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Screening of phytochemical and antibacterial potential of different organic solvent extracts of *Stoechospermum marginatum* (Ag) Kutz. from Manappad coast, Tuticorin District, South India

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ABSTRACT

The antibacterial activity of different extracts of hexane, chloroform, ethyl acetate, acetone and methanol extract of a brown alga, *Stoechospermum marginatum* (Ag) Kutz. against *Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella flexneri* and *Vibrio cholerae*. The extent of the inhibitory zone, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. The ethyl acetate extract of *S. marginatum* showed the highest antibacterial activity against all the bacterial strains tested than the other extracts. The mean zones of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.1 to 18.1 mm. The MIC were between 125 and 500 μg/ml, while the MBC were between 250 and 1000 μg/ml. The ethyl acetate extract of *S. marginatum* showed the presence of terpenoids, tannins, phenolic compounds and steroids strongly than the other solvent extracts. The highest mean of zone inhibition (18.1 mm) was observed in the ethyl acetate extract of *S. marginatum* against *B. subtilis*. These finding suggest that the ethyl acetate extract of *S. marginatum* can be used as an antibacterial substance for the treatment of bacterial infections.

KEY WORDS: Antibacterial activity, *Stoechospermum marginatum,* Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

INTRODUCTION

In recent years, multiple drug resistance has been developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases and has become a global public health problem (WHO, 2003). A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment. The overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes (Ang *et al.*, 2004). Antibiotics provide the main basis for the therapy of microbial infections. Since, the discovery of these antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infection diseases (Rosina *et al.*, 2009). However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi drug resistant (MDR) strains of several groups of micro-organisms (Harbottle *et al.*, 2006). In the light of the evidence of rapid global spread of resistant clinical isolates, the need to find a new antimicrobial agent is of paramount importance. However, the past record of rapid, wide spread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates *et al.*, 2002).

Bacteria are the leading cause of nosocomial disease, and viruses are a distant second. Occasionally, fungi cause disease but rarely protozoa are involved. Mostly nosocomial diseases are caused by Gram-negative bacilli like *Escherichia coli*. The use of therapeutic and diagnostic equipment (such as intravenous and urinary catheters), surgical procedure and transplantation has increased

the risk of nosocomial diseases (Emori and Gaynes, 1993). Bacteria have evolved numerous defenses against antimicrobial agents and drug-resistant pathogens. In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented (Jones *et al.*, 2004). Bacterial infection causes a high rate of mortality in the human population and aquaculture organisms (Kandhasamy and Arunachalam, 2008). For example, *Enterococcus faecalis* is the causative agent of inflammatory bowel disease (Balish and Warner, 2002), *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases such as mastitis, abortion, and upper respiratory complications, while *Salmonella* sp. causes diarrhea and typhoid fever (Jawetz *et al.*, 1995). *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness (Kandhasamy and Arunachalam, 2008). Infectious diseases caused by drug/antibiotic-resistant bacterial strains have always been a matter of clinical concern. Mortality due to urinary tract infections has been of almost clinical concern in the last four decades, with 81% in 1973 and 50% in 2006 due to MDR *P. aeruginosa*, for example (Pennington *et al.*, 1973; Giamarellos-Bourboulis *et al.*, 2006).

Antibacterial resistance of Enterobacteriaceae, especially the emergence of multiple drug resistant strains is an important clinical problem worldwide. 1, 3 β-lactam antibiotics are the most common prescribed antibiotics. The major mechanism of resistance to β-lactams, particularly in Gram-negative bacteria, is the production of extended spectrum β-lactamases. These multidrug-resistant bacteria have also created immense clinical problems in cancer and immunocompromised patients. Most important multidrug-resistant bacteria on the global scale include Gram-positive (methicillin-resistant *S. aureus* [MRSA], vancomycin resistant Enterococci) and Gram-negative bacteria (members of Enterobacteriaceae producing plasmid-mediated extended spectrum β-lactamases and others like *P. aeruginosa*, *Mycobacterium tuberculosis* (Medeiros, 1997; Sajduda *et al.*, 1998). *Klebsiella pneumoniae* carbapenemase enzyme and metallo β-lactamase are the common armamentaria of carbapenem resistance in Enterobacteriaceae (Kitchel *et al.*, 2009; Cendejas *et al.*, 2010). *Proteus mirabilis* Northern Kentucky University strain has been recorded as showing resistance to a large number of antibiotics, therefore, and their control is very difficult (Doublet *et al.*, 2010).

