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Anti-MRSA Activity of *Padina tetrastromatica, Padina gymnospora* from Gulf of Mannar Biosphere

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ABSTRACT

Nowadays coming out of bacterial resistance poses a significant clinical problem. Hence, the aim of this study was to explain the current susceptibility patterns of methicillin-resistant Staphylococcus aureus (MRSA).As well as to find out antimicrobial characteristics in the different organic solvents with increasing polarity *viz.,* hexane, chloroform, ethyl acetate, and methanol extracts of *Padina tetrastromatica, Padina gymnospora* marine macro algae belonging to the family Phaeophyta were studied. Their crude extracts were tested against *Staphylococcus aureus* (MTCC 737 & 7443), and three clinical isolates of MRSA were tested and has been shown to exhibit antibacterial activity against methicillin methicillin-resistant Staphylococcus aureus (MRSA). The Minimum inhibitory concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined. The ethyl acetate extracts of the seaweeds showed the presence of Photochemical, terpenoids, tannins, phenolic compounds and steroids strongly than the other solvent extracts. The highest activity was recorded in the ethyl acetate extract of *Padina tetrastromatica* than the other extract tested. The mean zone of inhibition produced by the extracts in disc diffusion assays against the tested bacterial strains ranged from 7.1 to 26.5 mm. The lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) values were observed in the ethyl acetate extract of *P. tetrastromatica* against *S. aureus* (737 & 7443), MRSA1 and MRSA3. Further separation of active principle from the potential seaweed extract as a source of antibacterial compound useful for the control of Methicillin resistant *S. aureus* is under progress.

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***Keywords*:** Antibacterial activity, MRSA, MIC, MBC, *Padina tetrastromatica, Padina gymnospora*

1. INTRODUCTION

Pathogenic bacteria with resistance to different drugs are a globalize problem causing increased concern in healthcare institutions. Certainly, multi-resistant *Staphylococcus aureus* (MRSA) infections are predictable to have defeated more than 20,000 people in the USA and UK exclusively during 2005 [1] Office for National Statistics 2007). These days, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics [2]. Enteric infections are major public health problems in developing countries and contribute to the death of 3.3-6.0 million children annually [3].

When the first antibiotics such as penicillin began making their way into clinical use, they were hailed as miracle drugs. By killing the bacteria that cause many of humankind's worst infectious diseases, such as tuberculosis and pneumonia, they saved countless lives, but not all miracles last forever [4]. Specifically, the infections caused by MRSA have been particularly hard to cure with normal antibiotics and have become a problem for clinical treatment [5].S. aureus has been shown to be susceptible to the earliest antimicrobial substances, most notably penicillin. However, the abuse of antibiotics resulted in a spread of staphylococcal resistance [6]. Resistance to treatment for methicillin-resistant *Staphylococcus aureus* (MRSA) causing nosocomial infection has become a serious medical issue and currently, there is no effective antibiotic against MRSA but vancomycin, teicoplanin and arbekacin. MRSA first emerged in the late 1970s [7].

Vancomycin has been used for the treatment of MRSA-related infections. With the increasing use of vancomycin, vancomycin-intermediate and -resistant S. aureus (VISA and VRSA) have been reported in a number of countries [8]. This resistance was overcome with penicillinase-stable methicillin and other derivatives (methicillin, oxacillin, cloxacillin, and flucloxacillin). However, widespread use of methicillin resulted in the appearance of methicillin-resistant S. aureus (MRSA) [9]. MRSA infections are extremely difficult to treat due to their multidrug-resistant properties, which are resistant to almost all available antibiotics, and MRSA is associated with the resurgence of multidrug resistance [10]. As most current antibiotics are only based on compounds from terrestrial organisms or existing synthetic antimicrobial agents, it may be difficult for researchers to discover new compounds with high antibiotic activity. Therefore, the use of bioactive compounds from marine resources to overcome and prevent antibiotic multidrug resistance will be an effective alternative strategy [11].

