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Antibacterial activity of *Ulva fasciata* against Multidrug Resistant Bacterial Strains

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ABSTRACT

The present study was conducted to evaluate the antibacterial activity of different organic solvent increasing polarity *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Ulva fasciata* (Chlorophyceae) were collected from Kanniyakummari, Gulf of Mannar biosphere Reserve, Tamilnadu, India. Marine green algae extracts of *U. fasciata* 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, Proteus mirabilis* and *P. vulgaris*. The ethyl acetate extracts of *U. fasciata* showed highest antibacterial activity against all the bacterial strains tested. The mean zone of inhibition produced by the extracts in disc diffusion assays were ranged from 7.1 mm to 15.0 mm. The Minimum Inhibitory Concentrations (MIC) were between 125 µg/ml and 500 µg/ml, while the Minimum Bactericidal Concentrations (MBC) were between 250 µg/ml and 1000 µg/ml. The highest mean of zone inhibition (15.0 mm) and lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extract of *U. fasciata* against *B. subtilis*. The ethyl acetate extract of the *U. fasciata* showed the presence of phytochemicals, terpenoids, tannins and phenolic compounds in *U. fasciata* than the other solvents extracts. The present results of the ethyl acetate extract of *U. fasciata* can be used as an antibacterial substance for the treatment of multi drug resistant bacterial infections.

Keywords: Antibacterial activity; *Ulva fasciata*; MDR Bacterial strains

1. INTRODUCTION

Chlorophyceae seaweeds, popularly known as green algae, are widely distributed in both inter-tidal and deep-water regions of the seas. These seaweeds are of immense pharmaceutical and agricultural value. *U. fasciata* Delile, a green alga (Division: Chlorophycota; Class: Ulvophyceae; Order: Ulvales), belonging to the family Ulvaceae, commonly known as ‘‘sea lettuce”. Plants to several decimeters tall; characteristically deeply lobed or divided with clefts often extending to near holdfast; divisions somewhat digitately arranged from broadened basal region; blades plane, margins simple, crisped, or slightly undulate, bright gold when reproductive, margins then eroding; blades mostly (25-) 45-110 µm thick in central part, thinner toward margins. Cells tending taller than broad (10-25 µm) in

many specimens, or more quadrate and of equal dimensions; near base of blades, extracellular material between two cell layers thickens blade; margins relatively smooth, but irregular from erosion of spent reproductive cells; with occasional coarse spines on basal portions. *U. fasciata* occupies a major share amongst different green algae in the coastal region of southern India (Selvin and Lipton, 2004).

The *Ulva* are a group of edible algae that are widely distributed along the coasts of the world's oceans (Wolf *et al.,* 2012), and they have an interesting chemical composition that makes their commercial exploitation attractive to produce functional or health promoting food (Messyasz and Rybak, 2010). Seaweed *U. fasciata* have showed antimicrobial activities against *S. aureus* and *P. aeruginosa* that are commonly found in human infections (Selvin and Lipton, 2004). In recent years, wide concern on the antioxidant effects of *U. fasciata* has been aroused from scholars all over the word (Chakraborty and Paulraj, 2010).

In recent years, the escalation of multidrug resistance in bacteria 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 multi drug resistant (MDR) strains the increasing of MDR incidence in the genetic and mechanisms of resistance evolved by bacteria, as such information could lead to strategies for counter acting the effect of antimicrobial resistance (Bonnet, 2004).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases, appearance of undesirable side effects of certain antibiotics, as well as the increasing development of resistance to the must be taken to control the use of antibiotic, to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs. There are different approaches to cure and control the infection caused by the MDR strains of bacteria (Cowan, 1999).

Hence the present investigation was made to evaluate the antibacterial activity of different extracts of *U. fasciata* against clinical and standard MDR bacterial strains.