Seaweeds contain various inorganic and organic substances which can benefit human health (Kuda *et al.*, 2002). Seaweeds are known to contain reactive antioxidant molecules, such as ascorbate and glutathione when fresh, as well as secondary metabolites, including carotenoids (α- and β-carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine), and catechins (e.g. catechin, epigallocatechin), gallate, phlorotannins (e.g. phloroglucinol), eckol and tocopherols (α-, χ-, δ- tocopherols) (Yuan *et al.*, 2005). Brown-algal polyphenols and phlorotannins worked as antioxidants and antibacterial compounds (Kuda *et al.*, 2007).

Phaeophyceae members are commonly called as brown alga constitutes the major component of the seaweed population of the tropical countries of the world hence *Stoechospermum marginatum* a monotypic genus belonging to the order Dictyotales of Phaeophyta was selected for the study. The plant is brown to yellowish brown in color, thallus is flat and foliaceous 5-55 cm long and 1-3 cm wide, irregularly branched into dichotomously strap-shaped segments with entire margins. Apical margins are involute and the growth of the thallus occurs by means of a marginal meristem. Hairs are scattered all over the surface of the thallus. Reproductive structures occur as marginal sori (Poonam, 2011). *S. marginatum* is very abundant brown seaweed, rich in sulfated fucans and known to possess spasmogenic activity (Wealth of India, 1985).

Marine macroalgae are the most interesting algal group because of their broad spectrum of biological activities such as antimicrobial (Chiheb *et al.*, 2009), antiviral (Bouhlal *et al.*, 2011; Kim and Karadeniz, 2011), antifungal (De Felício *et al.*, 2010), anticoagulant (Dayong *et al.*, 2008), anticancer (Kim *et al.*, 2011), antifouling (Bhadury and Wright, 2004) and antioxidant activities (Devi *et al.*, 2011). They produce a wide variety of chemically active metabolites in their surroundings as an aid to protect themselves against other settling organisms (Bhadury and Wright, 2004). Many chemically unique compounds of marine algae with antimicrobial activity have been isolated and a number of them are under investigation and/or are being developed as new pharmaceuticals such as brominated phenols, sterols, terpenoids, polysaccharides, peptides, proteins, acrylic acid, terpenes, chlorophyllides, phenols, and heterocyclic carbons etc. (Bhacuni and Rawat, 2005; Li *et al.*, 2007; Bouhlal *et al.*, 2011; Priyadharshini *et al.*, 2011).

Hence, the present study was made to evaluate the antibacterial activity of different extracts of *S. marginatum* against bacterial strains.

MATERIALS AND METHODS

Collection of Sample

S. marginatum (Ag) Kutz. (Dictyotaceae) was collected at Manappad, (Latitude 8°30′N; Longitude 78°8′E)

Tuticorin district, Gulf of Mannar Marine Biosphere Reserve, Tamil Nadu, India. The collections were made during the months of November and December 2012. The voucher specimen was deposited in the Herbarium (AUBOT#274), Department of Botany, Annamalai University, Annamalai Nagar.

Preparation of Crude Extracts

The algal sample was handpicked during low tide and manually cleaned from sand, epiphytes and animal waste. Then the sample was rinsed with seawater to remove associated debris, planktons and loosely attached microorganisms and kept in an ice box containing slush ice and transported to the laboratory. Further, the material were washed thoroughly with tap water to remove the salt on the surface of the samples and the water was drained off from the alga 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 solvents during the extraction process. The alga samples were shade dried followed by oven drying at 50°C for an hour and milled in an electrical blender. 500 g of finely ground algal powder material were packed in Whatman filter paper. The powdered samples were extracted with different organic solvents in a Soxhlet apparatus for 72 h with increasing polarity *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol. The extracts were concentrated to solvents free by evaporation in a rotary vacuum evaporator (Heidolph, Germany) at a temperature <40°C. The crude extracts thus obtained were kept at 4°C for further analysis.