Marine macroalgae are divided into three major groups Rhodophytae (red algae), Chlorophytae (green algae) and Pheaophytae (brown algae) depending on their nutrient and chemical composition. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites [12]. Marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic and cytotoxic agents and the bioactive substances included alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols [13].

Algae can produce biologically active compounds that are capable of killing bacteria or inhibiting bacterial growth [14]. Most brown seaweeds contain carotenoid pigment fucoxanthin, which is responsible for the predominant brown colouration. This also contains polysaccharides such as alginates, laminarin, fucans, cellulose etc apart from a range of components with unique secondary metabolites such as phlorotannins, phloroglucinol, terpenes and tocopherol [15]. Brown algae represent a major component of littoral and sublittoral zones in temperate and subtropical ecosystems. An essential adaptative feature of this independent eukaryotic lineage is the ability to couple oxidative reactions resulting from exposure to sunlight and air with the halogenation of various substrates, thereby addressing various biotic and abiotic stresses, i.e., defence against predators, tissue repair, holdfast adhesion and protection against reactive species generated by oxidative processes. The food reserves of brown algae are typically complex polysaccharides and higher alcohols [16]. Most brown seaweeds contain carotenoid pigment fucoxanthin, which is responsible for the predominant brown colouration. This also contains polysaccharides such as alginates, laminarin, fucans, cellulose etc apart from a range of components with unique secondary metabolites such as phlorotannins, phloroglucinol, terpenes and tocopherol. Several species of brown seaweeds contains wide range of applications with antimicrobial, anticancer, antioxidant, anti-diabetic and anti-inflammatory properties [17]. Marine algae produce a cocktail of metabolites structures exhibited by these compounds range from acyclic entities with a linear chain to complex polycyclic molecules and included bioactive terpenes, phenolic compounds, alkaloids, polysaccharides and fatty acids. Their medical and pharmaceutical application has been investigated for several decades. Many of these secondary metabolites are halogenated, reflecting the availability of chloride and bromide ions in seawater [18]. Since seaweeds are known to contain a wide variety of, bioactive compounds as such offering a rich source of new drugs with potentially lower toxicity.

Hence, the present studies were aimed to screen and evaluate the efficiency of hexane, chloroform, ethyl acetate, and methanol extracts of *Padina tetrastromatica*, *Padina gymnospora* as antibacterial agents against the MRSA.

2. MATERIALS AND METHODS

Sample collection

Preparation of Extracts

The algal species were handpicked during low tides and washed thoroughly with sea water to remove all unwanted impurities, epiphytes, animal casting, and adhering sand particles etc. 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 blotted and dried using sterile tissue paper. 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, and methanol for 72 hours. The extracts were pooled and the solvent were evaporated under vacuum in rotary evaporator (Heidolph,

Germany) at 4 °C and the dried extracts were stored at 4 °C in refrigerator for antibacterial assay.

Phytochemical screening

The hexane, chloroform, ethyl acetate, and methanol extracts of *Padina tetrastromatica, Padina gymnospora* were used for qualitative phytochemical studies. Phytochemicals like terpenoids, tannins, cardic glycosides, steroids, alkaloids, phenolic compounds, coumarins, and diterpenoids were carried out according to the method described by Harborne [19] and Trease and Evans [20].

Bacterial strains used

The bacterial strains *viz.,* of *Staphylococcus aureus* (MTCC 737 & 7443), were procured from Microbial Type Culture Collection (MTCC), Chandigarh. Three clinical isolates of MRSA strains were obtained from Department of Microbiology, Rajah Muthiah Medical College, and Hospital, Annamalai University, Annamalai nagar, Tamil Nadu, India. The stock cultures were maintained on nutrient agar (HiMediaM087) medium at 4 ºC. *In vitro* antibacterial activity was determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) for *S. aureus* (MTCC 7443) and MHA and MHB supplemented with 4% sodium chloride for MRSA. The media were obtained from Himedia, Mumbai.

