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Antimicrobial activity of various extracts of *Sargassum glaucescens* on the antibiotic resistant organisms

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Abstract

The antimicrobial activity of brown alga methanol, ethyl acetate, hexane, and chloroform extracts on gram positive and gram negative bacteria, and fungi was evaluated by using nutrient broth macro dilution test. *Sargassum glaucescense* was collected around the coastal area of Chabahar (south of Iran) the protected marine area of the Oman Sea in April and May 2015. Six pathogenic organisms including; *Enterococcus faecium* ATCC 51299, *Streptococcus mutans* ATCC 35668, *Shigella boydii* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Salmonella enteritidi* PTCC, 1709, *Candida albicans* ATCC 10231 and *Aspergillus fumigatus* PTCC 5009 were investigated by the broth dilution method. Methanolic extract of six strains showed good activity amongst eight strains. Hexane extract, after methanolic extract has a good effect on the antimicrobial activity against five strains. All bacterial strains in this survey showed resistance against ethyl acetate and chloroformic extracts. *S. glaucescens* using four various solutions extracts against eight different human pathogens showed an important antimicrobial and antifungal activity. However, more investigation has to be done on separation, purification and detection of the active ingredients in order to recognize their antifungal and antifungal activity.

Keywords: Antimicrobial activity, *Sargassum glaucescens*, Bacteria, Fungi, Oman Sea.

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Introduction

During the last decade, drug therapeutic failure happened due to the inappropriate and extensive use of therapeutic agents (Franceschi *et al*., 2004). In some areas, uninformed use of antibiotics and food additives in livestock animals, poultry and household cleaners caused spontaneous mutations and selection pressure in organisms that led to the creation of resistant isolates (Amin *et al*., 2012; Fallah *et al*., 2013; Samanta *et al*., 2014). Antibiotic resistance can occur both genetically and environmentally. Genetic resistance spreads both "vertically," when resistance elements are inherited, and "horizontally," when genetic material is transferred to other bacteria (Wright *et al*., 2007). Environmentally, antibiotic resistance spread occurs when microorganisms are transferred from place to place by airplanes, water and wind. Centers for Disease Control and Prevention (CDC) has assessed that at least two million illnesses and 23,000 deaths occur by antibiotic resistant organisms yearly in the USA (Heuer *et al*., 2011; Blair *et al*., 2015). There is a continuous and crucial need to discover new antimicrobial compounds with varied novel mechanisms of action and chemical structures because there has been an alarming increase in the occurrence of new and re-emerging infectious illnesses, as well as the increasing development of resistance to the antibiotics in current clinical use (Bhagavathy *et al*., 2011; Moellering, 2011). Therefore, actions must be taken to control the use of antibiotics, to better comprehend the genetic mechanisms of resistance and to continue studies on developing new drugs (Bhagavathy *et al*., 2011; Sasidharan *et al*., 2011; Savoia, 2012). The use of plant extracts and phytochemicals, both with known antimicrobial effects, can be of great significance in therapeutic treatments and control of the infections caused by the multidrug-resistance (MDR) strains (Ahmad and Beg, 2001; Nascimento *et al*., 2000). In the recent years, several studies have been directed in different countries to demonstrate such efficiency (Nascimento *et al*., 2000; Aqil *et al*., 2006; Betoni *et al*., 2006; Ahmad and Aqil, 2007; Joshi *et al*., 2011). Many plants have been used because of their antimicrobial characters, which are due to compounds synthesized in the secondary metabolism of the plant (Aqil *et al*., 2006; Betoni *et al*., 2006; Ahmad and Aqil, 2007; Joshi *et al*., 2011). These products are known by their active materials, for instance, the phenolic compounds which are part of the vital oils (Djeridane *et al*., 2006), as well as in tannin (Hoste *et al*., 2006). Algae as a source of valuable biological diversity has a lot of applications such as food, textile, paint, photography, cosmetic, medical, pharmacy,

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dental and microbiological media preparation (Cannell, 1993). They can be categorized as three groups, rhodophyta (red algae), phaeophyta (brown algae) and chlorophyta (green algae) according to their nutrient and chemical structure (Davis *et al.*, 2003). In addition, algae have valuable polysaccharides such as agar, carrageenan and alginate that can be used in economic industries (Pulz and Gross, 2004). A chemical study demonstrated compounds such as phenol, tannin, saponin, flavin and steroid in the algae structure (Kumar *et al*., 2015). *Laminaria* and *Sargassum* are two main types of the brown algae (Teas, 1892). *Sargassum glaucescens* (*S. glaucescens*) is one of the most important brown algae species from the Oman Sea especially in the Port of Chabahar in the Sistan and Baluchestan Province of Iran (Esmaeili *et al*., 2015). *S. glaucescens* has high maximum growth in late autumn-early winter (May-Lin and Ching-Lee, 2013). There are many reports on the antibacterial activity of *S. glaucescens* extract against aquatic bacteria, but little evidence was accessible for human pathogens (Ghaednia *et al*., 2011). The aim of the present study was to determine the antibacterial and antifungal activities of methanol, ethyl acetate, hexane, and chloroform extracts of *S. glaucescens*.

