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Antibacterial properties of various extracts of *Sargassum wightii* against Multidrug Resistant Bacterial Strains

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Abstract

In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, a search for new antimicrobial substances from other sources including marine plants is essential. The present study deals with antibacterial activity of extracts of *Sargassum wightii* from hexane, chloroform, ethylacetate, acetone and methanol against multi-drug resistant Standard and Clinical Bacterial strains *viz., Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholerae, Shigella flexneri, S. dysentriae, Proteus mirabilis* and *P. vulgaris.* The antibacterial activity was performed by disc diffusion, Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC). The mean zone of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.1 to 15.3 mm. The MIC were between 125 and 500 µg/ml, while the MBC were between 250 and 1000 µg/ml. The highest mean of zone inhibition (15.3 mm) and lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extract of *S. wightii* against *B. subtilis*. The ethyl acetate extract of the *S. wightii* showed strong phytochemicals such as terpenoids, tannins, phenolic compounds and steroids than the other solvents extracts. This study revealed that ethyl acetate extract of *S. wightii* is a source of antibacterial compounds for the treatment of multi drug resistant bacteria.

Key words: Antibacterial activity, *Sargassum wightii*, MDR Bacterial strain, MIC, MBC

Introduction

Seaweeds are low calorific values but contain vitamins, minerals and fibres. These are secondary metabolites characterized by antiviral, antibacterial and antifungal activities (Vallinayagam and Arumugam, 2009). Researchers have separated approximately 7000 marine natural products, 25 % which are from algae, 33 % from sponges and 24% from other invertebrates, mollusks echinoderms. In the past few decades, macro algae have been widely used in producers of a broad range of bioactive metabolites (Faulkner, 1995; Rosell and Srivasta, 1987). Pharmaceutical industries are interested in marine plants because of their rich and active molecules (Madhusudan *et al.* 2011).

Microorganisms have developed adaptation mechanisms against the action of antimicrobial drugs (Al-Haj *et al.*, 2009). This is a major concern and an urgent need for searching for new and safe antibacterial agents. Several studies have been investigated about the biological activities of algae extracts (Tringali, 1997). Different active molecules from seaweeds showed the antimicrobial activities against the pathogens such as *S. aureus* and *P. aeruginosa* that commonly cause infection in the human (Selvin and Lipton, 2004).

Sargassum wightii, one of the marine macro alga genera belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the family Sargassaceae and order Fucales. Plant dark-brown, 20-30 cm in height with a well marked holdfast, upper portion richly branched, axes cylindrical, glabrous, leaves 5-8 cm long and 2-9 mm broad, leaves tapering at the base and apex, midrib inconspicuous vesicles large, spherical or ellipsoidal being 5-8 mm long and 3-4 mm broad, stipe of the vesicle 5-7 mm long seldom ending into a long tip, receptacles in clusters and repeatedly. It is a large, economically important and ecologically dominant brown algae present in much of the tropics. It is found to be the most diverse genus among Phaeophyta in India and is represented by 38 species. *Sargassum wightii* is one of the important species belonging to the genus *Sargassum* and a wide range of bioactive properties have been reported from this species (Mizukoshi *et al.,* 1993). It is widely distributed on the southern coasts of Tamilnadu, India and many parts of Asia and it is reported to be used as animal feed, food ingredients and fertilizer. *S. wightii* shows a good amount of flavonoids in support of its antioxidant activity (Meenakshi *et al.,* 2009), indicate that this genus is an ideal target for investigating presence of bio-molecules for various medical and industrial applications. The search for plants with antimicrobial activity has gained increasing importance in recent years, due to a growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Cowan, 1999; Raskin *et al.*, 2002).

Bacterial infections especially those caused by multi-drug resistant (MDR) bacteria have become one of the great challenges for modern healthcare. Majority of scientists define MDR as ″resistance to at least 3 classes of antimicrobial agents″ (Falagas *et al.*, 2006). Antimicrobial agents have substantially reduced the threat posed by infectious diseases over a period of time

since their discovery in the 1940s (Lewis and Ausubel, 2006). However, the escalation of multidrug resistance in bacteria in recent years has seriously jeopardized these gains. This has gained worldwide attention due to the high impact on public health. Increased usage of antimicrobial agents to treat bacterial infections has lead to the emergence of MDR strains (Bonnet, 2004).

Since the ancient time, the idea of finding healing powers in plants has been noteworthy. Plants produce a vast array of secondary metabolites that in many cases, these substances act as defense mechanisms against predation by herbivores, microorganisms and insects; also similar substances can be produced by plants as a part of their normal growth and development or in response to stress (Mirjana *et al.*, 2004; Cowan, 1999). Hence in the present study antibacterial activity of different organic solvents extracts of *S. wighti* was examined against multi-drug resistant standard and clinical bacterial strains to search for new antibacterial agents.

Materials and Methods

Sample collection and Preparation of extracts

The marine brown alga *Sargassum wightii* (Phaeophyceae) were collected on November to January, 2012 from Manappad (Lat. 8°30′N; Long. 78°8′E), Tuticorin district, the Gulf of Mannar Marine biosphere, Tamil Nadu, India. Fresh algal samples were handpicked during low tide and manually cleaned from sand, epiphytes and animal waste. Then the samples were rinsed with sea water to remove associated debris, planktons and loosely attached microorganisms. Morphologically distinct thallus of algae was placed separately in new polythene bags and were kept in an ice box containing slush ice and transported to laboratory. Further, the material was washed thoroughly with tap water to remove the salt on the surface of the samples and the water was drained off from the algae and spread on the blotting paper to remove the excess water. The shade dried samples were again cleaned with sterile distilled water to remove the remaining salt on the surface of the samples to avoid pumping of the solvent during the extraction process. The algal samples were shade dried followed by oven drying at 50 °C for an hour and milled in an electrical blender. Five hundred grams of powdered samples were packed in Soxhlet apparatus and extracted with different solvents like hexane, chloroform, ethyl acetate, acetone and methanol for 72 hours. The extracts were pooled and the solvent were evaporated under vacuum in rotary evaporator (Heidolph, Germany) at 40 °C and the dried extracts were stored at 4 °C in refrigerator for antibacterial assay.