Antibiotic sensitivity test

Antibiotic sensitivity of the bacterial strains were determined by standard method [21] using 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), Antibacterial agents from different classes of antibiotics 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) (Himedia, Mumbai).

Detection of MRSA

Three isolates of MRSA were analyzed and confirmed by Gram's stain and conventional biochemical methods viz., gram stain, catalase test, mannitol test and coagulase test [22]. Methicillin resistance was detected by disc diffusion technique (23) using Oxacillin 1 µg/disc. Retesting was done using Methicillin 5 µg/disc. Zone of inhibition less than 10 mm or any discernible growth within the zone of inhibition was the indication of methicillin resistance.

Antibacterial assay

Inhibition Zone determination by Disc diffusion assay

The agar diffusion method [23] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 ml of MHA for *S. aureus* (MTCC 7443) and MHA (Hi-MediaM173) supplemented with 4 % sodium chloride for MRSA and allowed to solidify. MHA plates were inoculated by streaking the swab over the entire agar surface using bacterial suspensions containing 108 colony forming units (CFU) per ml and allowed to dry for 10

minutes. The crude extracts were dissolved in 10% DMSO (Hi-MediaRM5856) and under aseptic conditions; sterile discs were impregnated with 20 µl of different concentrations of extracts (1000, 500, 250 µg/ml). The discs with extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Methicillin (5µg/disc) was used as positive control and 10 per cent DMSO was used as blind control in these assays. Finally, the inoculated plates were incubated at 37 °C for 24 h S. aureus (MTCC 7443), 35 °C for 24 - 48 h (MRSA). The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated three times.

Determination of the Minimum inhibitory concentration (MIC)

The MIC of the crude extracts, a modified resazurin microtitre plate assay was used as reported by [24]. Sterile MHB (Hi-MediaM391) for *S. aureus* (MTCC 7443 & 737) and MHA supplemented with 4% sodium chloride for MRSA and was used in this assay. 50 µl of respective broth was transferred in to each well of a sterile 96-well micro titer plate (Hi-Media TPG 96). The plant extracts was dissolved in 10 per cent DMSO to obtain 2000 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 50 µl of the solution was transferred to the second well and in this way, the 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 (Hi-MediaRM125) solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water). Then 30 µl of Sterile MHB for *S. aureus* (MTCC 7443) and MHA supplemented with 4% sodium chloride for MRSA was added to each well. Finally, 10 µl of bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 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 S. aureus (MTCC 7443), 35 °C for 24 - 48 h (MRSA). The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colourless). The lowest concentration at which colour change occurred was taken as the MIC value.

3. RESULTS

The antibiotic resistance of bacterial strains of both clinical and standard strains is confirmed by CLSI-M100-2012 method. The slandered strains of tested, *S. aureus* (MTCC 7443) were found to be reference drug highly sensitive to all the antibiotics tested except AMP and *S. aureus* (MTCC 737) was found to be highly resistant to all antibiotics tested except GEN, S, TE, AK, E and C. The three clinical isolates of MRSA were highly resistant to all the antibiotics resistance tested except GEN, S, TE, AK, E, C, VAN, LIN, NX, NA, and OF.

The various solvents with rising polarity viz., hexane, chloroform, ethyl acetate, and methanol extracts of *P. tetrastromatica and P. gymnospora* were tested against two standard strains and three clinical isolates of *S. aureus* (737 & 7443). The mean values are presented in (Table 1 to 2).

