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SCREENING OF PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *SPATOGLOSSUM ASPERUM* (AGARDH, J.G.) AGAINST MULTIDRUG-RESISTANT BACTERIAL STRAINS

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ABSTRACT

Seaweeds are important source of bioactive molecules with known beneficial effects on human health. In the present study was carried out to investigate the antibacterial efficacy against some human pathogenic bacteria of various organic solvent extracts hexane, chloroform, ethyl acetate, and methanol *viz., S. asperum reacted* positively against selected human bacterial pathogens. *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholera, Shigella flexneri, Proteus mirabilis* and *Proteus vulgaris* by disc diffusion method. The antibacterial activity was performed by disc diffusion, Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC). The mean zone of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.1 to 17.8 mm. The MIC values were between 62.5 and 500 µg /disc, while the MBC values were between 250 and 1000 µg /disc. The highest mean of zone inhibition (17.8 mm) and lowest MIC (62.5 µg /disc) and MBC (250 µg /disc) values were recorded in ethyl acetate extract. The phytochemical analysis of hexane, chloroform, ethyl acetate and methanol extracts of *S. asperum* had showed the presence of terpenoids, tannins and steroids. This study revealed

that ethyl acetate extract of S. *asperum* is a source of antibacterial compounds for the treatment of human bacterial pathogens.

KEYWORDS: Antibacterial activity, *Spatoglossum asperum*, Phytochemicals, MIC, MBC.

INTRODUCTION

The algae over 1,50,000 species are established in intertidal zones and tropical seas of the oceans, accounting for 80% of the world's plant diversity, which makes the principal source of natural products (Paul and Devi, 2013). Marine macroalgae may live up to 270 metres deep and have been found on about 8000 different coasts throughout the world. There are red algae 350 species, green algae 25 species, brown algae 90 species and 90 species of brown algae in the world's shorelines, all of which have commercial value due to their biochemical composition (Chakraborty and Bhattacharya 2012). Seaweeds belong to a group of plants known as alga. Seaweeds are classified as *Rhodophyceae* (red algae), *Phaeophyceae* (brown algae) and *Chlorophyceae* (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit the human health (Guiry, 2024). Seaweeds are especially rich in a widespread range of structurally assorted secondary metabolites as well as polyphenols, alkaloids, terpenes and stilbenes with lots of these compounds individual halogenated (Watson and Cruz-Rivera, 2003). Marine macro algae are rich sources of structurally work of fiction and biologically active secondary metabolites. Approximately 2500 new metabolites were reported from a variety of oceanic organisms during the years from 1977 to 1987(Arul Senthil *et al.*, 2008). With many of secondary metabolites in seaweeds having bacteriocidal or bacteriostatic properties (Steinberg *et al.*, 1997). Seaweeds are a rich source of natural foodstuffs and May be incorporated into the human diet, be used in cosmetic products and as traditional medicine (Fisch *et al*., 2003). They fabricate primary or secondary metabolites which are potentially bioactive compounds of interest in pharmaceutical industries such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals (Joseph Selvin and Aaron Premnath Lipton 2004).

There have been a number of reports of antibacterial activity from marine plants (AL-Haj *et al.*,2010, Kolanjinathan *et al.*, 2009). Most of the compounds responsible for the antimicrobial activity of seaweed are primarily phenolic and polysaccharide components, and their mechanism of action could be stasis (growth inhibition of microorganisms) or cidal (direct destruction of microorganisms) (Davidson and Naidu, 2000). Phlolorotanins, a

category of polyphenol fashioned throughout the pathway of acetate malonate, are fashioned through brown seaweeds comes under secondary metabolite (Ummat *et al.,* 2020). Phlorotanins play a crucial role in the biological activities of algae and a number of critical mechanisms, including protection against oxidative damage brought on by various abiotic environmental stimuli. Scientists are interested in these phenolic compounds because of their possible biological properties, which include anticancer activity, anti-oxidant, antibacterial, antidiabetic, and anti-inflammatory properties (Rocha *et al.,* 2018).

In developing countries, bacterial infections are extensive, especially in informal settlements, due to poor sanitation and unhygienic conditions. Furthermore, diseases such as AIDS, malaria and tuberculosis, result in higher morbidity and mortality than those caused by susceptible pathogens; the global impact of increasing resistance is a major concern (Atif *et al*., 2020). Multi-drug resistant strains of bacteria have become a serious public health problem (Wright GD 2005) Most important multi-drug resistant bacteria on the global scale include Gram-positive (methicillin-resistant *Staphylococcus 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* (Sajduda *et al*., 1998). The synergistic effects of seaweed antimicrobial extracts in association with antibiotics can provide effective therapy against drug resistant bacteria, thus providing the pharmaceutical sector with novel products characterized by multiple mechanisms of action (Newman *et al.,* 2003). In the present study was made to evaluate the antibacterial activity of different extracts of *S. asperum* (Phaeophyceae) against various bacterial strains.