Materials and methods

Sampling algae and preparation of the plant extract

Brown algae, *S. glaucescense* was collected around the coastal area of Chabahar (in the south of Iran), the protected marine area of the Oman Sea in April and May 2015. All samples were transferred to the laboratory and washed by distilled water in order to separate sand and epiphytic organisms. Then, the algae was air-dried in the shade, at 25˚C, and ground to powder with a mortar and pestle. One hundred and fifty grams of each sample were successively extracted by mixing with 800 ml methanol, ethyl acetate, hexane, and chloroform at room temperature. Each extract was filtrated and the residue re-extracted. All filtrates were collected to be dried by evaporating under vacuum and re-dissolved in respective methanol, ethyl acetate, hexane, and chloroform.

Testing microorganisms

Antibacterial and antifungal activities of different algal extracts against six pathogenic bacteria (*Enterococcus faecium* ATCC 51299, *Streptococcus mutans* ATCC 3566, *Shigella boydii* ATCC25923, *Pseudomonas aeroginosa* ATCC27853, *Klebsiella pneumoniae* ATCC 13883 and *Salmonella enteritidis* PTCC1709) and two pathogenic fungi (*Candida albicans* ATCC10231, *Aspergillus fumigatus* PTCC5009) were investigated by the broth dilution

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method (Payghami *et al*., 2014). All isolates were obtained from the department of microbiology Tehran University of medical sciences, (Tehran, IR Iran).

Broth macrodilution test bacterial strain

The MIC values of the different extracts were determined using the broth dilution test as defined by Borah *et al*. (2013). The initial concentration (50 mg ml^{-1}) of the different algae extracts was diluted using double fold serial dilution by transferring 2.5 ml of the sterile different algae extracts stock solutions into 2.5 ml of sterile Mueller Hinton broth (Merck Co., Germany) to obtain a 25 mg ml^{-1} concentration. The above procedure was repeated several times to get other dilutions: 25 mg ml^{-1} (1:2), 12.5 mg ml^{-1} (1:4), 6.25 mg ml^{-1} (1:8), 3.12 mg ml^{-1} (1:16), 1.56 mg ml^{-1} (1:32), 0.8 mg ml^{-1} (1:64), 0.4 mg ml^{-1} (1:128), 0.2 mg ml^{-1} (1:256), 0.1 mg ml^{-1} (1:512), 0.05 mg ml^{-1} (1:1024) and finally 0.025 mg ml^{-1} (1:2048). In order to prepare different concentrations of extracts, each concentration was inoculated with 0.1ml of the standardized bacterial cell suspensions (0.5 Mc Farland) of bacteria in separate sets of tubes and incubation was done at 370 ^{0}C for 24 h. The lowest concentration of the different algae extracts that inhibits growth of the organisms, as detected by the lack of visual turbidity, was designated as the minimum inhibitory concentration (MIC). Two quality control test tubes were maintained for each test batch that included an antimicrobial control (the growth medium without inoculum and tube containing extract) and organism control (the inoculum and the tube containing the growth medium). The lowest concentration of the extract that completely inhibited bacterial growth (no turbidity) in comparison to the positive growth control test was observed as MIC. Gentamycin (0.62-5 mg ml^{-1}) was used as drug quality control for microorganisms assay.

For minimum bactericidal concentration (MBC) assessment of different extracts, 0.1ml of each tube content was cultured on the Mueller-Hinton agar plates. After incubation at 370 ^{0}C for 24 h, colony count was completed and compared to the number of colony forming units CFU ml^{-1} in the original inoculums. The lowest concentration of extracts that allowed less than 0.1% of the original inoculums to survive (i.e., 99% killing of bacterial isolates) was determined by MBC.

Fungal strain

Candida albicans

The MIC values were assessed by the visual broth macrodilution method. Fungal suspensions were diluted into RPMI-1640 medium without bicarbonate (pH 7.0 with 0.165 morpholine propane sulfonic acid) broth supplemented with

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glutamine, to a concentration of approximately 0.5×105 CFU ml^{-1}, verified by colony count in SDA. A two fold serial dilution of 0.2 ml each of different algae extracts was added to 1.8 ml of the RPMI-1640 medium. The concentrations were 50-0.025 mg ml^{-1}. No antifungal samples were used in the Control group. To compare the results with standard, fluconazole (0.016 to 256 mg ml^{-1}) was used. Tubes were defined as the lowest concentration which did not yield visual growth and MFC were determined as the lowest concentration of agent resulting in no growth.