Table 1. Antibacterial activity of different extracts of *Padina tetrastomatica.*

S. No.	Microbial strains/ solvents	Mean zone of inhibition[a] (mm)[b]				MIC (µg/mL)	MBC (µg/mL)
		Concentration of the extracts (g/disc)					
		1000	500	250	Methicillin (10 µg/disc)		
1	***Staphylococcus aureus* (MTCC 7443)**						
	Hexane	19.0±0.50	15.0±0.30	11.0±0.25	9.3 0.28	125	250
	Chloroform	19.5±0.30	16.5±0.25	12.1±0.10	12.1 0.28	125	250
	Ethyl acetate	26.5±0.50	19.0±0.20	14.1±0.50	8.8 0.76	62.5	125
	Methanol	16.0±0.50	14.3±0.43	10.4±0.07	11.0 0.50	125	250
2	***S. aureus* (MTCC 737)**						
	Hexane	13.0±0.80	10.5±0.60	8.3±0.50	9.3 0.57	250	500
	Chloroform	15.8±0.30	13.3±0.20	12.0±0.54	12.8 0.28	250	500
	Ethyl acetate	23.5±0.50	19.8±0.30	13.3±0.80	12.0 0.50	125	125
	Methanol	12.5±0.60	9.8±0.50	8.1±0.60	8.6 0.76	250	500
3	MRSA1						
	Hexane	14.7±0.97	12.6±0.95	9.1±0.69	12.0 0.50	250	500
	Chloroform	15.0±0.92	13.1±0.80	10.4±0.79	12.0 0.50	250	500
	Ethyl acetate	17.5±0.33	14.2±0.90	11.3±0.86	8.6 0.76	62.5	125
	Methanol	15.3±0.03	13.7±0.20	9.8±0.75	8.6 0.76	125	250
4	MRSA2						
	Hexane	12.6±0.96	11.2±0.86	9.0±0.57	12.1 0.28	250	500
	Chloroform	13.8±0.98	12.1±0.92	9.2±0.70	12.8 0.76	250	500
	Ethyl acetate	15.8±0.76	13.4±0.03	10.1±0.07	10.8 0.50	125	125
	Methanol	13.5±0.02	11.5±0.88	9.1±0.69	8.8 0.76	250	500
5	MRSA3						
	Hexane	12.3±0.94	10.8±0.83	8.6±0.65	7.3 0.57	250	500
	Chloroform	12.9±0.92	11.4±0.87	9.3±0.69	8.8 0.28	250	500
	Ethyl acetate	15.3±0.99	13.1±0.10	10.3±0.78	7.8 0.50	125	125
	Methanol	13.4±0.02	11.1±0.84	9.0±0.69	9.3 0.57	250	500

a - diameter of zone of inhibition (mm) including the disc diameter of 6 mm;
b - mean of three assays; ± - standard deviation.

Among the tested extracts, the ethyl acetate possessed notable activity against *S. aureus* (737 & 7443) strains tested. The ethyl acetate extract of *P. tetrastromatica* shows potential activity against all the bacterial strains tested. All the extracts of marine macro algae possessed significant antibacterial activity against *S. aureus* (737 &74443) and three isolates of MRSA tested when compared to the available antibiotics tested. There was no much variation among the standard and clinical bacterial strains towards the algal extracts tested. When the different extracts were assay against the test bacteria by disc diffusion assays, the mean zone of inhibition recorded were between 7.1 and 26.5 Methicillin (5 µg/disc) antibacterial positive control produced mean zone of inhibition ranged from 7.1 to 9.0 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The lowest MIC value of 62.5 µg/ml and MBC value of 125 µg/ml ware recorded in the ethyl acetate extracts of *P.tetrastromatica* against *S. aureus* (737 & 7443), MRSA1, and MRSA3.