Phytochemical Screening

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. marginatum* were subjected to qualitative phytochemical studies. Phytochemicals like terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds and coumarins were tested according to the method (Harborne, 1973; Trease and Evans, 1983).

Collection of Bacterial Strains

The standard bacterial strains *viz., Bacillus subtilis* (MTCC 441), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 741), *Proteus mirabilis* (MTCC 425), *Proteus vulgaris* (MTCC 426), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457) and *Vibrio cholerae* (MTCC 3906) were procured from Microbial Type Culture Collection (MTCC), Chandigarh. *In vitro* antibacterial activity was determined by using Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) obtained from Himedia, Mumbai.

Antibiotic Sensitivity Test

Antibiotic sensitivity of the bacterial strains was determined by standard CLSI disc diffusion method M100-S22, (CLSI, 2012) using different classes of antibiotics *viz.,* amikacin (30 µg/disc), ampicillin (AMP 10 µg/disc), cefixime (CFM 5 µg/disc), ceftazidime (CAZ 30 µg/disc), ciprofloxacin (5 µg/disc), chloramphenicol (30 µg/disc), erythromycin (E 15 µg/disc), gentamycin (10 µg/disc), norfloxacin (10 µg/disc), nalidixic acid (NA 30 µg/disc), ofloxacin (5 µg/disc), streptomycin (S 10 µg/disc) and tetracycline (TE 30 µg/disc) (Himedia, Mumbai).

Disc Diffusion Method

The antibacterial activity of crude extracts of *S. marginatum* was determined by disc diffusion method according to Bauer *et al.* (1966) with modifications. Petri dishes were prepared by pouring 20 ml of MHA. Then the plates were allowed to solidify and used in susceptibility test. The standardized inoculum using bacterial suspensions containing 10^8 colony forming units (CFU) per ml, were swabbed on the top of the solidified media and allowed to dry for 10 minutes. The extracts were dissolved in 10% dimethyl sulfoxide (DMSO) and under aseptic conditions sterile discs were impregnated with 20 µl of three different concentrations of the crude extracts (500, 250 and 125 µg/disc). 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. Ampicillin (10 µg/disc) was used as a positive antibacterial control, and 10% DMSO was used as a blind control in all the assays. Finally, the inoculated plates were incubated at 37°C for 24 h for all the bacterial strains tested. The zones of inhibitions were observed and measured in millimeters. The assay in this experiment was repeated 3 times.

Microdilution Broth Assay

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was determined for the crude extracts of *S. marginatum* in MHB by using a modified reaszurin microtiter plate assay was carried out according to methods of Sarker *et al.* (2007). 50 ml of Sterile MHB were transferred into each well of a sterile 96-well microtiter plate. The algae extracts were dissolved in 10% DMSO to obtain 2000 µg/ml stock solutions. A volume of 50 µl of crude extracts stock solution was added to the first well. After fine mixing of the crude extracts and 50 µl of the broth solution was transferred to the second

well and in this way, the serial dilution procedure was continued to a two-fold dilution to obtain concentrations like 1000 to 15.625 µg/ml of the crude 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 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well dissolved and homogenous solution). Finally, 10 µl of the 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 for all the bacterial strains tested. 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 crude extracts that inhibited the growth of the organisms.

Determination of the Minimum Bactericidal Concentration (MBC)

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

RESULTS AND DISCUSSION

Phytochemical Investigation

The phytochemical analysis of hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *S. marginatum* had showed the presence of terpenoids, tannins, phenolic compounds and steroids. Steroids were present in all the extracts tested except methanol extracts. Cardiac glycosides were present in chloroform and acetone extracts. Phenolic compounds were present in chloroform and ethyl acetate extracts. Alkaloids and coumarins are not present in all the extracts tested. The ethyl acetate extract of *S. marginatum* showed the presence of terpenoids, tannins, phenolic compounds and steroids strongly than the other solvent extracts.

Resistance Pattern of Multi Drug Resistant Bacteria

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

Antibacterial Potential

The different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. marginatum* were studied against bacterial strains. All the extracts of *S. marginatum* possessed significant antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. The mean values are presented in Table 1. When the different extracts were assayed against the test bacteria by agar diffusion assays, the mean zones of inhibition obtained were between 7.1 and 18.1 mm. Ampicillin (10 µg/disc) antibacterial positive control produced mean zones of inhibition ranged from 7.3 to 13.8 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. marginatum* ranged from 125 to 500 µg/ml, while the MBC values were between 250 and 1000 µg/ml.