2. MATERIALS AND METHODS

2. 1. Sample collection and Preparation of extracts

Ulva fasciata (Chlorophyceae) were collected from the rocky shores of Kanniyakumari at (Lat. 9°11′N; Long. 79°24′E) Kanniyakumari district, Gulf of Mannar Marine biosphere Reserve, Tamil Nadu, India. The collections were made from the month of October 2011. The fresh seaweed species were handpicked during low tide and washed thoroughly with sea water to remove any associated with impurities, epiphytes, animal casting, and adhering sand particles and other suspend materials. Morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in an ice box containing slush ice and transported to the laboratory. Then, the samples were blot dried using sterile tissue paper. The seaweed sample were shade dried followed by hot air oven drying (50 °C) and milled in an electric blender.

The algae were identified by Former Prof. R. Panneerselvam, Department of Botany, Annamalai University and the museum specimen was deposited in the Department of Botany, Annamalai University. Six hundred grams of powdered samples was 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 for antibacterial assay.

2. 2. Collection of bacterial strains

The antibacterial activity was tested using seaweed extracts from each individual against two strains of gram positive bacteria viz., *Bacillus subtilis* (MTCC 441), *Streptococcus pyogenes* (MTCC 442), gram negative bacteria viz., *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (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 Collection (MTCC), Chandigarh.

The Clinical isolates of bacterial strains *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. These strains were maintained on nutrient agar slant at 4 °C.

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

2. 3. Phytochemical screening

The qualitative phytochemical analyses studies hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Ulva fasciata* Phytochemicals like terpenoids, tannin, cardic glycosides, steroids, alkaloids, phenolic compounds and coumarins were carried out according to the method described by (Harborne 1973).

2. 4. Antibiotic sensitivity test

Antibiotic sensitivity of the bacterial strains were determined by standard CLSI disc diffusion method (M100-S22, 2012). Antibacterial agents from different classes of antibiotics viz., Methicilin (ME 5 µg/disc), Oxacillin (OX µg/disc), Linezolid (LIN 30 µg/disc), Vancomycin (VAN 30 µg/disc), 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 5 µg/disc), Streptomycin (S 10 µg/disc) and Tetracycline (TE 30 µg/disc), were obtained from Himedia, Mumbai.

2. 5. Anti-bacterial assay

2. 5. 1. Disc Diffusion Method

The antibacterial activity of extracts of U. *fasciata* was determined by disc diffusion method according to Bauer *et al.* (1966) with modifications. Petri plates were prepared by pouring 20 ml of sterilized molten MHA. Then the plates were allowed to solidify and used in susceptibility test. MHA plates were inoculated by streaking the swab over the entire agar surface using bacterial suspensions containing 10^8 colony forming units (CFU) per ml and allowed to dry for 10 minutes. The crude extracts were dissolved in 10 % DMSO and under aseptic conditions sterile discs were loaded with different extracts impregnated with 20 µl of three different concentrations (500, 250, 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 (10 µg/disc) was used as positive control and 10 per cent DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24 h. The zone of inhibition was recorded in millimeters. The assay in this experiment was repeated for three times.

2. 5. 2. Determination of the Minimum inhibitory concentration (MIC)

The MIC of the *U. fasciata* crude extracts, a modified reaszurin microtitre plate assay was carried out according to methods of Sarker *et al.* (2007). 50 µl of Sterile MHB were transferred in to each well of a sterile 96-well micro titer plate (Hi-Media TPG 96). The *U. fasciata* extracts was dissolved in 10 per cent DMSO to obtain 1000 µg/ml stock solution. 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 wells. To each well 10 µl of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270 mg tablet in 40 mL 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 the standardized bacterial suspensions to each well to achieve a concentration of approximately 5 $\times 10^5$ CFU/ml were transferred in to all wells. 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 color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). In this study, the MIC was the lowest concentration of *U. fasciata* extracts that exhibited the growth of the organisms in the values by visual reading.

2. 5. 3. Determination of the Minimum Bactericidal Concentration (MBC)

MBC of the *U. fasciata* extracts were determined by plating 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.

2. 5. 4. Statistical Analysis

The results were 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.