Table 2. Antibacterial activity of different extracts of *Padina gymnospora*

S. No.	Microbial strains/ solvents	Mean zone of inhibition[a] (mm)[b]				MIC (µg/mL)	MBC (µg/mL)
		Concentration of the extracts (g/disc)					
		1000	500	250	Methicillin (10 µg/disc)		
1	***Staphylococcus aureus* (MTCC 7443)**						
	Hexane	12.0±0.50	11.0±0.30	10.0±0.25	9.3 0.28	125	250
	Chloroform	14.5±0.30	13.5±0.25	11.1±0.10	12.1 0.28	125	250
	Ethyl acetate	18.5±0.5	14.0±0.20	11.5±0.50	8.8 0.76	62.5	125
	Methanol	12.5±0.50	13.3±0.43	10.5±0.07	11.0 0.50	125	250
2	***S. aureus* (MTCC 737)**						
	Hexane	11.5±0.50	10.0±0.30	8.5.0±0.25	8.3 0.57	250	500
	Chloroform	12.5±0.30	11.5±0.25	9.1±0.10	12.8 0.28	250	500
	Ethyl acetate	16.5±0.5	13.0±0.20	10.5±0.50	12.0 0.50	125	125
	Methanol	11.5±0.50	10.3±0.43	8.3±0.07	8.6 0.76	250	500
3	MRSA1						
	Hexane	11.3±0.28	9.3±0.30	7.3±0.33	12.0 0.50	250	500
	Chloroform	13.3±0.33	10.6±0.33	9.0±0.57	12.0 0.50	250	500
	Ethyl acetate	15.6±0.33	13.3±0.33	11.6±0.33	8.6 0.76	62.5	125
	Methanol	12.8±0.16	11.6±0.33	8.0±0.28	8.6 0.76	125	250
4	MRSA2						
	Hexane	13.6±0.28	11.8±0.16	7.5±0.28	12.1 0.28	250	500
	Chloroform	14.8±0.15	13.0±0.08	8.7±0.14	12.8 0.76	250	500

	Ethyl acetate	17.2±0.14	15.0±0.17	11.5±0.28	10.8 0.50	125	125
	Methanol	14.0±0.11	12.0±0.12	8.0±0.08	8.8 0.76	250	500
5	MRSA3						
	Hexane	14.3±0.11	12.3±0.14	10.4±0.30	7.3 0.57	250	500
	Chloroform	16.1±0.15	13.3±0.15	10.7±0.16	8.8 0.28	250	500
	Ethyl acetate	17.8±0.37	16.1±0.05	13.3±0.20	7.8 0.50	125	125
	Methanol	15.0±0.12	13.2±0.17	11.9±0.05	9.3 0.57	250	500

a - diameter of zone of inhibition (mm) including the disc diameter of 6 mm;
b - mean of three assays; ± - standard deviation.

Algae secrete some of compounds with a broad range of biological activities for example as antibiotics. As exhibited by the results, the seaweeds under investigation produced large biomass and also showed high biological activity Phaeophyceae were the most active in comparison with chlorophyceae and rhodophyceae [25] (Oumaskour *et al.*, 2012). These strong activities related to brown algae may be due to the phenolic compounds such as phlorotannins, eckol and eckol- related compounds that have strong bactericidal activity [26] (Nagayama *et al.*, 2002). Revealed that the ethyl acetate was to the best solvent for isolation of antimicrobial activity from the tested marine algae followed by methanol which in coincide to our results.

In this study, the different solvents *viz.*, hexane, chloroform, ethyl acetate, and methanol extracts of *Padina tetrastromatica, Padina gymnospora* have possessed antibacterial activity against all bacterial strains tested. In this study, ethyl acetate extract of *Padina tetrastromatica* showed the highest anti MRSA activity with a mean zone of inhibition of 26.5 mm against *S. aureus* (MTCC7443). The lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) values of the ethyl acetate extracts of *Padina tetrastromatica* against *S. aureus* (7443), were recorded. Thillairajasekar *et al.* [27] reported that the ethyl acetate extract of *Ulva lactuca* and *G. verrucosa* showed the highest antimicrobial activity against *E. coli, K. pneumoniae,* MRSA and *B. subtilis* and also identified the presence of myristic and palmitic acid, linoleic acid, oleic acid, lauric, stearic and myristic acid, from ethylacetate extracts. Salem *et al*. [28] reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *Caulerpa racemosa, Sargassum dentifolium, Padina gymnospora* and methanolic extracts of *Sargassum hystrix*, *C. racemosa, C. fragile, S. dentifolium* and *Cystoseria myrica*. These results, contrast with the study of Lavanya and Veerappan [29] who reported that the methanol, chloroform, ethyl acetate and aqueous extracts of *Codium decorticatum, Caulerpa* scalpelliformis, *Gracilaria crassa, Acanthophora* spicifera, *S. wightii* and *Turbinaria conoides* were more active than the acetone, diethyl ether and hexane extracts against the bacterial pathogens.