Materials and Methods

Collection of samples

Spatoglossum asperum J. Agardh (Phaeophyceae) were collected from the low tidal region of the Muyal theevu, Tuticorin district, the Gulf of Mannar Marine biosphere, Tamil Nadu, India. The sample were cleaned with fresh seawater and there in distilled water to remove all the unwanted impurities, epiphytes and adhering sand particles morphologically distinct thallus of algae were placed in new polythene bag and kept in an ice box containing slush ice and transported to the laboratory. Further they were washed thoroughly using tap water to remove the salt on the surface of the seaweed then dried the water off and samples were spread on the blotting paper to remove excess of water. They were then dried samples were in

the room temperature. 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. The dried samples were chopped into fine fragment with the help of mixer. The powder samples were stored in refrigerator for further use.

Preparation of extracts

The dried seaweed materials were blended into a coarse powder before extraction portions of the powdered samples Two hundred grams of powdered samples was packed inside a Soxhlet apparatus and the successive extraction was carried out using 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 40 °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 *S. asperum* were subjected to qualitative phytochemical studies. Phytochemical 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 cholera* (MTCC 3906) were procured from Microbial Type Culture Collection (MTCC), Chandigarh. *In vitro* antibacterial activity was determined by using.

Antibiotic Sensitivity Tests

Antibiotic sensitivity of the bacterial strains was determined by standard Clinical Laboratory Standards Institute (CLSI) disc diffusion method (CLSI, 2012) using different classes of antibiotics *viz.,* amikacin (AK, 3 µg/disc), ampicillin (AMP 10 µg/disc), cefixime (CFM 5 µg/disc), ceftazidime (CAZ 30µg/disc), ciprofloxacin (CIP 5µg/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 10µg/disc) and tetracycline (TE 30µg/disc) (Himedia, Mumbai, Maharashtra, India).

Anti-bacterial Assay

Disc diffusion method

The antibacterial activity of different extracts of *S. asperum* was determined by disc diffusion method according to Bauer *et al*. (1966) with modifications. Petri dishes were prepared by pouring 20 mL of Muller Hinton Agar. 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 min. The algal extracts was dissolved in 10% dimethyl sulfoxide (DMSO) and under aseptic conditions, sterile discs were impregnated with 20 µl of three different concentrations of the algae extracts (1000, 500, and 250 µg/disc). The discs with algae extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. AMP (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 bacterial strains tested. The zones of inhibitions were observed and measured in millimeters. The assay in this experiment was repeated 3 times.

Micro dilution Broth Assay

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was determined for the crude extracts of *S. asperum* in MHB by using a modified reaszurin micro titer 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 micro titer 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 reaszurin indicator solution was added (The reaszurin 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.

RESULTS AND DISCUSSION

The antibiotic resistance of bacterial strains of standard strains was confirmed by CLSI-M100-S22, 2012 method. The standard strains of *B. subtilis, K. pnemoniae* and *P. vulgaris* were sensitive to all the antibiotics tested except CFM, AMP and CAZ. The standard strains of *S. flexneri* and *P. mirabilis* were the antibiotics tested except AMP. The standard strains of *S. pyogenes* were resistant to CFM, AMP, CAZ, NA and E and sensitive to all antibiotics tested. The standard strains of *E. coli* were sensitive to all antibiotics tested except AMP and NA. The standard strains of *P. aeruginosa* were resistant to CFM, AMP and TE and sensitive to all other antibiotics tested. The standard strains of *S. typhimurium* were sensitive to all antibiotics except AMP and E. The standard strains of *V. cholera* were resistant AMP and intermediate resistant to S and sensitive to all other antibiotics tested. The phytochemical analysis of hexane, chloroform, ethyl acetate and methanol extracts of *S. asperum* had showed the presence of terpenoids, tannins and steroids. Alkaloids and coumarins are not present in all the extracts tested. Steroids were present in all the extracts tested except methanolic extracts. Cardiac glycosides were present in chloroform and acetone extracts. Phenolic compounds were present in chloroform and ethyl acetate extracts.