3. RESULTS

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *U. fasciata* were used analysed for phytochemicals, terpenoid, tannin, cardiac glycosides, steroid, alkaloids, phenolic compounds and coumarins. The ethyl acetate extract of *U. fasciata* contained presence of phytochemicals terpenoids, tannins, and phenolic compounds than the other solvent extracts. Among the phytochemicals, cardiac glycosides were present in all the extracts except acetone and methanolic extracts. Steroids, alkaloids and coumarins were absent in all the extracts of *U. fasciata.*

The multi drug resistance resistance profile, of bacterial strains of both clinical and standard strains was confirmed by CLSI-M100-2012 method. The standard strains of *B. subtilis, K. pneumoniae* 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 sensitive to all the antibiotics tested except AMP. The standard strains of *S. pyogenes* were resistant to CFM, AMP, CAZ, NA and E and sensitive to all other 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.vulgaris* were sensitive to all antibiotics tested and resistant to AMP, CFM, GEN, NX, NA and OF. In the present study different solvents of hexane, chloroform, ethylacetate, acetone, and methanol extracts of *U. fasciata* were studied against multidrug resistant both clinical and standard bacterial strains. The highest activity was displayed by ethyl acetate extract of *U. fasciata* against *B. substilis* with the mean zones of inhibition (15.0 mm) followed by *S. pyogenes* (14.0 mm), *E. coli* (13.6 mm) and *P. mirabilis* (13.3 mm). All the extracts of marine macro algae 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 Tables 1 and 2. When the different extracts were assayed against the test bacteria by disc diffusion assays, the mean zone of inhibition obtained were between 7.1 and 14.5 mm. Ampicillin (30 µg/disc) antibacterial positive control produced mean zone of inhibition ranged from 7.1 to 12.8 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The highest mean of zone inhibitions (15.0 mm), and the lowest MIC (125µg/ml) and MBC values (250 µg/ml) were observed in ethyl acetate extracts of *U. fasciata* against *B. subtilis*.

Table 1. Antibacterial activity of *Ulva fascita* against Multidrug Resistant standard Bacterial Strains.

S. No	Bacterial strains/ Seaweed Extracts	Mean zone of inhibition[a] (mm)[b]				MIC (µg/ml)	MBC (µg/ml)
		Concentration of the disc (µg/disc)					
		500	250	125	Ampicillin (10 µg/disc)		
1	*Bacillus subtilis* (MTCC 441)						
	Hexane	10.8 ± 0.76	9.5 ± 0.50	7.3 ± 0.57	11.8 ± 0.76	500	1000
	Chloroform	13.0 ± 0.50	10.1 ± 0.28	7.5 ± 0.50	11.0 ± 0.50	250	500
	Ethyl acetate	15.0 ± 0.50**	10.8 ± 0.76	8.5 ± 0.50	12.5 ± 0.50	125	250
	Acetone	12.6 ± 0.76	9.5 ± 0.50	7.8 ± 0.76	10.6 ± 0.76	500	1000
	Methanol	11.5 ± 0.50	9.6 ± 0.76	7.1 ± 0.28	9.3 ± 0.57	500	1000
2	*Streptococcus pyogenes* (MTCC 442)						
	Hexane	10.1 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	10.3 ± 0.57	500	1000
	Chloroform	12.5 ± 0.50	9.6 ± 0.28	7.8 ± 0.76	11.6 ± 0.76	250	500
	Ethyl acetate	13.5 ± 0.50**	10.0 ± 0.50	8.1 ± 0.28	12.1 ± 0.28	250	500
	Acetone	12.0 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	11.6 ± 0.76	500	1000
	Methanol	11.1 ± 0.28	9.6 ± 0.28	7.1 ± 0.28	7.3 ± 0.28	500	1000
3	*Escherichia coli* (MTCC 443)						
	Hexane	10.1 ± 0.28	9.0 ± 0.50	7.1 ± 0.28	12.8 ± 0.28	500	1000
	Chloroform	12.5 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	12.0 ± 0.50	250	500
	Ethyl acetate	13.1 ± 0.28	10.0 ± 0.50	8.6 ± 0.57	9.3 ± 0.57	250	500
	Acetone	11.5 ± 0.50	9.6 ± 0.76	7.3 ± 0.57	7.3 ± 0.28	500	1000
	Methanol	10.8 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	9.3 ± 0.57	500	1000
4	*Klebsiella pneumoniae* (MTCC109)						
	Hexane	10.8 ± 0.2	9.1 ± 0.28	7.3 ± 0.57	10.3 ± 0.28	500	1000
	Chloroform	11.5 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	9.3 ± 0.57	500	1000
	Ethyl acetate	12.1 ± 0.50	10.3 ± 0.28	8.3 ± 0.28	7.3 ± 0.28	500	1000
	Acetone	10.6 ± 0.76	9.6 ± 0.76	7.1 ± 0.28	12.0 ± 0.50	500	1000
	Methanol	10.3 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	8.6 ± 0.76	500	1000
5	*Proteus mirabilis* (MTCC 425)						
	Hexane	11.0 ± 0.50	9.6 ± 0.76	7.1 ± 0.28	12.0 ± 0.86	500	1000
	Chloroform	11.5 ± 0.50	9.8 ± 0.28	7.6 ± 0.57	8.8 ± 0.76	500	1000
	Ethyl acetate	13.3 ± 0.28	10.0 ± 0.50	8.5 ± 0.50	7.3 ± 0.57	250	500
	Acetone	11.0 ± 0.50	9.6 ± 0.76	7.3 ± 0.28	8.6 ± 0.57	500	1000
	Methanol	11.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.28	8.6 ± 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). Antibacterial activity of *Ulva fascita* against Multidrug Resistant standard Bacterial Strains.