Phytochemical analysis

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. wightii* were subjected to qualitative phytochemical studies. Phytochemicals like terpenoids, tannins, cardic glycosides, steroids, alkaloids, phenolic compounds, coumarins, unsaturated sterols and diterpenoids were carried out according to the method described by Harborne (1973) and Trease and Evans, (1983).

Microorganisms tested

The following Standard Bacterial strains used for assay viz., *Bacillus subtilis* (MTCC 441), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Klebsiella pneumonia* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 741), *Proteus mirabilis* (MTCC 425), *P. vulgaris* (MTCC 426), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457) and *Vibrio cholera* (MTCC 3906) were procured from Microbial Type Culture Collections (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

The Clinical isolates of bacterial strains were as following *viz., S. pyogenes, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, P. aeruginosa, S. typhimurium, S. dysentriea, S. flexneri,* and *V. cholerae* were obtained from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The strains were maintained in the laboratory by regular sub-culturing onto nutrient agar slants.

In vitro antibacterial activity was determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) was obtained from Himedia, Mumbai

Antibiotic sensitivity test

Antibiotic sensitivity of the bacterial strains was determined by standard CLSI disc diffusion method (M100-S22, 2012). Antibacterial agents from different classes of antibiotics viz., Amikacin (AK 30 µg/disc), Ampicillin (AMP 10 µg/disc), Cefixime (CFM 5 µg/disc), Ceftazidime (CAZ 30 µg/disc), Ciprofloxacin (CIP 5 µg/disc), Chloramphenicol (C 30 µg/disc), Erythromycin

(E 15 µg/disc), Gentamycin (GEN 10 µg/disc), Norfloxacin (NX 10 µg/disc), Nalidixic acid (NA 30 µg/disc), Ofloxacin (OF 5µg/disc), Streptomycin (S 10 µg/disc) and Tetracycline (TE 30 µg/disc) were obtained from Himedia, Mumbai.

Antibacterial activity assay

Disc Diffusion Method

The antibacterial activity of extracts of *S. wightii* was determined by disc diffusion method according to Bauer *et al.* (1966) with modifications. The Petri plates were prepared by pouring 20 ml of sterilized molten MHA. All bacterial isolates were suspended in saline to a turbidity equivalent to 0.5 McFarland (1.5 x 10^8 CFU/ml) and 0.1ml standardized inoculum suspension was swabbed uniformly in MHA plates. The crude extracts were dissolved in 10% DMSO and under aseptic conditions Sterile HiMedia paper disc (6mm) were impregnated with 20 µl of different concentrations (500, 250 and 125 µg/disc) of crude extracts. The discs with extract were placed on the surface on the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Ampicillin (10µg/disc) was used as positive control and a disc loaded with 10 % DMSO alone served as the blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of the disc). The assay in this experiment was repeated three times.

Determination of the Minimum Inhibitory Concentration (MIC)

MIC of the *S. wightii* crude extracts, a modified reaszurin microtitre plate assay was carried out according to methods of Sarker *et al.* (2007). Fifty microliter of Sterile MHB was transferred in to each well of a sterile 96-well micro titer plate (Hi-Media TPG 96). The *S. wightii* extracts was dissolved in 10 per cent DMSO to obtain 2000 µg/ml stock solution. A volume of 50 µl of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth 50µl of the solution was transferred to the second well and in this way, the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 1000 to 15.625 µg/ml of the extract in each well. To each well 10 µL of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270 mg tablet in 40mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution). Then 30 µl of MHB was added to each well. Finally, 10 µl of bacterial suspension was added to each well to achieve a concentration of approximately 5 $\times 10^5$ CFU/ml. Each plate had a set of controls: a column with all solutions with the exception of the crude extracts; a column with all solutions with the exception of the bacterial solution adding 10 µl of MHB instead and a column with 10 % DMSO solution as a negative control. The plates were incubated at 37 °C for 24 h for all bacterial strains. The colour change was then assessed visually. The growth was indicated by colour changes from purple to pink (or colourless). The lowest concentration at which colour change occurred was taken as the MIC value.

Determination of the Minimum Bactericidal Concentration (MBC)

MBC of the *S. wightii* extracts were determined by plating a loop full of bacterial solution from each MIC assay well with growth inhibition into freshly prepared MHA. The plates were incubated at 37 °C for 24 h for all bacterial strains. The MBC was recorded as the lowest concentration of the extract that did not permit any visible bacterial growth after the period of incubation.

Statistical Analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant difference between different extracts for *in vitro* antibacterial assays. Comparison of means for *in vitro* antibacterial assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. P value < 0.05 was considered statistically significant.