In the present study, the different extracts of *Padina tetrastromatica, Padina gymnospora* showed potential anti MRSA activity. Similar observation Kim *et al.* [30] had screened hexane, chloroform, ethyl acetate and methanol extracts of *Ulva lactuca* showed the antimicrobial activity against *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Candida albicans* and three MRSA strains.

The results revealed that the highest mean zones of inhibition were recorded with the ethyl acetate extract against *B. subtilis* (15 mm), *M. luteus* (12 mm), *S. aureus* (18 mm), *E. coli* (17 mm), *K. pneumoniae* (8 mm), *P. aeruginosa* (8 mm), *S. typhimurium* (18 mm), *Vibrio parahemolytics* (18 mm), *E. tarda* (18 mm) and three MRSA strains with the mean zones of inhibition ranged between 18.0 and 27.0 mm. Lee *et al.* [31] reported that the ethyl acetate-soluble fraction of *Ecklonia stolonifera* and *Ecklonia cava* exhibited the strongest anti-MRSA activity. Dieckol has been isolated from *Ecklonia stolonifera* and *E. cava* is a known antibacterial substance with activity against MRSA.

In this study, the different extracts of *Padina tetrastromatica, Padina gymnospora* against bacterial strains tested, among these, *Staphylococcus aureus* (7443) was most susceptible to the ethyl acetate extract of *Padina tetrastromatica* with the lowest MIC value of (62.5 µg/mL). Chandrasekaran *et al.* [32, 33] reported that the highest antibacterial activity were recorded in the brown alga, *S. marginatum* against MRSA and Vancomycin resistant *Enterococcus faecalis* in the ethyl acetate extracts when compared to other solvents extracts. Shanmughapriya *et al.* [34] reported that the *S. marginatum* extracts inhibited the growth of multi drug resistant *Klebsiella pneumoniae, Proteus mirabilis, Micrococcus luteus, Escherichia coli* and *Enterococcus faecalis*. The extract of *S. marginatum* has also exhibited strong antifungal activity [35]. In the present study, the ethyl acetate extracts of *Padina tetrastromatica, Padina gymnospora* possessed the antibacterial activity due to the presence of phytochemicals, terpenoids, tannins, phenolic compounds, and steroids. 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 [36]. Zubia *et al.* [37] reported that 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. Steroid glycosides are a class of widespread natural products having either terrestrial or marine origins. Several cardiac glycosides are used therapeutically in the treatment of cardiac failure and arrhytmias, and many glycoside compounds, belonging to other structural groups, cytotoxic, antimicrobial, hypocholesterolemic and other biological activities [38]. Tannins are well known to possess general antimicrobial properties [39].

4. CONCLUSION

Finally, it can be concluded that ethyl acetate extracts of *P. tetrastromatica* was found to be the most effective antiMRSA agent. This study recommends that ethyl acetate extracts of *P. tetrastromatica* can be used as an antibacterial substance for treating MRSA infections after further scientific validation.

References

[1] Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 298 (2007) 1763-1771.

[2] Lavanya R, Veerappan N Antibacterial Potential of Six Seaweeds Collected from Gulf of Mannar of the Southeast Coast of India. *Advances in Biological Research* 5 (1) (2011) 38-44.

[3] DH Tambekar, SB Dahikar. Antibacterial activity of some Indian ayurvedic preparations against enteric bacterial pathogens .*Journal of advanced pharmaceutical technology & research* 2 (1) (2011) 24–29.

[4] Lee DS, Isolation and characterization of anti-MRSA (methicillin-resistant Staphylococcus aureus) substances from marine organisms. Ph.D. Dissertation, Department of Microbiology, Pukyong National University, Busan. Chapter 1 (2009) 2-23.