S. No	Bacterial strains/ Seaweed Extracts	Mean zone of inhibition[a] (mm)[b]				MIC (µg/ml)	MBC (µg/ml)
		Concentration of the disc					
		500	250	125	Ampicillin (10 µg/disc)		
6	*Proteus. Vulgaris* (MTCC 426)						
	Hexane	10.0 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	7.8 ± 0.76	500	1000
	Chloroform	12.3 ± 0.28	9.5 ± 0.50	7.5 ± 0.50	7.3 ± 0.28	500	1000
	Ethyl acetate	12.5 ± 0.50	10.1 ± 0.28	8.1 ± 0.28	8.6 ± 0.57	250	500
	Acetone	11.5 ± 0.50	9.1 ± 0.28	7.3 ± 0.57	7.3 ± 0.57	500	1000
	Methanol	10.1 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	8.6 ± 0.57	500	1000
7	*Pseudomonas aeruginosa* (MTCC 741)						
	Hexane	11.0 ± 0.50	9 ± 0.50	7.1 ± 0.28	7.3 ± 0.28	500	1000
	Chloroform	12.0 ± 0.50	10.0 ± 0.50	7.8 ± 0.76	11.6 ± 0.76	500	1000
	Ethyl acetate	12.8 ± 0.76	10.0 ± 0.50	8.3 ± 0.57	11.0 ± 0.76	250	500
	acetone	11.5 ± 0.50	9.6 ± 0.28	7.5 ± 0.50	11.6 ± 0.76	500	1000
	Methanol	11.1 ± 0.28	9.1 ± 0.28	7.3 ± 0.28	9.3 ± 0.57	500	1000
8	*Salmonella typhimurium* (MTCC 98)						
	Hexane	10.8 ± 0.28	9.3 ± 0.76	7.1 ± 0.28	8.6 ± 0.76	500	1000
	Chloroform	12.1 ± 0.28	9.5 ± 0.50	7.8 ± 0.76	9.3 ± 0.57	500	10 00
	Ethyl acetate	13.0 ± 0.50	10.0 ± 0.50	8.3 ± 0.57	8.8 ± 0.76	250	500
	Acetone	11.3 ± 0.28	9.0 ± 0.50	7.3 ± 0.57	8.0 ± 0.50	500	1000
	Methanol	11.6 ± 0.28	9.3 ± 0.57	7.1 ± 0.28	8.6 ± 0.76	500	1000
9	*Shigella flexneri* (MTCC 1457)						
	Hexane	11.0 ± 0.50	9.1 ± 0.28	7.5 ± 0.50	11.0 ± 0.50	500	1000
	Chloroform	10.8 ± 0.76	9.5 ± 0.50	7.5 ± 0.50	12.1 ± 0.28	500	1000
	Ethyl acetate	12.5 ± 0.50	9.8 ± 0.76	7.6 ± 0.76	12 .8 ± 0.76	250	500
	Acetone	11.5 ± 0.50	9.3 ± 0.57	7.5 ± 0.50	11.0 ± 0.50	500	1000
	Methanol	10.0 ± 0.50	8.5 ± 0.50	7.1 ± 0.28	12.1 ± 0.28	500	1000
10	*Vibrio cholera* (MTCC 3906)						
	Hexane	11.5 ± 0.50	9.3 ± 0.57	7.5 ± 0.28	10.3 ± 0.28	500	1000
	Chloroform	11.1 ± 0.28	9.6 ± 0.76	7.6 ± 0.76	12.1 ± 0.28	500	1000
	Ethyl acetate	13.1 ± 0.28	11.0 ± 0.50	8.6 ± 0.57	10.3 ± 0.28	250	500
	Acetone	11.5 ± 0.50	9.3 ± 0.28	7.3 ± 0.57	11.6 ± 0.76	500	1000
	Methanol	10.0 ± 0.50	8.8 ± 0.28	7.5 ± 0.57	9.3 ± 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. Antibacterial activity of *Ulva fascita* against Multidrug Resistant clinical Bacterial Strains.