Results

The antibiotic resistance of bacterial strains of both clinical and standard strains was confirmed by CLSI-M100-S22, 2012 method. The standard strains of *B. subtilis, K. pnemoniae* and *P. vulgaris* were sensitive to all the antibiotics tested except CFM, AMP and CAZ. The standard strains of *S. flexneri* and *P. mirabilis* were the antibiotics tested except AMP. The standard strains of *S. pyogenes* were resistant to CFM, AMP, CAZ, NA and E and sensitive to all antibiotics tested. The standard strains of *E. coli* were sensitive to all antibiotics tested except AMP and NA. The standard strains of *P. aeruginosa* were resistant to CFM, AMP and TE and sensitive to all other antibiotics tested. The standard strains of *S. typhimurium* were sensitive to all antibiotics

except AMP and E. The standard strains of *V. cholerae* were resistant AMP and intermediate resistant to S and sensitive to all other antibiotics tested.

The clinical isolates of *S. pyogenes* were sensitive to all antibiotics tested and resistant to CFM, AMP, CAZ, OF and E. The clinical isolates of *E. coli* were sensitive to all the antibiotics tested and resistant to CFM, AMP, CAZ and GEN. The clinical isolates of K. *pneumoniae* were resistant to all the antibiotics tested and sensitive to GEN, S, TE, AK and E. The clinical isolates of *P. aeruginosa* were sensitive to all the antibiotics tested and resistant to CFM, AMP, CAZ and E. The clinical isolates of *S. typhimurium* were sensitive to all antibiotics tested and resistant to AMP, CFM and OF. The clinical isolates of *V. cholerae* were sensitive to all antibiotics tested and resistant to AMP, CFM, CAZ, NX and E. The clinical isolates of *S. flexneri* were sensitive to all antibiotic tested and resistant to AMP, CFM, CAZ, NX, OF and NA. The clinical isolates of *S. dysentriea* were sensitive to all antibiotics tested and resistant to AMP, CFM and OF. The clinical isolates of *P. mirabilis* were sensitive to all antibiotics tested and resistant to AMP, CFM, GEN, NX, NA and E. The clinical isolates of *P. mirabilis* were sensitive to all antibiotics tested and resistant to AMP, CFM, GEN, NX, NA and OF.

The phytochemical analysis of hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. wightii* had showed the presence of terpenoids, tannins, unsaturated sterols and diterpenoids. Alkaloids and coumarins are not present in all the extracts tested. Steroids were present in all the extracts tested except methanolic extracts. Cardiac glycosides were present in chloroform and acetone extracts. Phenolic compounds were present in chloroform and ethyl acetate extracts.

The five different organic solvents of hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. wightii* were tested against MDR both clinical and standard bacterial strains and results are presented in (Table 1&2). All the extracts of *S. wightii* possessed significant antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. There was no much variation among the clinical and standard bacterial strains towards the algal extracts tested. The mean values are presented in Table 1 and 2. When the different extracts were assayed against the test bacteria by agar diffusion assays, the mean zone of inhibition obtained were between 7.1 and 15.3 mm. Ampicillin (30 µg/disc) antibacterial positive control produced mean zone of inhibition ranged from 7.1 to 15.3 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The MIC values of the different extracts of *S. wightii* ranged between 125 and 500 µg/ml, while the MBC values were between 250 and 1000 µg/ml. The highest mean of zone inhibition (15.3 mm) and lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extract of *S. wightii* against *B. subtilis*.

Table – 1: Antibacterial activity of *Sargassum wightii* against Multidrug Resistant standard Bacterial Strains

Sl. No	Seaweed extracts/ solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc **(µg/disc)**				**MIC** (**µ**g/ml)	M**BC** (**µ**g/ml)
		500	**250**	**125**	**Ampicillin (10mg/disc)**		
1	*Bacillus subtilis* (MTCC 441)						
	Hexane	12 .0 ± 0.50	10.1 ± 0.28	8.0 ± 0.50	8 .0 ± 0.50	250	250
	Chloroform	13 .3 ± 0.57	10.3 ± 0.57	8.3 ± 0.57	7.3 ± 0.57	250	500
	Ethyl acetate	15.3 ± 0.57**	13.0 ± 0.50	9.5 ± 0.50	8 .1 ± 0.28	125	250
	Acetone	11.5 ± 0.50	9.3 ± 0.57	7.3 ± 0.57	7.5 ± 0.50	500	1000
	Methanol	11.1 ± 0.28	9.1 ± 0.57	7.1 ± 0.28	7.8 ± 0.76	500	1000
2	*Streptococcus pyogenes* (MTCC 442)						
	Hexane	12.5 ± 0.50	9.5 ± 0.50	7.1 ± 0.28	8.0 ± 0.50	500	1000
	Chloroform	12.8 ± 0.76	9.6 ± 0.57	7.5 ± 0.50	8.3 ± 0.57	250	1000