In the present study, the ethyl acetate extract of *S. marginatum* showed the highest antibacterial activity than other extracts against *B. subtilis* and *S. pyogenes.* Chandrasekaran *et al.* (2014a); Chandrasekaran *et al.* (2014b) reported that the highest antibacterial activity were recorded in the brown alga, *S. marginatum* against MRSA and vancomycin resistant *E. faecalis* in ethyl acetate extracts when compared to other solvents extracts.

In the present study, the ethyl acetate extract of *S. marginatum* showed the highest activity than other extracts. Thillairajasekar *et al.* (2009) reported that the ethyl acetate extracts of *Ulva lactuca* and *Gracilaria 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 ethyl acetate extracts. Salem *et al.* (2011) reported that the higher antibacterial activity was recorded for ethyl acetate extracts of *Caulerpa racemosa*, *Sargassum dentifolium,*

Table 1: Antibacterial activity of *Stoechospermum marginatum* against bacterial strains

Bacterial strains/seaweed extracts prepared with different solvents	Mean zone of inhibitiona (mm)[b]					
	Concentration of the disc					
	500 (μg/disc)	250 (μg/disc)	125 (μg/disc)	Ampicillin (10 μg/disc)	MIC (μg/ml)	MBC (μg/ml)
Bacillus subtilis						
Hexane	13.1±0.28	11.0±0.50	8.1±0.28	9.3±0.57	250	250
Chloroform	14.5±0.50	11.5±0.50	9.1±0.28	8.6±0.76	250	500
Ethyl acetate	18.1±0.28**	14.0±0.50	10.5±0.50	8.3±0.57	125	250
Acetone	11.0±0.50	9.5±0.50	7.3±0.57	8.0±0.50	500	1000
Methanol	10.5±0.50	9.5±0.50	7.1±0.28	10.6±0.76	500	1000
Streptococcus pyogenes						
Hexane	10.1±0.28	9.0±0.50	7.1±0.28	10.8±0.76	250	1000
Chloroform	11.5±0.50	9.5±0.50	7.1±0.28	8.6±0.76	250	1000
Ethyl acetate	14.0±0.50**	11.5±0.50	9.5±0.50	9.3±0.57	250	500
Acetone	10.6±0.28	9.5±0.50	7.5±0.50	9.3±0.57	500	1000
Methanol	10.5±0.50	9.3±0.57	7.1±0.28	11.6±0.76	500	1000
Escherichia coli						
Hexane	12.0±0.50	10.1±0.50	7.5±0.50	9.3±0.57	500	1000
Chloroform	12.6±0.76	10.5±0.50	8.0±0.58	7.3±0.28	500	1000
Ethyl acetate	13.5±0.50	11.3±0.28	8.6±0.76	11.0±0.76	250	500
Acetone	12.3±0.28	9.5±0.5	7.8±0.76	9.3±0.57	500	1000
Methanol	10.6±0.76	9.0±0.5	7.1±0.28	7.3±0.28	500	1000
Klebsiella pneumoniae						
Hexane	11.3±0.28	9.5±0.50	7.1±0.28	9.3±0.28	500	1000
Chloroform	12.0±0.50	9.8±0.76	7.5±0.50	12.8±0.57	500	1000
Ethyl acetate	13.3±0.28	10.1±0.28	8.5±0.50	12.0±0.50	250	500
Acetone	11.0±0.50	9.1±0.28	7.3±0.28	9.3±0.57	500	1000
Methanol	10.0±0.50	9.1±0.28	7.3±0.57	8.3±0.28	500	1000
Proteus mirabilis						
Hexane	12.0±0.50	9.8±0.28	7.3±0.57	8.6±0.76	500	1000
Chloroform	12.5±0.50	9.5±0.50	7.5±0.50	9.8±0.28	250	1000
Ethyl acetate	14.0±0.50	10.5±0.50	9.0±0.50	8.8±0.28	250	500
Acetone	11.6±0.57	9.5±0.50	7.1±0.28	11.0±0.50	500	1000