The different organic solvents of hexane, chloroform, ethyl acetate and methanol extracts of *S. asperum* was tested against MDR bacterial strains. All the extracts of *S. asperum* possessed significant antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. There was no much variation among the standard bacterial strains towards the algal extracts 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 zone of inhibition obtained were between 7.1 and 17.8 mm. Ampicillin (10 µg/disc) antibacterial positive control produced mean zone of inhibition ranged from 7.1 to 17.8 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. asperum* ranged between 62.5 and 500 µg/ml, while the MBC values were between 250 and 1000 µg/ml. The highest mean of zone inhibition (17.8 mm) and lowest MIC (62.5 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extract of *S. asperum* against *B. subtilis*. In the present study, the different solvents *viz.,* hexane, chloroform, ethyl acetate and methanol extracts of *S. asperum* possessed antibacterial activity against standard bacterial strains tested. The ethyl acetate extract of *S. asperum* showed the highest antibacterial activity than other extracts against *B. subtilis* and followed by all bacterial strains tested.

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

The present study different extracts were assayed against the test bacteria by agar diffusion assays, the mean zone of inhibition obtained were between 7.1 and 17.8 mm. Ampicillin (10 µg/disc) antibacterial positive control produced mean zone of inhibition ranged from 7.1 to 17.8 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. asperum* ranged

between 62.5 and 500 µg/ml, while the MBC values were between 250 and 1000 µg/ml. The highest mean of zone inhibition (17.8 mm) and lowest MIC (62.5 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extract of *S. asperum* against *B. subtilis*. Similar results were observed Adaikala Raj *et al.* (2024) evaluated hexane, chloroform, ethyl acetate, and methanol extracts against multidrug-resistant bacteria using the disc diffusion method. The ethyl acetate extract of *Dictyota dichotoma* showed the highest antibacterial activity, with a 26.5 mm inhibition zone and the lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) against *Staphylococcus aureus*. The main chemical components of algal cells are believed to be botanical compounds including phenol, flavonoids, and tannin substances, which, relying on their concentration and setting, may either activate or inhibit the growth of bacteria. The human microbes *S. aureus, S. mutans,* and *E. coli* are prevented from growing by the brown algae *P. australis* (Singkoh et al., 2021).

The brown algae *P. boergesenii* and *C. racemosa* has the ability to inhibit the growth of both gram positive and negative microbes and it shows the highest activity towards. the lowest activity of *C. racemosa* against *P. aeruginosa* 23.6 ± 0.3, *S. aureus* 22.0 ± 0.8 and *P. boergesenii* shows *K. pneumoniae* 18.2± 0.3, *B. subtilis* 19.7 ± 0.2 (Ragunath *et al.,* 2020). Salem *et al*. (2011) reported that higher antibacterial activity was recorded for ethyl acetate extracts of *C. racemosa*, *Sargassum dentifolium, Padina gymnospora* methanolic extracts of *S. hystrix, C. racemosa, C. fragile, S. dentifolium* and *Cystoseria myrica.* Immanuel *et al.* (2004) showed the antibacterial properties of the n-butanolic extracts of four ayurvedic herbals and two seaweeds *Ulva lactuca* and *S. wightii* against shrimp pathogen *Vibrio parahaemolyticus*. Bibiana *et al.* (2012) reported that the highest activity of diethyl ether extract of *S. wightii* and *K. alwarezii* showed a good antibacterial activity against *Streptococcus faecalis, S. pneumoniae, B. subtilis, Bacillus aureus, Vibrio cholera, V. parahaemolyticus E. coli, S. aureus K. pneumoniae, P. aeruginosa, Proteus, Citrobacter, Microsporum gypsum, Aspergillus fumigatus, A niger, A. flavus* and *Trichophyton rubrum*.

Table 1. Antibacterial activity of different extracts of *Spatoglossum asperum*.

S. No.	Microbial strains/ solvents	Mean zone of inhibition[a] (mm)[b]				MIC (µg/mL)	MBC (µg/mL)
		Concentration of the extracts (µg/disc)					
		1000	500	250	Amphicillin		