Sl. No	Bacterial strains/ Seaweed Extracts	Mean zone of inhibition[a] (mm)[b]				MIC (µg/ml)	MBC (µg/ml)
		Concentration of the disc					
		500	250	125	Ampicillin (10 µg/disc)		
1	*Streptococcus pyogenes*						
	Hexane	10.1 ± 0.28	8.5 ± 0.50	7.1 ± 0.28	13.1 ± 0.28	500	1000
	Chloroform	12.5 ± 0.50	9.6 ± 0.28	7.5 ± 0.50	11.6 ± 0.76	250	500
	Ethyl acetate	14.0 ± 0.50**	10.0 ± 0.50	8.1 ± 0.28	12.1 ± 0.28	250	500
	Acetone	12.0 ± 0.50	9.5 ± 0.50	7.3 ± 0.57	11.6 ± 0.76	500	1000
	Methanol	11.1 ± 0.28	9.6 ± 0.28	7.1 ± 0.28	11.8 ± 0.76	500	1000
2	*Escherichia coli*						
	Hexane	11.5 ± 0.50	9.1 ± 0.28	7.3 ± 0.57	8.8 ± 0.76	500	1000
	Chloroform	13.1 ± 0.57	10.0 ± 0.50	7.8 ± 0.76	9.0 ± 0.86	250	500
	Ethyl acetate	13.6 ± 0.76	10.3 ± 0.28	8.3 ± 0.57	9.3 ± 0.57	250	500
	Acetone	12.1 ± 0.28	9.8 ± 0.76	7.3 ± 0.57	7.3 ± 0.28	500	1000
	Methanol	11.0 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	9.1 ± 0.28	500	1000
3	*Klebsiella pneumoniae*						
	Hexane	10.1 ± 0.28	9.0 ± 0.50	7.3 ± 0.57	10.8 ± 0.76	500	1000
	Chloroform	11.3 ± 0.57	9.5 ± 0.50	7.5 ± 0.50	11.6 ± 0.76	500	1000
	Ethyl acetate	12.0 ± 0.50	10.1 ± 0.28	7.6 ± 0.57	11.0 ± 0.50	500	1000
	Acetone	10.5 ± 0.50	9.3 ± 0.28	7.1 ± 0.28	12.0 ± 0.50	500	1000
	Methanol	10.8 ± 0.70	9.0 ± 0.50	7.1 ± 0.28	8.6 ± 0.76	500	1000
4	*Proteus mirabilis*						
	Hexane	11.0 ± 0.50	9.6 ± 0.76	7.3 ± 0.57	12.0 ± 0.86	500	1000
	Chloroform	11.5 ± 0.50	9.1 ± 0.28	7.6 ± 0.57	8.8 ± 0.76	500	1000
	Ethyl acetate	13.3 ± 0.28	10.0 ± 0.50	8.1 ± 0.57	9.3 ± 0.57	250	500
	Acetone	11.0 ± 0.50	9.6 ± 0.76	7.1 ± 0.28	8.6 ± 0.57	500	1000
	Methanol	11.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.28	8.8 ± 0.76	500	1000
5	*Proteus vulgaris*						
	Hexane	10.0 ± 0.50	9.1 ± 0.28	7.3 ± 0.57	9.1 ± 0.28	500	1000
	Chloroform	12.3 ± 0.28	9.5 ± 0.50	7.5 ± 0.50	7.3 ± 0.28	500	1000
	Ethyl acetate	12.5 ± 0.50	10.1 ± 0.28	7.8 ± 0.76	8.6 ± 0.76	250	500
	Acetone	11.5 ± 0.50	10.1 ± 0.28	7.3 ± 0.57	7.3 ± 0.57	500	1000
	Methanol	10.1 ± 0.28	8.5 ± 0.50	7.3 ± 0.57	8.6 ± 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). Antibacterial activity of *Ulva fascita* against Multidrug Resistant clinical Bacterial Strains.

