Among them, some of the compounds are recognized as insecticides. Eggs collected from ICMR were reared under laboratory conditions and Larvicidal assay was performed with three different solvent extracts against second, third and fourth instar larvae of *A.aegypti*. On comparing the mortality percentage with different polar and non-polar solvent extracts, at 24, 48 and 72 hours of exposure, methanol extract was highly potential and showed minimum LC_{50}

and LC_{90} values (the lethal concentration at 24h was, 181.28µgmL^{-1}, 154.71µgmL^{-1}and 231.07µg mL^{-1} for 2^{nd,} 3rd and 4th instar respectively) when compared with acetone (207.10µgmL^{-1} 205.70µgmL^{-1} and 257.35µgmL^{-1} for 2nd,3rd and4th instar respectively) and diethyl ether (256.20µgmL^{-1}, 237.49 µgmL^{-1} and 290.95 µgmL^{-1} for 2nd, 3rd and 4th instar respectively) against *A.aegypti*. The $LC_{50}$and LC_{90} values (of 2^{nd,} 3rd and 4th instar) for the methanol extract, at different time exposure are listed in table 2.

Mortality rate of more than 90% was recorded at 350ppm (Table 3) for all the instars with methanol extract following acetone and diethyl ether extracts. 100% mortality was observed within 24 hours during the exposure to the chemical insecticide, malathion (5ppm concentration). The results of regression analysis revealed that the mortality rate (Y) is positively correlated with the period of exposure (X) having a regression coefficient close to one in each case. The results revealed that LC_{50} and LC_{90} values gradually decreased with the exposure time having the lowest value at 72 hours of exposure to third instar larvae, followed by second and fourth instar larvae (Figure 3.).

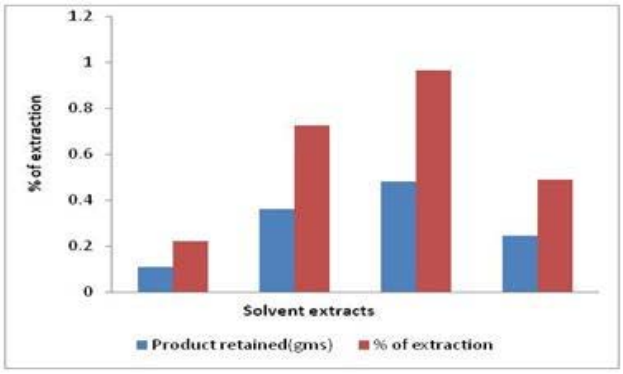


Figure 1: Percentage of Solvent extracts from *S.wightii*

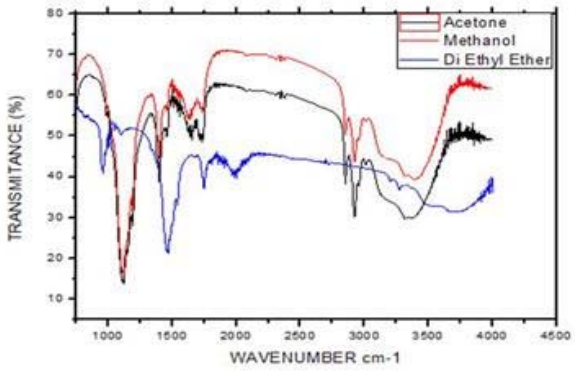
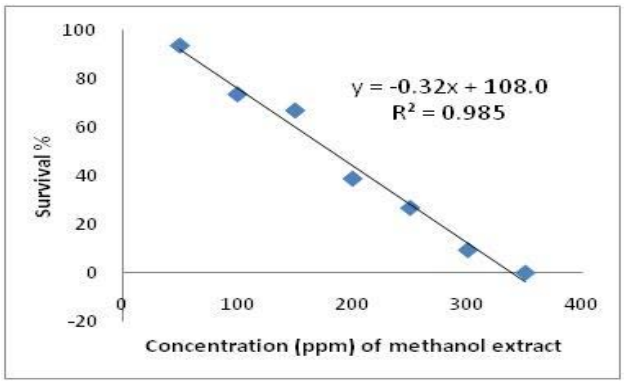
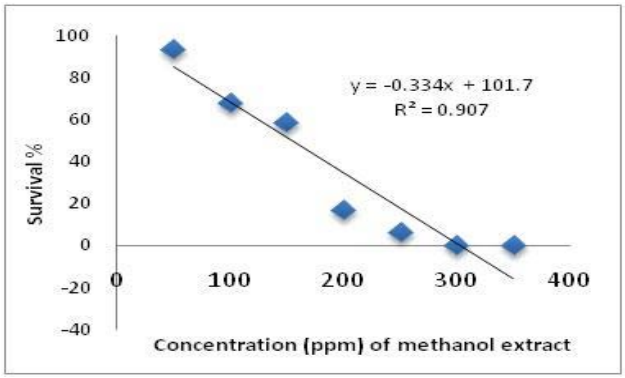