					(10 µg/disc)		
1	***Staphylococcus aureus***						
	Hexane	10.6±0.15	7.5±0.50	7.6±0.15	9.5 ± 0.42	125	250
	Chloroform	12.9±0.12	10.7±0.12	9.4±0.16	12.3 ± 0.38	125	250
	Ethyl acetate	17.8 ±0.15	13.7±o.o5	11.4±0.20	9.0 ± 0.75	62.5	125
	Methanol	10.6±0.35	9.2±0.20	7.4±0.30	11.0 ± 0.50	125	250
2	***Streptococcus pyogenes***						
	Hexane	9.3±0.50	8.1±0.30	7.6±0.25	9.1 ± 0.57	250	500
	Chloroform	10.5±0.65	9.8±0.25	8.4±0.35	12.6 ± 0.28	250	500
	Ethyl acetate	13.6±0.27	11.6±0.10	10.6±0.26	12.3 ± 0.50	125	125
	Methanol	11.8±0.15	10.5±0.25	9.8±0.30	8.5 ± 0.76	250	500
3	***Bacillus substilis***						
	Hexane	8.6±0.30	7.10±0.15	7.6±0.13	12.0 ± 0.50	250	500
	Chloroform	10.3±0.15	9.6±0.25	8.7±0.25	12.0 ± 0.50	250	500
	Ethyl acetate	16.5±0.15	12.8±0.05	11.1±0.40	9.6 ± 0.76	62.5	125
	Methanol	9.5±0.50	7.3±0.20	6.5±0.28	8.5 ± 0.76	125	250
4	***Escherichia coli***						
	Hexane	10.2±0.30	8.4±0.15	7.3±0.20	12.1 ± 0.28	250	500
	Chloroform	11.7±0.28	10.1±0.21	7.9±0.21	12.8 ± 0.76	250	500
	Ethyl acetate	13.2±0.31	10.9±0.45	8.5±0.35	10.8 ± 0.50	125	125
	Methanol	10.4±0.50	8.7±0.25	7.1±0.20	8.8 ± 0.76	250	500
5	***Proteus vulgaris***						
	Hexane	10.3±0.15	9.5±0.21	7.3±0.05	8.5 ± 0.57	250	500
	Chloroform	11.5±0.15	10.2±0.10	8.1±0.10	8.8 ± 0.28	250	500
	Ethyl acetate	13.6±0.26	10.6±0.12	8.6±0.04	9.8 ± 0.70	125	125
	Methanol	10.7±0.42	9.3±0.13	7.15±0.06	9.5 ± 0.65	250	500

No.	**Microbial strains/ solvents**	**Mean zone of inhibition[a] (mm)[b]**				**MIC (g/mL)**	**MBC (g/mL)**
		Concentration of the extracts (g/disc)					
		1000	**500**	**250**	**Amphicillin**		

					(10 g/disc)		
6	***Pseudomonas aeruginosa***						
	Hexane	9.8±0.12	8.02±0.14	7.0±0.14	12.5 ± 0.76	250	500
	Chloroform	10.9±0.12	9.46±0.09	7.36±0.07	10.0± 0.50	250	500
	Ethyl acetate	13.5±0.09	10.38±0.05	8.08±0.05	11.6 ± 0.76	125	125
	Methanol	9.8±0.32	8.94±0.18	6.9±0.15	12.0 ± 0.5	250	500
7	***Vibrio cholera***						
	Hexane	10.5±0.22	8.8±0.23	7.1±0.05	11.6 ± 0.76	250	500
	Chloroform	11.3±0.05	10.02±0.33	8.16±0.13	12.1± 0.28	250	500
	Ethyl acetate	13.2±0.09	11.6±0.06	9.52±0.18	9.8± 0.57	125	125
	Methanol	10.6±0.13	9.8±0.05	11.9±0.11	11.6± 0.57	250	500
8	***Shigella flexneri***						
	Hexane	11.4±0.09	9.2±0.10	7.1±0.08	13.1± 0.28	250	500
	Chloroform	12.2±0.08	10.06±0.07	8.6±0.11	12.8 ± 0.76	250	500
	Ethyl acetate	14.6±1.94	11.9±0.05	9.1±0.08	11.0 ± 0.5	250	500
	Methanol	11.1±0.04	8.6±0.07	7.8±.0.53	10.5 ± 0.50	500	1000
9	***Proteus mirabilis***						
	Hexane	10.3±0.16	9.36±0.07	7.1±0.04	10.3 ± 0.28	250	500
	Chloroform	12.6±0.07	10.05±0.05	7.5±0.05	9.3 ± 0.57	250	500
	Ethyl acetate	13.5±0.05	11.13±0.05	8.3±0.05	10.8 ± 0.50	250	500
	Methanol	10.07±0.05	9.4±0.10	7.92±0.11	8.8 ± 0.76	250	500
10	***Salmonella typhimurium***						
	Hexane	11.18±0.05	9.64±0.08	7.1±0.05	9.8 ± 0.76	250	500
	Chloroform	12.30±0.32	10.8±0.05	8.6±0.09	9.6 ± 0.76	250	500
	Ethyl acetate	13.6±0.06	12.6±0.07	9.1±0.13	10.3 ±0.28	125	125
	Methanol	11.02±0.09	9.18±0.08	8.72±0.21	12.0 ± 0.86	250	500

[a]Diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]Mean of three assays; ± - Standard deviation;[*]Significant at $P<0.05$.