Sl. No	Bacterial strains/ Seaweed Extracts	Mean zone of inhibition[a] (mm)[b]				MIC (µg/ml)	MBC (µg/ml)
		Concentration of the disc					
		500	250	125	Ampicillin (10 µg/disc)		
6	*Pseudomonas aeruginosa*						
	Hexane	11.5 ± 0.50	9.5 ± 0.50	7.1 ± 0.28	9.0 ± 0.86	500	1000
	Chloroform	12.3 ± 0.28	10.0 ± 0.50	7.8 ± 0.76	8.8 ± 0.76	500	1000
	Ethyl acetate	13.3 ± 0.76	10.1 ± 0.28	8.3 ± 0.57	11.6 ± 0.76	250	500
	acetone	12.1 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	12.1 ± 0.28	500	1000
	Methanol	11.3 ± 0.28	9.1 ± 0.28	7.3 ± 0.28	12.8 ± 0.28	500	1000
7	*Salmonella typhimurium*						
	Hexane	10.8 ± 0.28	9.3 ± 0.76	7.1 ± 0.28	9.1 ± 0.28	500	1000
	Chloroform	12.1 ± 0.28	9.5 ± 0.50	7.5 ± 0.50	9.3 ± 0.57	500	10 00
	Ethyl acetate	13.0 ± 0.50	10.0 ± 0.50	7.8 ± 0.76	8.8 ± 0.76	250	500
	Acetone	11.3 ± 0.28	9.0 ± 0.50	7.3 ± 0.57	7.3 ± 0.28	500	1000
	Methanol	11.6 ± 0.28	9.3 ± 0.57	7.1 ± 0.28	8.6 ± 0.76	500	1000
8	*Shigella dysentrieae*						
	Hexane	10.0 ± 0.50	9.3 ± 0.28	7.5 ± 0.50	10.3 ± 0.28	500	1000
	Chloroform	12.0 ± 0.50	10.3 ± 0.57	7.6 ± 0.57	12.1 ± 0.28	500	1000
	Ethyl acetate	13.5 ± 0.50	10.5 ± 0.50	7.8 ± 0.76	10.3 ± 0.28	250	500
	Acetone	11.8 ± 0.76	9.8 ± 0.28	7.6 ± 0.76	11.6 ± 0.76	500	1000
	Methanol	11.1 ± 0.28	9.3 ± 0.57	7.6 ± 0.28	9.3 ± 0.57	500	1000
9	*Shigella flexneri*						
	Hexane	11.0 ± 0.50	9.1 ± 0.28	7.1 ± 0.28	11.0 ± 0.50	500	1000
	Chloroform	11.5 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	12.1 ± 0.28	500	1000
	Ethyl acetate	12.5 ± 0.50	9.8 ± 0.76	7.6 ± 0.76	12 .8 ± 0.76	250	500
	Acetone	10.5 ± 0.50	9.3 ± 0.57	7.3 ± 0.57	11.0 ± 0.50	500	1000
	Methanol	10.0 ± 0.50	8.5 ± 0.50	7.1 ± 0.28	12.1 ± 0.28	500	1000
10	*Vibrio cholera*						
	Hexane	11.5 ± 0.50	9.6 ± 0.76	7.3 ± 0.57	11.8 ± 0.76	500	1000
	Chloroform	11.1 ± 0.28	9.8 ± 0.28	7.6 ± 0.76	11.6 ± 0.57	500	1000
	Ethyl acetate	13.1 ± 0.28	11.0 ± 0.50	8.1 ± 0.28	10.0 ± 0.50	250	500
	Acetone	11.5 ± 0.50	9.3 ± 0.28	7.3 ± 0.57	11.6 ± 0.76	500	1000
	Methanol	10.0 ± 0.50	8.8 ± 0.28	7.1 ± 0.28	9.3 ± 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 $p < 0.05$