	Ethyl acetate	13.6 ± 0.28	10.0 ± 0.50	8.8 ± 0.28	9.3 ± 0.57	250	500
	Acetone	10.6 ± 0.57	9.1 ± 0.28	7.1 ± 0.28	8.6 ± 0.76	500	1000
	Methanol	11.6 ± 0.57	9.5 ± 0.5	7.3 ± 0.57	9.3 ± 0.57	500	1000
3	*Escherichia coli* (MTCC 443)						
	Hexane	10.8 ± 0.57	9.1 ± 0.28	7.1 ± 0.28	12.8 ± 0.28	500	1000
	Chloroform	11.5 ± 0.50	9.6 ± 0.28	7.3 ± 0.57	12.0 ± 0.50	500	10 00
	Ethyl acetate	12.6 ± 0.57	9.6 ± 0.28	8.5 ± 0.50	9.3 ± 0.57	250	500
	Acetone	10.5 ± 0.50	9.3 ± 0.28	7.3 ± 0.57	7.3 ± 0.28	500	1000
	Methanol	10.1 ± 0.28	9.1 ± 0.28	7.1 ± 0.28	7.3 ± 0.28	500	1000
4	*Klebsiella pneumoniae* (MTCC109)						
	Hexane	11 ± 0.50	9.1 ± 0.28	7.5 ± 0.50	8.6 ± 0.76	500	1000
	Chloroform	12 ± 0.50	10.1 ± 0.28	7.6 ± 0.50	9.3 ± 0.57	500	1000
	Ethyl acetate	12.8 ± 0.28	10.3 ± 0.28	8.0 ± 0.50	7.3 ± 0.28	250	500
	Acetone	10.5 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	7.8 ± 0.76	500	1000
	Methanol	10.5 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	9.3 ± 0.57	500	1000
5	*Proteus mirabilis* (MTCC 425)						
	Hexane	11.1 ± 0.28	9.3 ± 0.57	7.3 ± 0.57	11.6 ± 0.76	500	1000
	Chloroform	12.0 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	11.0 ± 0.50	500	1000
	Ethyl acetate	13.0 ± 0.50	10.1 ± 0.76	8.0 ± 0.50	12.1 ± 0.28	250	500
	Acetone	11.1 ± 0.28	9.3 ± 0.57	7.5 ± 0.50	9.3 ± 0.57	500	1000
	Methanol	10.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.28	11.6 ± 0.76	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm;[b]-mean of three assays; ± - standard deviation. ** significant at $p < 0.05$

Table – 1: continued

Sl. No	Seaweed extracts/ solvents	Mean zone of inhibition[a] (mm)[b]				**MIC** (µg/ml)	M**BC** (µg/ml)
		Concentration of the disc					
		500	**250**	**125**	**Ampicillin (10mg/disc)**		
6	*Proteus. vulgaris* (MTCC 426)						
	Hexane	9.8 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	10.3 ± 0.28	500	1000
	Chloroform	12.1 ± 0.28	10.0 ± 0.50	7.5 ± 0.50	11.0 ± 0.50	500	1000
	Ethyl acetate	12.8 ± 0.57	10.5 ± 0.50	7.8 ± 0.76	12.8 ± 0.28	250	500
	Acetone	10.5 ± 0.50	9.0 ± 0.50	7.3 ± 0.57	8.6 ± 0.76	500	1000
	Methanol	10.5 ± 0.50	9.0 ± 0.50	7.1 ± 0.28	10.3 ± 0.57	500	1000
7	*Pseudomonas aeruginosa* (MTCC 741)						
	Hexane	10.1 ± 0.28	9.1 ± 0.28	7.6 ± 0.76	10.3 ± 0.28	500	1000

	Chloroform	11.8 ± 0.76	9.8 ± 0.50	7.5 ± 0.50	12.0 ± 0.86	500	1000
	Ethyl acetate	13.1 ± 0.28	10.6 ± 0.28	8.5 ± 0.50	10.8 ± 0.76	250	500
	Acetone	10.6 ± 0.76	9.1 ± 0.28	7.1 ± 0.35	8.6 ± 0.76	500	1000
	Methanol	10.6 ± 0.28	9.1 ± 0.28	7.1 ± 0.28	10.3 ± 0.28	500	1000
8	*Salmonella typhimurium* (MTCC 98)						
	Hexane	11.3 ± 0.86	8.8 ± 0.76	7.3 ± 0.57	7.8 ± 0.76	500	1000
	Chloroform	11.8 ± 0.57	9.5 ± 0.50	7.5 ± 0.50	8.8 ± 0.76	500	10 00
	Ethyl acetate	13.3 ± 0.28	10.1 ± 0.76	8.6 ± 0.57	11 ± 0.50	250	500
	Acetone	10.6 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	8.8 ± 0.76	500	1000
	Methanol	10.6 ± 0.57	8.8 ± 0.28	7.1 ± 0.28	10.3 ± 0.57	500	1000
9	*Shigella flexneri* (MTCC 1457)						
	Hexane	10.5 ± 0.50	9.1 ± 0.28	7.5 ± 0.5	7.8 ± 0.76	500	1000
	Chloroform	12.0 ± 0.50	10.1 ± 0.50	7.6 ± 0.76	8.8 ± 0.76	500	1000
	Ethyl acetate	13.1 ± 0.57	10.3 ± 0.28	8.5 ± 0.50	10.3 ± 0.28	250	500
	Acetone	11.1 ± 0.28	9.5 ± 0.50	7.6 ± 0.76	10.8 ± 0.76	500	1000
	Methanol	10.8 ± 0.28	9.0± 0.50	7.3 ± 0.28	11.0 ± 0.5	500	1000
10	*Vibrio cholerae* (MTCC 3906)						
	Hexane	10.5 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	12.0 ± 0.5	500	1000
	Chloroform	12.0 ± 0.50	10.0 ± 0.28	7.6 ± 0.57	11.6 ± 0.76	500	1000
	Ethyl acetate	13.5 ± 0.50	10.1 ± 0.28	8.1 ± 0.28	10.3 ± 0.28	250	500
	Acetone	11.1 ± 0.28	9.5 ± 0.50	7.3 ± 0.57	10.3 ± 0.28	500	1000
	Methanol	10.5 ± 0.50	8.8 ± 0.28	7.1 ± 0.28	12.1 ± 0.28	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation. ** significant at $p < 0.05$

Table – 2: Antibacterial activity of *Sargassum wightii* against Multidrug Resistant clinical Bacterial Strains