Aspergillus fumigatus

The activity of different algae extracts against *A. fumigatus* was determined by the broth macrodilution method (Arikan *et al*., 2001). Dilutions of extracts (50, 25, 12.5, 6.25, 3.12, 1.56, 0.8, 0.4, 0.2, 0.1, 0.05 and 0.025 mg ml^{-1}) were prepared in RPMI 1640 Medium in 2.5 ml volumes in test tubes. 2.5 ml *A. fumigatus* with turbidity of 2.5×103 CFU ml^{-1} was added to each test tube. After 48h incubation, MIC and MFC were determined. MIC values were determined as the lowest concentration of agent resulting in the maintenance or reduction of the inoculum and MFC were determined as the lowest concentration of agent resulting in no growth and then compared with the results of itraconazole (0.002 to 32 mg ml^{-1}) (Alizadeh *et al.*, 2014).

HPLC analysis

Methanolic and hexane extracts of *S. glaucescens,* due to high antimicrobial and cytotoxic effects, were respectively selected for HPLC analysis. These extracts of *S. glaucescens* were centrifuged at ×3000 rpm for 12 min and then filtered by Whatmann No.1 filter paper using high pressure vacuum pump. The specimen is diluted to 1:10 with the same solvents. HPLC method was done on a SHIMADZU LC-10AT VP HPLC system (Shimadzu, Kyoto, Japan), equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10AT. A Hypersil BDS C-18 column (4.6× 250 mm, 5 µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 ml min^{-1} at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µL while the wavelength of the UV-Vis detector was set at 365 nm (Brkljaca and Urban, 2014).

Results

Four different extracts were evaluated against two gram-positive, four gram-negative and two fungi species. Some extracts had a significant activity for gram-positive bacteria but not on gram negative bacteria.Methanolic extract for six strains showed good activity

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amongst eight strains. Hexane extract, after methanolic extract had good effect on antimicrobial activity against five strains. All bacterial strains in this survey showed resistance against ethyl acetate and chloroformic extracts. *K. pneumoniae* ATCC 13883 and *S. enteritidis* PTCC 1709 were also resistant to all extracts. Furthermore, only methanolic extract had antibacterial activity against *P. aeroginosa* ATCC 27853. Methanolic extract showed an MIC of 1.56 mg ml^{-1} for gram-positive bacteria, while for gram-negative bacteria it showed an MIC of 12.5 mg ml^{-1} (Table 1). Two fungal *C. albicans* ATCC 10231 and *A. fumigatus* PTCC 5009 strains had a good response to all extracts, although *A. fumigatus* PTCC 5009 in comparison with *Candida albicans* ATCC 10231 had higher MIC and MFC for all extracts. Ethyl acetate extract had the lowest MIC (0.4 and 1.56 mg ml^{-1}) and MBC (0.8 and 3.12 mg ml^{-1}) for *C. albicans* ATCC10231 and *A. fumigatus* PTCC5009 (Table 2). Our findings showed that methanolic extract had a superior effect among four extracts. All strains indicated that they had an MIC range for the quality of control drugs.

Table 1: MIC and MBC (mg ml^{-1}) of various extracts *of Sargassum glaucescens* and antibiotics.

Antimicrobials			*Microbial isolation*					
			Enterococcus faecium **ATCC 51299**	***Streptococcus mutans*** **ATCC 35668**	***Shigella boydii*** **ATCC25923**	***Pseudomonas aeroginosa*** **ATCC27853**	***Klebsiella pneumoniae*** **ATCC 13883**	***Salmonella enteritidis*** **PTCC1709**
Plant extracts	Chloroformic Extract	MIC	-	-	-	-	-	-
		MBC	-	-	-	-	-	-
	Methanolic Extract	MIC	1.56	1.56	12.5	12.5	-	-
		MBC	3.12	3.12	25	25	-	-
	Hexane Extract	MIC	6.25	6.25	-	-	-	-
		MBC	12.5	12.5	-	-	-	-
	Ethyl acetate Extract	MIC	-	-	-	-	-	-
		MBC	-	-	-	-	-	-
Antibiotics	Piperacillin/ Tazobactam	MIC	-	-	2.1	32	4	2
		MBC	-	-	3.12	68	8	4.1
	Gentamycin	MIC	0.25	0.125	-	-	-	-
		MBC	2	2.15	-	-	-	-

Table 2: MIC and MFC (mg ml^{-1}) of various *Sargassum glaucescens* extracts and antifungals.