4. DISCUSSION

In present study different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *U. fasciata* possessed antibacterial activity against all the clinical and standard bacterial strains tested. The ethyl acetate extract of *U. fasciata* showed the highest antibacterial activity than other extracts against *B. subtilis, S. pyogenes*, *E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, P. aeruginosa, S. typhimurium, S. dysentriea, S. flexneri* and *V. cholerae*. The highest mean of zone inhibition (15.0 mm) and lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extract of *U. fasciata* against *B. subtilis*. This may indicate that the extraction method had definite effects on the isolation of bioactive principles. This may be due to the solvent to extract the different constituents having antibacterial activity.

In present work, different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *U. fasciata* possessed antibacterial activity against all the clinical and standard bacterial strains tested. Choudhury *et al.* (2005) reported that methanol extract of *Enteromorpha compressa* and *U. fasciata* shows no activity against *Enterobacter aerogenes., Vibrio alginolytics, Aeromonas hydrophila*. In controversy, Lima- filho *et al.*(2002) reported that *U. fasciata* has not any antimicrobial activity against tested microorganisams. 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 *U. fasciata* showed the antibacterial activity may due to the presence of phytochemicals, terpenoids, tannins, phenolic compound, and steroids. A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids, have been reported from various marine green algae (Blunt *et al.,* 2006). 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). Tannins are well known to possess general antimicrobial properties reported by Scalbert, (1991). Several cardiac glycosides are used therapeutically in the treatment of cardiac failure and atrial arrhytmias and many glycoside compounds, belonging to other structural groups, show cytotoxic, antimicrobial, hypocholesterolemic and other biological activities (Ivanchina *et al.,* 2011).

In the present study, the different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *U. fasciata* possessed antibacterial activity against all the clinical and standard bacterial strains tested. The ethyl acetate extracts of *U. fasciata* showed the highest antibacterial activity than other extracts against *B. subtilis*. Chakraborty and Paulraj, (2010) have reported that isolation of five sesquiterpens were isolated from methanol extract of *U. fasciata* such as 2,5,5-trimethyl-4-(4/-methyl-3/-pentenyl)-2- cycloexen-1-ol, 4-isopentyl-3,4,5,5-tetramethyl-2-cyclohexen-1-ol, two diastereoisomeric compounds), 6-isopentyl-1,5,5,6-tetramethyl-1-cyclohexene, and 3,4,5,5-tetramethyl-4-(3/-oxopentyl)-2-cyclohexen-1-one. Two guaiane sesquiterpene derivatives, viz., guai-2-en-10a-ol and guai-2-en-10a-methanol isolated from the chloroform and methanolic extracts of *U. fasciata.* The latter has been acetylated to furnish guai-2-en-10a-methyl methanoate compounds were evaluated for their potential antimicrobial properties against marine aquacultural pathogens, *Vibrio parahaemolyticus, V. alginolyticus* and *V. vulnificus* (Chakraborty *et al.,* 2010).