Sl. No	Seaweed extracts/ solvents	Mean zone of inhibition[a] (mm)[b]				**MIC** (µg/ml)	M**BC** (µg/ml)
		Concentration of the disc **(µg/disc)**					
		500	**250**	**125**	**Ampicillin (10mg/disc)**		
1	*Streptococcus pyogenes*						
	Hexane	12.5 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	7.3 ± 0.28	500	1000
	Chloroform	13.0 ± 0.50	9.6 ± 0.57	7.8 ± 0.76	8.3 ± 0.57	250	1000
	Ethyl acetate	13.0 ± 0.50	10.0 ± 0.50	8.0 ± 0.50	9.3 ± 0.57	250	500
	Acetone	10.6 ± 0.57	9.1 ± 0.28	7.3 ± 0.57	8.6 ± 0.76	500	1000
	Methanol	11.6 ± 0.57	9.5 ± 0.50	7.1 ± 0.28	9.1 ± 0.28	500	1000
2	*Escherichia coli*						
	Hexane	10.3 ± 0.28	9.0 ± 0.50	7.1 ± 0.28	12.8 ± 0.28	500	1000

	Chloroform	11.0 ± 0.50	9.6 ± 0.28	7.3 ± 0.57	12.0 ± 0.50	500	10 00
	Ethyl acetate	12.5 ± 0.50	10.0 ± 0.50	7.5 ± 0.50	13.1 ± 0.28	250	500
	Acetone	10.1 ± 0.28	9.1 ± 0.28	7.3 ± 0.57	11.6 ± 0.57	500	1000
	Methanol	10.0 ± 0.28	9.0 ± 0.50	7.1 ± 0.28	11.8 ± 0.76	500	1000
3	*Klebsiella pneumoniae*						
	Hexane	10.1 ± 0.28	9.0 ± 0.50	7.3 ± 0.57	8.8 ± 0.76	500	1000
	Chloroform	11.1 ± 0.28	9.6 ± 0.76	7.5 ± 0.50	9.3 ± 0.57	500	1000
	Ethyl acetate	12.6 ± 0.76	10.1 ± 0.28	7.8 ± 0.76	10.3 ± 0.28	250	500
	Acetone	10.0 ± 0.50	9.1 ± 0.28	7.1 ± 0.50	7.3 ± 0.57	500	1000
	Methanol	10.3 ± 0.57	9.0 ± 0.50	7.1 ± 0.28	9.3 ± 0.57	500	1000
4	*Proteus mirabilis*						
	Hexane	11.1 ± 0.28	9.5 ± 0.50	7.3 ± 0.57	10.8 ± 0.76	500	1000
	Chloroform	12.0 ± 0.50	9.3 ± 0.57	7.5 ± 0.50	10.3 ± 0.28	500	1000
	Ethyl acetate	13.6 ± 0.28	10.1 ± 0.76	8.3 ± 0.57	8.8 ± 0.76	250	500
	Acetone	11.1 ± 0.28	9.5 ± 0.50	7.3 ± 0.57	9.3 ± 0.57	500	1000
	Methanol	10.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.28	11.6 ± 0.76	500	1000
5	*Proteus vulgaris*						
	Hexane	9.8 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	11.6 ± 0.76	500	1000
	Chloroform	12.1 ± 0.28	10.0 ± 0.50	7.5 ± 0.50	10.3 ± 0.28	500	1000
	Ethyl acetate	12.8 ± 0.57	10.5 ± 0.50	7.6 ± 0.76	9.1 ± 0.28	250	500
	Acetone	10.5 ± 0.50	9.0 ± 0.50	7.3 ± 0.57	9.3 ± 0.57	500	1000
	Methanol	10.5 ± 0.50	9.0 ± 0.50	7.1 ± 0.28	10.3 ± 0.57	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation. ** significant at $p < 0.05$

Table – 2: continued

Sl. No	Seaweed extracts/ solvents	Mean zone of inhibition[a] (mm)[b]				**MIC** (µg/ml)	M**BC** (µg/ml)
		Concentration of the disc **(µg/disc)**					
		500	**250**	**125**	**Ampicillin (10mg/disc)**		
6	*Pseudomonas aeruginosa*						
	Hexane	11.5 ± 0.50	9.8 ± 0.57	7.5 ± 0.50	10.3 ± 0.57	500	1000
	Chloroform	13.0 ± 0.50	10.1 ± 0.76	7.8 ± 0.76	12.0 ± 0.86	250	500
	Ethyl acetate	13.0 ± 0.50	10.5 ± 0.50	8.3 ± 0.57	11.6 ± 0.76	250	500
	Acetone	10.6 ± 0.76	8.8 ± 0.76	7.1 ± 0.28	12.1 ± 0.28	500	1000
	Methanol	11.0 ± 0.50	9.1 ± 0.28	7.5 ± 0.50	12.8 ± 0.28	500	1000
7	*Salmonella typhimurium*						
	Hexane	11.3 ± 0.86	8.8 ± 0.76	7.3 ± 0.57	8.6 ± 0.76	500	1000