Fungal isolation	Plant extracts								Antifungals			
	Chloroformic Extract		Methanolic Extract		Hexane Extract		Ethyl acetate Extract		Fluconazole		Itraconazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Candida albicans **ATCC10231**	12.5	25	6.25	12.5	6.25	12.5	0.4	0.8	0.64	0.5	-	-
Aspergillus fumigatus **PTCC5009**	25	50	12.5	25	12.5	25	1.56	3.12	-	-	0.32	0.64

The qualitative HPLC fingerprint profile of Hexane extracts of *S. glaucescens* were selected at a wavelength of 365 nm due to sharpness of the peaks and proper baseline. Hexane extract prepared by cold extraction was subjected to HPLC for the isolation and identification of constituents present in the *S. glaucescens*. Four

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compounds were separated at different retention time viz., 6.636, 8.818, 9.167 and 11.267 respectively. The profile displayed one prominent peak at a retention time of 8.818 min and some moderate peaks were also observed at a retention time of 11.267 min, and 9.167 min respectively (Fig. 1). Methanolic extracts of *S. glaucescens* were illustrated with three compounds with the retention time of 16.193, 9.535 and 6.791 min respectively. The profile displayed one prominent peak at a retention time of 16.193 min (Fig. 2).

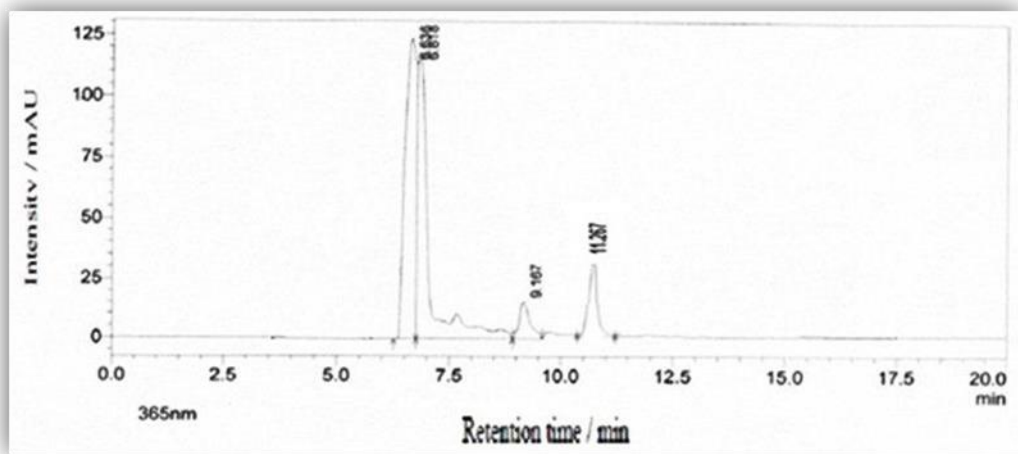

Figure 1: HPLC chromatogram of the Hexane extract of *Sargassum glaucescens.*

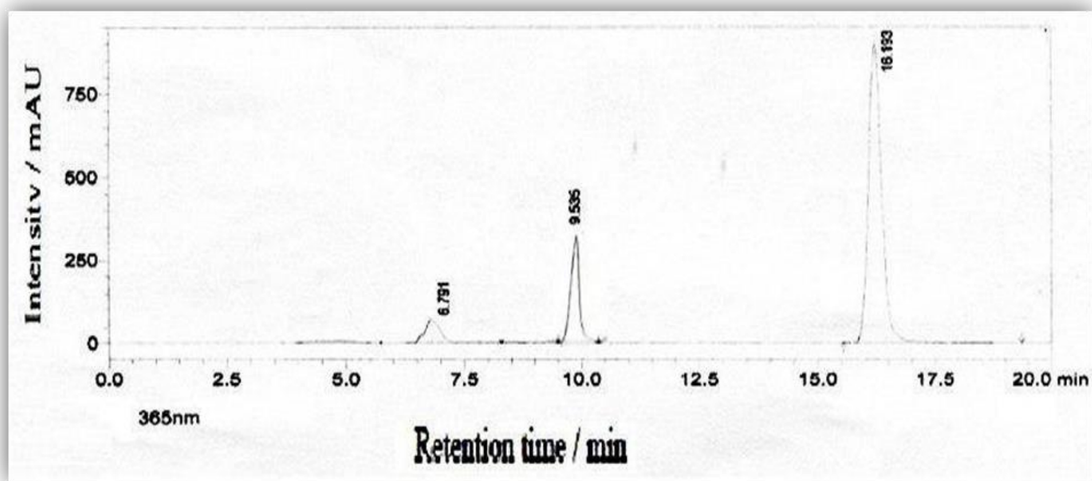

Figure 2: HPLC chromatogram of the Methanolic extract of *Sargassum glaucescens.*