Methanol	11.1±0.76	9.3±0.57	7.1±0.28	12.1±0.28	500	1000
Proteus vulgaris						
Hexane	10.0±0.50	9.1±0.28	7.1±0.28	12.0±0.57	500	1000
Chloroform	12.0±0.50	10.3±0.28	7.6±0.50	12.1±0.57	250	500
Ethyl acetate	13.5±0.50	10.5±0.50	8.1±0.28	11.5±0.57	250	500
Acetone	11.0±0.50	9.1±0.28	7.6±0.28	10.3±0.57	500	1000
Methanol	10.0±0.50	8.8±0.76	7.1±0.28	9.6±0.57	500	1000
Pseudomonas aeruginosa						
Hexane	11.3±0.57	9.1±0.28	7.3±0.57	12.0±0.86	500	1000
Chloroform	11.5±0.50	10.3±0.28	8.1±0.28	10.3±0.28	500	1000
Ethyl acetate	13.0±0.50	10.6±0.28	9.8±0.76	11.6±0.76	250	500
Acetone	12.0±0.76	10.1±0.28	8.0±0.50	8.6±0.76	500	1000
Methanol	10.3±0.50	9.1±0.28	7.1±0.28	10.3±0.28	500	1000
Salmonella typhimurium						
Hexane	10.0±0.50	8.5±0.50	7.1±0.28	12.1±0.28	500	1000
Chloroform	11.6±0.76	9.8±0.76	7.3±0.57	12.8±0.76	250	500
Ethyl acetate	13.6±0.76	10.1±0.28	8.1±0.28	11.6±0.76	250	500
Acetone	11.5±0.50	9.6±0.28	7.6±0.28	10.3±0.57	500	1000
Methanol	11.0±0.50	9.5±0.50	7.1±0.28	9.3±0.57	500	1000
Shigella flexneri						
Hexane	10.5±0.50	9.0±0.50	7.3±0.57	11.6±0.76	500	1000
Chloroform	11.5±0.50	9.6±0.28	7.8±0.76	12.1±0.28	500	1000
Ethyl acetate	14.3±0.57**	11.0±0.50	9.1±0.28	12.8±0.57	250	500
Acetone	11.1±0.28	9.6±0.50	7.5±0.50	11.6±0.57	500	1000
Methanol	10.1±0.28	8.6±0.76	7.1±0.28	11.0±0.50	500	1000
Vibrio cholerae						
Hexane	12.0±0.50	9.8±0.28	7.5±0.50	10.3±0.28	500	1000
Chloroform	12.8±0.56	10.5±0.50	7.6±0.57	12.1±0.28	250	500
Ethyl acetate	14.1±0.28	10.8±0.76	8.6±0.28	10.3±0.28	250	500
Acetone	11.8±0.28	9.5±0.50	7.1±0.28	10.3±0.57	500	1000
Methanol	10.3±0.28	8.8±0.28	7.1±0.28	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. **Significant at $P<0.05$

Padina gymnospora; methanol extracts of *Sargassum hystrix*, *S. dentifolium, C. racemosa*, *C. fragile* and *Cystoseria myrica*.

The different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Ulva fasciata* against multi-drug resistant bacterial strains *viz., B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium,V. cholerae, S. flexneri, P. mirabilis,* and *P. vulgaris* (Chandrasekaran *et al.,* 2014c). Chandrasekaran *et al.* (2014d) examined that the antibacterial activity of different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts *Sargassum wightii* against multi-drug resistant bacterial strains *viz., B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, S. dysentriae, P. mirabilis* and *P. vulgaris.* Adaikala Raj *et al.* (2015) investigated that the antibacterial activity of different *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Caulerpa chemnitiza* against bacterial strains *viz., B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. mirabilis* and *P. vulgaris, P. aeruginosa, S. typhimurium, S. flexneri* and *V. cholerae*.