Figure 2: FT-IR analysis of crude solvent extracts of *S.wightii*

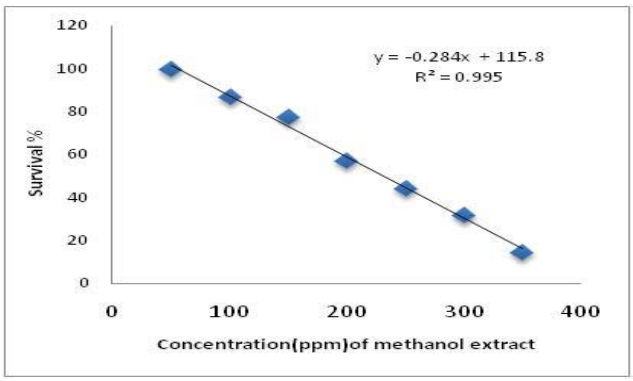
The secondary compounds make up a vast repository with a wide range of biological activities. The reports have indicated the presence of active compounds as steroidal Saponins. Saponins are freely soluble in both organic solvents and water, and they work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which is the most probable reason for larval death.[10,11] reported that saponin extracted from the fruit of *Balanites aegyptica* showed 100% mortality against larvae of *Stegoymia aegypti*. The larvicidal property of a saponin mixture isolated from *Cestrum diurnum* was also evaluated against *Anopheles stephensi* mosquito.[12] Aluminium chloride obtained from alder leaf, known for its phenolic complexing activity, is also reported to have the larvicidal activity against *S. aegypti.*[13] Isoflavonoids from tubers of *Neoraut aneniamitis* had a larvicidal effect against, the malaria and filariasis transmitting mosquitoes, *Anopheles gambiae* and *culex quinquefaciatus*, respectively.[14] Essential oils extracted from Brazilian plants exhibited larvicidal activity against *S. aegypti*, with LC_{50} values ranging from 60 to 538 ppm.[15]



a) 2nd instar



b) 3rd instar



c) 4th instar

Figure 3: Graphical estimation of LC_{50} of methanol extract at 24th h

Table 1: Preliminary phytochemical analysis of crude Solvent extracts of *S.wightii.*

Test	Hexane extract	Diethyl Ether Extract	Acetone extract	Methanol extract
Carbohydrate	-	-	-	-
Proteins	-	-	-	-
Aminoacids	-	-	-	-
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	-	+	+	-
Alkaloids	-	-	+	+
Flavonoids	+	+	+	+
Tannins	-	-	+	+
Saponins	-	+	+	+

Table 2: Larvicidal potentiality of different concentrations of methanol extract of *S.wightii* on 2nd, 3rd and 4th instar larvae of *A.aegypti* (mean of three experiments)

Instar	concentration ppm	Mean mortality (%)		
		24h	48h	72h
2nd	50	6.66	18.66	26.66
	100	26.66	45.33	65.33
	150	33.33	64.0	81.3
	200	61.33	81.33	94.66
	250	73.33	86.66	98.66
	300	90.66	94.66	100
	350	100	100	100
3rd	50	6.66	25.33	25.33
	100	32.0	46.66	66.66
	150	41.33	62.66	81.33
	200	82.66	94.66	100
	250	93.33	100	100
	300	100	100	100
	350	100	100	100
4th	50	0	0	0
	100	13.33	21.33	21.33
	150	22.66	41.33	48.0
	200	42.66	57.33	64.0
	250	56.0	72.0	72.0
	300	68.0	82.66	89.33
	350	85.33	93.33	93.33

The larvicidal activity of crude leaf extract of *Gymnema sylvestre* showed the highest mortality in the concentration of 1,000 ppm against the larvae of *Aedes subpictus* (LC_{50}=166.28 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50}=186.55 ppm), and the maximum efficacy was observed in gymnemagenol compound isolated from leaf extract of *G. sylvestre* using petroleum ether.[16] It showed LC_{50}values against the larvae of *A. subpictus* at 22.99 ppm and against *C.quinquefasciatus* at 15.92 ppm.

The present study indicated that solvent extracts of *S.wightii* offers, potential Biocontrol activity against *A. aegypti*. The highest larvicidal activity was noted with methanol extract. However, further studies on the identification of the active principles involved and their mode of action and field trials are needed to be analyzed.