In the present study, the gram positive bacteria were more susceptible than the gram negative bacteria. Taskin *et al.* (2001) reported that 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). The resistance of gram

negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharides molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside (Shan *et al.,* 2007).

5. CONCLUSION

Based on the present study, the different crude extracts of *U. fasciata* were possessed antibacterial activity. The use of this plant in folk medicine for the treatment of human gastrointestinal tract and various diseases whose symptoms might involve bacterial infections. The ethyl acetate extract of *U. fasciata* highest antibacterial activity against MDR bacterial pathogens. This study recommended seaweed extracts as antibacterial substance for treating multi drug resistant microbes causing acquired infection.

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Reference

[1] A.W. Bauer, W.M.M. Kirby, J.C. Scherris, M. Turck, *American Journal of Clinical Pathology* 45 (1966) 493-496.

[2] J. W. Blunt, B.R. Copp, M.H.G. Munro, P.T. Northcote, M.R. Prinsep, *Natural Product Reports* 2 (2006) 26-78.

[3] R. Bonnet, *Antimicrobial Agents and Chemotherapy* 48 (2004) 1-14.

[4] K. Chakraborty, A. Lipton, P. Paulraj, R. Rekha, D. Chakraborty, *European Journal of Medicinal Chemistry* 45 (2010) 2237-2244.

[5] K. Chakraborty, R. Paulraj, *Food Chemistry* 122 (2010) 31-41.

[6] S. Choudhury, A. Sree, S.C. Mukherjee, P. Pattnaik, M. Bapuji, *Asian Fisheries Science* 18 (2005) 285-294.

[7] Clinical Laboratory Standards Institute (formerly NCCLS): Performance Standards for Antimicrobial disk susceptibility tests: M100-S22 (2012) CLSI Vol. 32.

[8] M.M. Cowan, *Clinical Microbiology Reviews* 12 (1999) 564-582.

[9] I.B. Harborne, Phytochemicals methods: A guide to modern techniques of plant analysis. 2nd edn, Chapman and Hall, New York (1973) 88-185.

[10] N.V. Ivanchina, A. A. Kicha, V.A. Stonik, *Steroids* 76 (2011) 425-454.

[11] J.V.M. Lima-filho, A.F.F.U. Carvalho, S.M. Freita, V.M.M. Melo, *Brazilian Journal of Microbiology* 33 (2002) 311-314.

[12] B. Messyasz, A. Rybak, *Aquatic Ecology* 45 (2010) 75- 87.

[13] E.A. Paz, M.P. Cerdeiras, J. Fernandez, F. Ferreira, P. Moyna, M. Soubes, A. Vazquez, S. Vero, L. Zunino, *Journal of Ethnopharmacology* 45 (1995) 67-70.

[14] C.A. Reguant, L. Bordons Arola, N. Roze, *Journal of Applied Microbiology* 88 (2000) 1065-1071.

[15] S.D. Sarker, L. Nahar, Y. Kumarasamy, *Methods* 42 (2007) 321-324.

[16] A. Scalbert, 1991. Antimicrobial properties of tannins. *Phytochemistry* 30 (1991) 3875-3883.

[17] J. Selvin, A.P. Lipton, *Journal of Marine Science and Technology* 12 (2004) 1-6.

[18] B. Shan, Y.Z. Cai, J.D. Brooks, H Corke, *International Journal of Food Microbiology* 117 (2007) 112-119.

[19] E. Taskin, M. Ozturk, O. Kurt, *African Journal of Biotechnology* 6 (2001) 2746-2751.

[20] M.A. Wolf, K. Sciuto, C. Andreoli, I. Moro, *Journal of Phycology* 48 (2012) 1510-1521.

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