	Chloroform	11.8 ± 0.57	9.5 ± 0.50	7.5 ± 0.50	8.8 ± 0.76	500	10 00
	Ethyl acetate	13.3 ± 0.28	10.1 ± 0.76	7.6 ± 0.57	10.3 ± 0.28	250	500
	Acetone	10.6 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	8.8 ± 0.76	500	1000
	Methanol	10.6 ± 0.57	8.8 ± 0.28	7.1 ± 0.28	10.3 ± 0.57	500	1000
8	*Shigella dysentrieae*						
	Hexane	11.6 ± 0.76	9.5 ± 0.50	7.3 ± 0.57	12.0 ± 0.50	500	1000
	Chloroform	12.1 ± 0.28	10.0 ± 0.50	7.5 ± 0.50	11.6 ± 0.76	500	1000
	Ethyl acetate	12.6 ± 0.76	10.1 ± 0.28	7.6 ± 0.76	8.8 ± 0.76	250	500
	Acetone	11.5 ± 0.50	10.5 ± 0.50	7.3 ± 0.57	10.3 ± 0.28	500	1000
	Methanol	11.0 ± 0.50	9.5 ± 0.50	7.3 ± 0.57	10.8 ± 0.76	500	1000
	Shigella flexneri						
	Hexane	10.5 ± 0.50	9.1 ± 0.28	7.5 ± 0.50	7.8 ± 0.76	500	1000
	Chloroform	12.0 ± 0.50	10.1 ± 0.50	7.8 ± 0.76	8.8 ± 0.76	500	1000
	Ethyl acetate	13.1 ± 0.57	10.3 ± 0.28	8.5 ± 0.50	10.3 ± 0.28	250	500
	Acetone	11.1 ± 0.28	9.5 ± 0.50	7.6 ± 0.76	10.8 ± 0.76	500	1000
	Methanol	10.8 ± 0.28	9.0± 0.50	7.3 ± 0.28	11.0 ± 0.50	500	1000
10	*Vibrio cholerae*						
	Hexane	10.5 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	9.3 ± 0.57	500	1000
	Chloroform	12.0 ± 0.50	9.5 ± 0.50	7.6 ± 0.57	11.0 ± 0.50	500	1000
	Ethyl acetate	13.5 ± 0.50	10.1 ± 0.28	8.1 ± 0.28	12.1 ± 0.28	250	500
	Acetone	11.1 ± 0.28	9.8 ± 0.76	7.3 ± 0.57	10.0 ± 0.50	500	1000
	Methanol	10.5 ± 0.50	8.8 ± 0.28	7.1 ± 0.28	12.1 ± 0.28	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation. ** significant at $p < 0.05$

Discussion

The marine environment representing approximately half of the global biodiversity is an enormous resource for new compounds. Seaweeds are potential sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents (Kotnala and Chatterji, 2009). Many studies were reported about the biological activities of algal extracts from different coastal regions around the world (Vijayabaskar and Shiyamala, 2011). In the present study, the different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. wightii* possessed antibacterial activity against all the clinical and standard bacterial strains tested. The ethyl acetate extract of *S. wightii* showed the highest antibacterial activity than other extracts against *B. subtilis* and followed by all bacterial strains tested.

Salem *et al*. (2011) reported that higher antibacterial activity was recorded for ethyl acetate extracts of *Caulerpa racemosa, Sargassum dentifolium, Padina gymnospora*; methanolic extracts of *Sargassum hystrix, C. racemosa, C. fragile, S. dentifolium* and *Cystoseria myrica*. Immanuel *et al.* (2004) showed the antibacterial properties of the n-butanolic extracts of four ayurvedic herbals and two seaweeds *Ulva lactuca* and *Sargassum wightii* against shrimp pathogen *Vibrio parahaemolyticus*. Bibiana *et al.* (2012) reported that the maximum activity of diethyl ether extract of *S. wightii* and *Kappaphyus alwarezii* showed a good antibacterial activity against *Streptococcus faecalis, S. pneumoniae, B. subtilis, Bacillus aureus, Vibrio cholerae V. parahaemolyticus E. coli S. aureus K. pneumonia, P.aeruginosa, Proteus, Citrobacter, Microsporum gypsum, Aspergillus fumigatus, A niger, A. flavus* and *Trichophyton rubrum*.

In this study, ethyl acetate extracts of *S. wightii* showed antibacterial activity against *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, P. mirabilis*, and *P. vulgaris*. Ibtissam *et al.* (2009) reported non efficiency of the methanol extract of *Sargassum vulgare*, which did not show antibacterial activity against *E. coli* and *S. aureus* growth. Kandhasamy and Arunachalam (2008) reported that extracts prepared with methanol showed the best activity. Vijayabaskar and Shiyamala (2011) reported that *Turbinaria ornata* and *S. wightii* extracts were active against nine pathogens such as *Aeromonas hydrophila, B. subtilis, Enterococcus faecalis, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. flexneri* and *Staphylococcus aureus*. These results indicate that the extracts contained different antibacterial substances and reflect the variety of secondary metabolites. Zubia *et al.* (2008), suggested by the great variation observed in the potential antimicrobial components in seaweeds could be due to the external environmental factors such as herbivory, light, depth, salinity and nutrients of their growing environment.

In this study, different extracts of *S. wightii* showed highest mean zone of inhibition (15.3 mm) and the lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extracts of *S. wightii* against *B. subtilis.* Kim and Lee (2008) used methanolic extracts of *Esiena bicyclis* (B32) and *Sargassum* sp. (B36) which showed strong antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, *Vibrio parahemolyticus*. Kolanjinathan and Stella (2009) indicated that acetone was the best solution for extracting the effective antimicrobial materials from *Sargassum myricystum, Turbinaria conoides, Hypnea musiformis, Gracilaria edulis* and *Halimedia gracilis*. Fatty acid methyl ester extracts of *Sargassum longifolium* and *S. teretifolium* showed antibacterial activity against *B. subtilis, S. aureus, Micrococcus luteus, Salmonella typhimurium, P. aeruginosa, K. pneumoniae* and *E. coli* (Anantharaj *et al.*, 2005). Tuney *et al.* (2006) reported the antibacterial activity of the methanolic extracts of *Ulva rigida, Enteromorpha Linza, Padina pavonica, Cystoseria sniosa, Dictyopteris linearis, D. membranacea, C. mediterranea, E siliculous, Ceramium rubrum, Gracilaria gracilis Acanthophora nojadiformis* inhibited the growth *E. coli*. Kolanjinathan and Stella, (2002) and Mansuya *et al.* (2010) reported that the aqueous and methanolic extract of all selected algal species were screened and showed antibacterial activity against *E. coli, P. aeruginosa, S. typhi, Staphylococcus epidermis and S. pyogenes.*