Antimicrobial activity

To date, many chemically unique compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals. The cell extracts and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria [17]. The crude extracts (diethyl ether, methanol and acetone) of *S.wightii* were screened for antimicrobial activities against human pathogens. Methanol extract of *S.wightii* showed highest zone of inhibition against *Staphylococcus aureus* (16±1.3) mm followed by *Bacillus subtilis* (15.3±0.2 mm) and *K. pneumonia* (14.6±0.9 mm).Moderate zone of inhibition was observed against *P. aeruginosa* (14±0.5 mm). Similarly, acetone extract showed moderate zone of inhibition against *Bacillus subitilis* (12±1.4 mm), followed by *P. aeruginosa* (12±0.6mm), *K. pneumoniae* (12±1.4mm) and *S. aureus* (11±1.6mm). Diethyl ether extracts showed highest zone of inhibition against *S. aureus* (16.3±1.2 mm), followed by *B. subtilis* (14±1.2mm), *K. Pneumoniae* (11±1.8mm) and moderate zone of inhibition against *P. aeruginosa*(10±.02mm). No zone of inhibition was observed against *Salmonella typhi* for all the three extracts (Table 4).

The antifungal activity was studied from three solvent extracts of *S. wightii* against *Aspergillus niger, Candida albicans and Aspergillus flavus*. Methanol extract showed maximum antifungal activities against *C. albicans* (14±0.9mm) followed by *A. flavus* (12.6±0.2mm), *A. niger* (12±1.9mm). Acetone extract showed maximum antifungal activities against *C. albicans* (13±0.7mm) followed by *A. niger* (11.3±0.4mm) and *A. flavus* (9±0.7mm). No antifungal activity was observed with the extracts of diethyl ether (Table 5). The brown algal extracts showed higher activity than the extracts of red algae.[18] Earlier investigations showed higher antibacterial activity in the species of Phaeophyta and Rhodphyta of.[19-20] The extracts from brown algae shown higher degrees of antibacterial activity than extracts obtained from red and green algae.[21-22]

The results revealed that the methanol extract showed a strong antimicrobial activity against both gram positive and gram negative bacteria. Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth

according to their constitution and concentration.[23] The solvent extracts of brown seaweed *S.wightii* were active against bacterial and fungal pathogens. This may be due to active components which are present in the seaweed extracts. The results indicate that the extracts contained different antibacterial substances and reflect variety of secondary metabolites. However, the exact mechanism and the compound responsible for the antimicrobial activities were currently unclear. Therefore, it is suggested that the further works were performed on the isolation and characterization of the compound.

Table 3: Lethal concentration values of methanol extract of *S.wightii* against *A.aegypti* at 24, 48 &72 hours

Mosquito Larval Instars	Exposure hours	Regression equation	R^2	LC 50 (μgmL^{-1})	LC90 (μgmL^{-1})
II	24	Y= -0.32X+108.01	0.9853	181.28	306.28
	48	Y=-0.2686X+ 82.863	0.9213	122.34	271.26
	72	Y= -0.2191X+62.873	0.7603	58.75	241.31
III	24	Y= -0.3343X+101.72	0.9078	154.71	247.36
	48	Y= -0.2629X+76.96	0.8514	102.54	254.69
	72	Y= -0.2208X+62.251	0.7208	55.48	236.64
IV	24	Y= -0.2848X+115.81	0.995	231.07	371.52
	48	Y= -0.3095X+109.33	0.9814	191.69	320.93
	72	Y= -0.3143X+107.43	0.9571	182.72	309.99

Table 4: Antibacterial activity of crude extracts of *S.wightii* obtained in different solvents

Bacterial Strains	Standard (Ampicillin) (100µg/ml)	Negative control (100µg/ml)	Solvent Extracts (1000µg/ml) (Zone of inhibition – mm)		
			Acetone	Diethyl Ether	Methanol
B. subtilis	18±0.66	R	15±1.4	14±1.2	12.3±0.2
P .aeruginosa	20±0.54	R	12±0.6	10±0.2	14±0.5
S.aureus	18±0.46	R	13±1.6	16.3±1.2	16±1.3
S. typhi	24± 0.7	R	R	R	R
K. pneumonia	19±1.8	R	12±1.4	11±1.8	14.6±0.9

Table 5: Antifungal activity of crude extracts of *S.wightii* obtained in different solvents

Fungal Pathogens	Standard (Amphotericin) (100µg/ml)	Negative control (DMSO)	Solvent extracts (1000µg/ml) (Zone of inhibition – mm)		
			Acetone	Diethyl Ether	Methanol
C.albicans	22 ± 1.2	R	13 ± 0.7	R	14.6 ± 0.9
A.niger	20 ± 0.0	R	11.3 ± 0.4	R	12.33 ± 1.9
A.flavus	18 ± 1.8	R	9 ± 0.7	R	12.6 ± 0.2

CONCLUSION

Solvent extraction and phytochemical characterization of *S.wightii* was performed. Mosquito larvicidal activity was carried out with dengue viral vector, *A.aegypti*. Antimicrobial activity was carried out with bacterial and fungal pathogens. The organic solvent extracts of *S.wightii* were found to be a potent larvicide against second and third instar larvae of *A.aegypti*. The results of the study indicate that *S.wightii* is a potential candidate for the eco-congenial larvicide as an alternative to chemical insecticides.

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