In this study, ethyl acetate extracts of *S. asperum* showed antibacterial activity against *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholera, S. flexneri, P. mirabilis,* and *P. vulgaris.* Rangaiah *et al* (2010) reported that the aqueous extract of marine brown alga *S. ilicifolium* showed higher antibacterial activity against the pathogenic bacteria such as *P. aeruginosa, K. pneumoniae, S. aureus* and *B. subtilis.* They also reported higher antibacterial activity in the marine brown algae *P. tetrastromatica* against the following species of *B. subtilis, S. aureus* and *K. pneumoniae*. The present study shows lesser inhibitory activity against the above bacterial species, so this study was contrasted to Rangaiah *et al* (2010) reported more activity in the methanolic extract of marine brown algae *S.ilicifolium* and *P. tetrastromatica* against *B. subtilis, K. pneumonia, S. aureus, B. subtilis* and *S. aureus*. The present study was contrasted to Xavier *et al* (2012) reported the methanolic extract of marine brown algae *P. gymnospora* and *S. wightii* showed no activity against the bacterial species of *B. subtilis, S. aureus, K. pneumonia, P. aeruginosa* and *S. typhi.*

In this study, different extracts of *S. asperum* showed highest mean zone of inhibition (16.7 mm) and the lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in ethyl acetate extracts of *S. asperum against B. subtilis.* Kim and Lee (2008) used methanolic extracts of *Esiena bicyclis* (B32) and *Sargassum* sp. (B36) which showed strong antibacterial activity against Methicillin-resistant *S. aureus* (MRSA) strains, *Vibrio parahemolyticus*. Kolanjinathan and Stella (2009) indicated that acetone was the best solution for extracting the effective antimicrobial materials from *S. myricystum*, *Turbinaria conoides*, *Hypnea musiformis*, *G. edulis* and *Halimedia gracilis*. Fatty acid methyl ester extracts of *S. longifolium* and *S. teretifolium* showed antibacterial activity against *B. subtilis, S. aureus, Micrococcus luteus, S. typhimurium, P. aeruginosa, K. pneumoniae* and *E. coli* (Anantharaj *et al.,* 2005). Tuney *et al.* (2006) reported the antibacterial activity of the methanolic extracts of *Ulva rigida*, *Enteromorpha Linza*, *Padina pavonica*, *Cystoseria sniosa*, *Dictyopteris linearis*, *D. membranacea*, *C. mediterranea*, *E siliculous*, *Ceramium rubrum*, *G. gracilis Acanthophora nojadiformis* inhibited the growth *E. coli*. Kolanjinathan and Stella, (2002) and Mansuya *et al.* (2010) reported that the aqueous and methanolic extract of all selected algal species were screened and showed antibacterial activity against *E. coli, P. aeruginosa, S. typhi, Staphylococcus epidermis* and *S. pyogenes.*

Manilal *et al.* (2009) and Rangaiah *et al.* (2010) showed that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate which in contrast to our results. However, the result obtain by the aforementioned author suggest by the same species varies remarkable difference may be due to several factors. The variation in antibacterial activity may be due to the method of extraction, solvents used in extraction and season at which samples were collected.

The results of the present study revealed that Gram positive bacteria were more susceptible than Gram negative bacteria. Gram positive bacteria were more effectively controlled by the extracts of algae used in their study compared to Gram negative bacteria. Taskin and Ozturk Kurt (2001), revealed similar observations, indicating that the more susceptibility of Gram-positive bacteria to the algal extract was due to the differences in their cell wall structure and their composition. In Gram negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics (Tortora *et al.,* 2001). The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors (Martin, 1995). Since ethyl acetate extract of *S. asperum* showed potential antibacterial activity against all the MDR bacterial strains tested. Moreover, it was indicated the potential source of a variety of biologically active marine organisms and it is hope that the present results will provide a starting point for investigations aimed at exploiting new natural antibacterial substances present in the *S.asperum.*

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CONFLICTS OF INTEREST: The authors declare to competing interest.

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