Chiheb *et al*. (2009) reported methanol extract of *Sargassum vulgare* did not show antibacterial activity against *E. coli* and *S. aereus*. Manilal *et al.* (2009) and Rangaiah *et al.* (2010) showed that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate which in contrast to our results. However, the result obtain by the aforementioned author suggest by the same species varies remarkable difference may be due to several factors. The variation in antibacterial activity may be due to the method of extraction, solvents used in extraction and season at which samples were collected.

In the present work, the ethyl acetate extract of *S. wightii* showed the maximum antibacterial activity probably be due to the presence of phytochemicals, terpenoids, tannins, phenolic compound, and steroids. Seaweeds provide a rich source of structurally diverse secondary metabolites. Several studies have demonstrated that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols and carotenoids has exhibited different biological activities (Rodriguez-Bernaldo de Quiros *et al.*, 2010). Steroids, phenolic groups, saponins, tannins, flavonoids, carbohydrates, carboxylic acid, coumarins, and xantoproteins were detected in the extracts of *S. wightii* (Vijayabaskar and Shiyamala, 2011). Many tannin containing drugs are used in medicine as astringent. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, antiinflammatory, antiulcer and antioxidant property for possible therapeutic applications (Kolodziej and Kiderlen, 2005). Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposome (Raquel and Epand, 2007). 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 (Reguant *et al.*, 2000). Zapata and McMillan (1979) reported that the role of phenolic compounds present in seagrasses could also enhance the antimicrobial activity.

The results of the present study revealed that Gram positive bacteria were more susceptible than Gram negative bacteria. Tuney et al. (2006) also reported that Gram positive bacteria were more effectively controlled by the extracts of algae used in their study compared to Gram negative bacteria. Taskin and Ozturk Kurt (2001), revealed similar observations, indicating that the more susceptibility of Gram-positive bacteria to the algal extract was due to the differences in their cell wall structure and their composition (Paz et al., 1995). In Gram negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics (Tortora *et al.*, 2001). The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors (Martin, 1995).

Since ethyl acetate extract of *S. wightii* showed potential antibacterial activity against all the MDR bacterial strains tested. Moreover, it was indicated the potential source of a variety of biologically active marine organisms and it is hope that the present

results will provide a starting point for investigations aimed at exploiting new natural antibacterial substances present in the *S. wightii*.

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References

Ananatharaj, M., V.Venkatesalu, M. Chandrasekaran, and Sivasankari, S, 2005. Screening of fatty acid methyl ester of marine alga for antibacterial activity. Seaweed. Res. Util., 26: 87-92.

Al-Haj, N. A., N.I. Mashan, M.N. Shamsudin, H. Mohamad, C. S. Vairappan, and Z. Sekawi, 2009. Antibacterial activity in marine alga *Eucheuma denticulatum* against *Staphylococcus aureus* and *Streptococcus pyogenes.* Res. J. Biol. Sci., 4: 519-524.

Arputha Bibiana, M., K. Nithya, M.S. Manikandan, P. Selvamani, and S. Latha, 2012. Antimicrobial evaluation of the organic extracts of *Sargassum wightii* (brown algae) and *Kappaphycus alwarezii* (red algae) collected from the coast of Meemesal, Tamilnadu. Int. J. Pharm. Chem. Biol. Sci. 2(4): 439-446.

Bauer, A.W., W.M.M. Kirby, J.C. Scherris, and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. American J. Clin. Pathol., 45: 493–496.

Bonnet, R. 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother., 48: 1–14.

Chiheb, I., R. Hassane, Martinez- L. José, D.S.J. Francisco, G.V.J. Antonio, B. Hassan and K. Mohamed, 2009. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. Afr. J. Biotechnol., 8(7): 1258-1262.

Clinical Laboratory Standards Institute (formerly NCCLS): Performance Standards for Antimicrobial disk susceptibility tests: M100-S22, CLSI, **32** (3) 2012.

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clinic. Microbiol. Rev., 12: 564 - 582.

Falagas, M.E., P.K. Koletsi, and I.A. Bliziotis, 2006. The diversity of definitions of multidrugresistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa.* J. Med. Microbiol., 55:1619-29.

Faulkner, J. 1995 Chemical riches from the oceans. *Chem. Brit.*, 31: 680-684.

Harborne I.B. 1973. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn, Chapman and Hall, New York, 88-185.

Ibtissam, C., H. Radio, J. Martinez-Lopez, S.J.F. Dominguez, V.J.A. Gomez, and H. Bouziane, 2009. Screening of antibacterial activity in marine green brown macroalgae from the coast of Morocco. Afr. J. Biotechnol., 8: 1258-1262.

Immanuel, G., V. C. Vincybai, V. Sivaram, A. Palavesam, and M. P. Marian, 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquacul., 236: 53–65.

Kandhasamy, M. and K.D. Arunachalam, 2008. Evaluation of in vitro antibacterial property of seaweeds of southeast coast of India. Afr. J. Biotechno., 7: 1958-1961.