Five saturated fatty acids were isolated from the chloroform and methanol extracts of *S. marginatum* such as myristate, pentadecylate, palmitate, margarate and non-adecylate and four unsaturated fatty acids *viz.,* tetradecatrienoate, pentadecenoate, hiragonate, and oleate, three sterols *viz.,* cholesterol (1), 24-methylene cholesterol (2) and 24-methyl cholesterol (3) four diterpenes *viz.*, 19-acetoxy-5 (R), 15, 18 (R and S)-tetrahydroxyspata-13, 16 (E)-diene, 5 (R), 15, 18 (R and S), 19- tetrahydrowspata-13, 16 (E)-diene (5), 5 (R), 18-dihydroxyspata-13, 16 (E)-dime (6) and 5 (R), 16-dihydroxyspata-13, 17-diene (7) and D-mannitol. All these diterpenes showed strong antibacterial activity against three Gram-positive and six Gram-negative bacteria (Shiaikh *et al.*, 1990). The active constituents were found to be a mixture of monoacetates belonging to the spatane diterpenoids, the structures of which have been elucidated (Gerwick *et al.*, 1981).

The brown alga *Ecklonia kurome* contained phenolic compounds such as phlorotannins, eckol and eckol-related compounds that have strong bactericidal activity Nagayama *et al.* (2002). Polyphenols were reported to have microbicidal activity against many pathogenic bacteria (Scalbert, 1991; Cowan, 1999). Various plant phenolics, including flavonoids and tannins, have been shown to have antibacterial effects (Mitscher *et al.*, 1980; Kolodziej *et al.*, 1999), and some flavonoids and xanthones are effective against MRSA (Sakagami *et al.*, 1998; Iinuma *et al.*, 1996). Phenolic compounds which play a major role in antibacterial and antifungal activities are found abundantly in brown seaweeds when compared with the green and red seaweeds (Chkhikvishvili and Ramazanov, 2000).

Zapta and McMillan (1979) reported that the role of phenolic compounds present in seagrasses could also enhance the antimicrobial activity. 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 (Alberto *et al.*, 2001; Reguant *et al.*, 2000).

In the present study, the highest zones of inhibition were recorded at the concentration of 500 µg/disc of all the extracts. As the disc dosage level increased, the inhibitory effect also increased. Similar observations were reported with fatty acid methyl ester extracts of some marine macro algae (Ananatharaj *et al.*, 2004) and leaves of *Ipomoea pes-caprae* (Chandrasekaran *et al.*, 2005).

In the present study, different extracts of *S. marginatum* possessed antibacterial activity against all the bacterial strains tested. Shanmughapriya *et al.* (2008) have reported that *S. marginatum* extracts inhibited the growth of multi drug resistant *K. pneumoniae, P. mirabilis, Micrococcus luteus, E. coli* and *E. faecalis*. In addition, the extracts from *S. wightii* and *S. marginatum* showed antibacterial activity against Gram-negative bacteria.

In the present study, the Gram-positive bacteria were more susceptible than the Gram-negative bacteria. The greater resistance of Gram-negative bacteria to plant extracts has been documented previously for seeds of *Syzygium jambolanum* (Chandrasekaran and Venkatesalu, 2004a) and bark of *Cassia siamea* (Chandrasekaran and Venkatesalu, 2004b).

The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). The resistance of Gram-negative bacteria toward antibacterial substances is related to lipopolysaccharides in their outer membrane (Sawer *et al.*, 1997; Gao *et al.*, 1997). The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms (Arias *et al.,* 2004).

In the present study, the ethyl acetate extracts of *S. marginatum* showed the antibacterial activity that may be due to the presence of strong phytochemicals, steroids, terpenoids, tannins, and phenolic compounds.

Seaweeds extracts are considered to be a rich source of phenolic compounds (Heo *et al*., 2005). In general, phenolic compounds possess specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, antifeedant, antiviral, anticancer and vasodilatory actions (Rievere *et al*., 2009). 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 were used therapeutically as antiviral, antibacterial, antiulcer, and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent (Kolodziej and Kiderlen, 2005). Steroids of plant origin are known to be important for insecticidal, antimicrobial, antiparasitic and cardiotonic properties. Steroids also play an important role in nutrition, herbal medicine and cosmetics (Okwu, 2001).

CONCLUSION

In this study, as the ethyl acetate extract of *S. marginatum* showed potential natural antibacterial action against the tested human pathogenic bacterial strains, it can be recommended that the species can be used as antibacterial substance for treating infection caused by bacterial strains.

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