[5] Kim, H.I., D. Lee, H.S. Lee, J. Ha, B. Ha, S. Kim and J. Lee, Antibacterial activity of *Ulva lactuca* againt methicillin-resistant *Staphylococcus aureus* (MRSA). *Biotechnology and bioprocess Engineering* (2007) 579-582.

[6] Finland, M. Emergence of antibiotic resistance in hospitals, 1935-1975. *Reviews of infectious diseases* 1(1) (1979) 4.

[7] Brumfitt, W. & J. M. T. Hamilton-Miller: The worldwide problem of methicillin-resistant *Staphylococcus aureus*. *Drugs Under Experimental and Clinical Research* 16(5) (1990) 205-214.

[8] [Isnansetyo, A. and Y. Kamei MC21-A, a bactericidal antibiotic produced by a new marine bacterim, Pseudoalteromonas phenolica sp. nov. O-BC30T, against methicillin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotheraphy* 47 (2003) 2-488.

[9] Eom SH, Kim DH, Lee SH, Yoon NY, Kim JH, Kim TH, Chung YH, Kim SB, Kim YM, Kim HW, Lee MS, Kim YM. *In vitro* antibacterial activity and synergistic antibiotic effects of phlorotannins isolated from Eisenia bicyclis against methicillin resistant *Staphylococcus aureus*. *Phototherapy Research* 27 (2013) 1260–1264.

[10] Liu C, Graber CJ, Karr M. A population-based study of the incidence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* disease in San Francisco, 2004–2005. *Clinical Infectious Diseases* 46 (2008) 1637-1646.

[11] Eom SH, Park JH, Yu DU, Choi JI, Choi JD, Lee MS, Kim YM Antimicrobial activity of brown alga Eisenia bicyclis against methicillin resistant *Staphylococcus aureus*. *Fisheries and Aquatic Sciences* 14 (2011) 251-256.

[12] Smit, A.J.Medicinal and pharmaceutical uses of seaweed natural products. A review. *Journal of Applied Phycology* 16 (2004) 245-262.

[13] Cabrita, M., Vale, C., Rauter, A. Halogenated compounds from marine algae. *Marne Drugs* 8 (2010) 2301-2317.

[14] Bennamara, A., A. Abourriche, M. Berrada, M. Charrouf, N. Chaib, M. Boudouma, and F.X. Garneau. Methoxybifurcarenone: an antifungal and antibacterial meroditerpenoid from the brown alga *Cystoseira tamariscifolia*. *Phytochemistry* 52(1) (1999) 37-40.

[15] Nagayama, K., Y. Iwamura, T. Shibata, I. Hirayama, and T. Nakamura. Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *Journal of Antimicrobial Chemotherapy* 50 (2002) 889-893.

[16] Nunnery JK, Mevers E, Gerwick WH. Biologically active secondary metabolites from marine cyanobacteria. *Current Opinion in Biotechnology* 21 (2010) 787-793.

[17] Mayer AM, Rodriguez AD, Berlinck RG, Fusetani N. Marine pharmacology in 2007-8: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis and antiviral activities; affecting the immune and nervous system and other miscellaneous mechanism of action. *Comparative Biochemistry and Physiology* 153 (2011) 191-222.

[18] Cabrita MT, Vale C, Router AP. Halogenated compounds from marine algae. *Marain Drugs* 8 (2010) 2301-2317.

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

[20] Trease, G.E. and Evans, W.C.Textbook of pharmacognosy. 12th Edition, Tindall and Co., London, (1983) 343-383.

[21] CLSI (Clinical Laboratory Standard Institute) (formerly NCCLS), Performance standards for antimicrobial disk susceptibility tests: Wayne, PA, USA. 100-22: 32-3.

[22] Colle J.G., A.G. Frasher, B.P. Marmiom, A. Simmon Makie and McCartney's Practical Medical Microbiology, 14th edn., Churchill Livingstone, (1996) 254-256: 796-8.