Kim, I.H. and J. H. Lee, 2008. Antimicrobial activities against methicillin-resistant *Staphylococcus aureus* from macroalgae. J. Ind. Eng. Chem., 14: 568-572.

Kolanjinathan, K. and D . Stella, 2009. Antibacterial activity of marine macro algae against human pathogens. Recent Res. Sci. Technol., 1(1): 20-22.

Kolodziej, H. and A.F. Kiderlen, 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania parasitised* RAW 264.7 cells. Phytochem., 66 (17): 2056-2071.

Kotnala, S., A. and A. Chatterji, 2009. Screening for the presence of antimicrobial activity in few Indian seaweeds. Pertanika J. Trop. Agric. Sci., 32:69-75.

Lewis, K. and F. M. Ausubel, 2006. Prospects of plant derived antibacterials. J. Nat. Biotechnol., 24: 1504-1507.

Madhusudan, C., S. Manoj, K. Rahul, and C.M. Rishi, 2011. Seaweeds: A diet with nutritional, medicina1 and industrial value. Res. J. Med. Plant., 5:153-157.

.

Manilal, A., S. Sujith, J. Selvin, C. Shakir and G. S. Kiran, 2009. Antibacterial activity of *Falkenbergia hillebrandii* (Born) from the Indian coast against human pathogens. Yton., 78: 161-166.

Mansuya, P., P. Aruna, S. Sridhar, J. Suresh Kumar and S. Babu, 2010. Antibacterial Activity and Qualitative Phytochemical Analysis of Selected Seaweeds from Gulf of Mannar Region. J. Exp. Sci., 8: 23-26.

Martin, G.J. 1995. Ethnobotany: A Methods Manual, Champan and Hall, London.

Meenakshi, S., G.D. Manicka, S. Tamilmozhi, M. Arumugam and T, Balasubramanian, 2009. Total flavanoid and in vitro antioxidant activity of two seaweeds of Rameshwaram Coast, Global J. Pharmacol., 3: 59-62.

Mirjana, S., B Nada, and D. Valerija, 2004. Variability of *Satureja cuneifolia* : ten essential oils and their antimicrobial activity depending on the stage of development. Eur .Food. Technol. 218: 367-71.

Mizukoshi, S., H. Matsuoka, H. Katou and H. Noda, 1993. Search for bioactive substances from marine algae, Bulletin Fac. Bioresource Mie Univ. Miedia Seibutsushigen Kiyo, 8: 27-34.

Paz, E. A., R.N. Lacy and M. Bakhtian, 1995. The B-Lactum antibiotics penicillin and cephalosporin in perspective. Hodder strong, London. 324.

Rangaiah, S.G., P. Lakshmi and E. Manjula, 2010. Antimicrobial activity of seaweeds *Gracillaria*, *Padina* and *Sargassum sps.* on clinical and phytopathogens. J. Chem. Anal. Sci., 1(6):114-117.

Raquel, F. and F. Epand, 2007. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. Biochim. Biophys. Acta., 2500–2509.

Raskin, I., D. Ribnicky, S. Komarnytsky, N. Ilic, A. Poulev, N., Borisjuk, A. Brinker, A. Moreno, C. Ripoll, N. Yakoby, T. Cornwell, I. Pastor and B. Fridlender, 2002. Plants and human health in the twenty-first century, Trends Biotechnol., 20 (12): 522-531.

Reguant, C., A. Bordons and N. Arola Roze., 2000. Influence of phenolic compounds on the physiology of *Oenococcus oeni*. J. Appl. Microbiol., 88: 1065-1071.

Rodriguez-Bernaldo de Quiros, A., M.A. Lage-Yusty and J. Lopez- Hernandez, 2010. Determination of phenolic compounds in macroalgae for human consumption. Food Chem., 2010; 121: 634-638.

Rosell, K.G., and L. M. Srivasta, 1987. Fatty acids as antimicrobials substance in brown algae. Hydrobiologia., 151/152: 471-475.

Salem, W. M., H. Galal and F. Nasr El-deen, 2011. Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). Afr. J. Microbiolo. Rese., 5(15): 2160-2167.

Sarker, S.D., L. Nahar and Y. Kumarasamy, 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemical. Methods., 42: 321-324.

Selvin, J. and A.P. Lipton, 2004. Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the Peninsular Coast of India. J. Mar. Sci. Technol., 12: 1-6.

Taskin, E. and M. Ozturk Kurt, 2001. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr. J. Biotechnol., 6: 2746-2751.

Tortora, G.J. and C.L. Funke, and B.R Case, 2001. Microbiology: An Introduction. Benjamin Cummings. San Francisco, p. 88.
Trease, G.E. and W. C. Evans, 1983. Evans Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London. 343-383.
Tringali, C. 1997. Bioactive metabolites from marine algae: recent results. Current Organic Chem., 1: 375-394.
Tuney, I., B. H. Cadirci, D .Unal, and A. Sukatar, 2006. Antimicrobial activities of the extracts of marine algae from the Coast of Urla (zmir, Turkey).Tur. J. Biol., 30: 1-5.
Vallinayagam, K. and R. Arumugam, 2009. Antibacterial activity of some selected Seaweeds from Pudumadam Coastal regions. *Global J. Pharmacol.*, 3(1): 50-52.

Vijayabaskar, P., V. Shiyamala, 2011. Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar Biosphere Reserve. Adv. Biol. Res., 5 (2): 99-102.

Zapta, O. and C. Mc millan, 1979. Phenolic acid in seagrasses. Aquatic. Bot ., 7: 307- 317.

Zubia, M., C. Payri and E. Deslandes, 2008. Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti French Polynesia. J. Appl. Phycol., 20: 1033-1043.