[23] Bauer A.W., W.M.M. Kirby, J.C. Scherris, M. Turck. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45 (1966) 493-496.

[24] Sarker S. D., L. Nahar, Y. Kumarasamy Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods* 42 (2007) 321-324.

[25] Oumaskour, K., N. Boujaber, S. Etahiri and Assobhei, O. Screening of antibacterial and antifungal activities in green and brown algae from the coast of Sidi Bouzid (El Jadida, Morocco). *African Journal of Biotechnology* 11 (104) (2012) 16831-16837.

[26] Nagayama, K., Y. Iwamura, T. Shibata, I. Hirayama, and Nakamura, T. Bactericidal activity phlorotannins from the brown alga Ecklonia kurome. *Journal of Antimicrobial Chemotheraphy* 50 (2002) 889-893.

[27] Thillairajasekar K., V. Duraipandiyan, P. Perumal, S. Ignacimuthu Antimicrobial activity of *Trichodesmium erythraeum* (Ehr) (microalaga) from South East Coast of Tamil Nadu, India. *International Journal of Integrative Biology* 5 (2009) 167-170.

[28] Salem W. M., H. Galal, F. Nasr El-deen Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). *African Journal of Microbiology Research* 5(15) (2011) 2160-2167.

[29] Lavanya R. and N. Veerappan Antibacterial Potential of Six Seaweeds Collected from Gulf of Mannar of Southeast Coast of India. *Advances in Biological Research* 5 (1) (2011) 38-44.

[30] Kim, I.H., S.H. Lee, J.M. Ha, B.J. Ha, S.K. Kim and J.H. Lee, Antibacterial activity of *Ulva lactuta* against methicillin- resistant *Staphylococcus aureus* (MRSA*). Biotechnology and Bioprocess Engineering* 12 (2007) 579-582.

[31] Lee, D.S., M.S. Kang, H.J. Hwang, S.H. Eom, J.Y. Yang, M.S. Lee, W.J. Lee, Y.J. Jeon, J.S. Choi and Y.M. Kim, Synergistic effect between deckle from *Ecklonia stolonifera* and β-lactams against methicillin-resistant *Staphylococcus aureus. Biotechnology Bioprocess Engineering* 13 (2008) 758-764.

[32] Chandrasekaran, M., V. Venkatesalu and G. Adaikala Raj, 2014a. Antibacterial activity of selected marine macro algae against Vancomycin resistant *Enterococcus faecalis. Journal of Coastal Life Medicine*. 2(12) (2014) 940-946.

[33] Chandrasekaran, M., V. Venkatesalu and G. Adaikala Raj, 2014b. Anti-MRSA activity of brown and red algae from Gulf of Mannar coast, South India. *Journal of Life Sciences and Technology*. 7(4) (2014) 22-31; 0974-5335.

[34] Shanmughapriya, S., A. Manilal, S. Sujith, J. Selvin, G. Seghal kiran and K. Nataraja seenivasan, Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Annals of Microbiology* 58 (3) (2008) 535-541.

[35] Usmanghani, K. and M. Shameel, Studies on the antimicrobial activity of certain seaweeds from Karachi coast. *In:* R. Ahmad and A.S. Pietro (Eds.), Prospects for Biosaline Research, *Proc. US-Pak. Biosal. Res. Works*, Karachi, pp. 519-526.

[36] Reguant C., A. Bordons Arola, L N. Roze Influence of phenolic compounds on the physiology of *Oenococcus oeni*. *Journal of Applied Microbiology* 88 (2000) 1065–1071.

[37] Zubia M., C. Payri, E. Deslandes Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti (French Polynesia). *Journal of Applied Phycology* 20(6) (2008) 1033-1043.

[38] Ivanchina N.V., A. A. Kicha, V.A. Stonik Steroid glycosides from marine organisms. *Steroids* 76 (2011) 425-454.

[39] Scalbert A. Antimicrobial properties of tannins. *Photochemistry, Phyfochemisrry,* 30 (12) (1991) 3875-3883.