Discussion

Sargassum species (phaeophyceae) are economically significant brown algae in Sistan O Baloochestan coastline, southern parts of Iran. Marine algae produce a wide range of new secondary metabolites with numerous biological activities (Noormohammadi *et al*., 2011). The previous study proved that the *Sargassum* species were the best sources for components like polysaccharides, flavonoids, tannins, bromophenols, carotenoids and phenolic acids which display different biological activities (14-17). Nowadays, various chemically unique compounds sourced from *Sargassum* species with different biological activities have been identified and some of them are under examination and are being used to improve novel pharmaceuticals (García-Ríos *et al*., 2012; Michalak and Chojnacka, 2015). The different cell extracts and active components of several brown algae have been demonstrated to have an in vitro antibacterial (Ibtissam *et al*., 2009), antifungal (Moreau *et al*., 1988) and antiviral activity (Barbosa *et al*., 2004). We evaluated antibacterial and antifungal activities of four extracts of *S. glaucescens* against eight strains using macrodilution broth. Rare data existed from broth dilution of antimicrobial effect of *S. glaucescens* extract against pathogenic microorganism. However, *Turbinaria ornata* and *Sargassum wightii* are two brown algae that have shown good activities against nine microbial pathogens such as *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Shigella flexneri, Aeromonas hydrophila* and *Proteus vulgaris* (Vijayabaskar and Shiyamala, 2011). Out of eight strains, 6 strains were susceptible to methanol extract. Our results indicated gram negative bacteria in comparison with gram positive were more resistant to all extracts, because gram-positive bacteria have more peptidoglycan in their cell wall structure while gram-negative bacteria have only a thin layer of peptidoglycan and most of their cell structure is lipoprotein and lip-polysaccharides (Tassou and Nychas, 1995; Ghalem and Mohamed, 2008). Brown algae show different antimicrobial activities because these activities depend on their solubility and polarity in different solvents (Salama and Marraiki, 2010). The methanol extract of *S. glaucescens* exhibited the strongest antibacterial activity against microorganism. This property is due to the presence of phenolic, alkaloids and amino acids in methanolic *S. glaucescens* extract which may be responsible for the antimicrobial and antifungal activity (Cox *et al.*, 2010; Srivastava *et al*., 2010). Mahianeh et al reported that *Vibrio harveyi* was resistant to extract n-hexane of *S. glaucescens*, Sub-critical to methanol and chloroform extract and sensitive to extract ethanol, but *S. aureus* was sensitive to extracts n-hexane, chloroform, methanol and ethanol, Also *B. cereus* was sensitive to extract methanol and ethanol (Mahianeh *et al*., 2014). They indicated that ethanol extracts of *S. glaucescens* possess the highest antibacterial activity against all microorganisms. These results were consistent with our results. Plaza *et al*. (2008) reported that the methanol extract of a species of *Sargassum* has an antibacterial activity against both gram positive and negative bacteria (Plaza *et al*., 2008). Both *Enterococcus faecium* ATCC 51299 and *Streptococcus mutans* ATCC 35668 were found to be susceptible to the methanolic and Hexane extract of *S. glaucescens* at both the concentrations 1.56 mg ml^{-1}. MIC value of the methanolic extract for *Shigella boydii* ATCC25923 and *Pseudomonas aeruginosa* ATCC27853 was 12.5 mg ml^{-1}. According to table 2, the ethyl acetate extracts of *S. glaucescens* have high effect against two fungal strains with MIC 0.4 mg ml^{-1} and 1.56 mg ml^{-1}. The two fungal strains were susceptible to all extracts of *S. glaucescens*, however *Klebsiella pneumoniae* ATCC 13883 and *Salmonella enteritidis* PTCC1709 were resistant to all solution extracts. The previous studies demonstrated that ethanol extract of seaweed species of *S. lanceolatum*, *S. ilicifolium* and *S. tenerrimum* has good effect against root infecting fungi (Ambreen *et al*., 2012). Bhaskar *et al*. (2005) found antibacterial activity of brown algae of *Padinatetra tomatica* (46). Our results similar to Manilal *et al*. (2009) and Rangaiah *et al*. (2010) clarified that methanol extraction yielded higher antimicrobial and antifungal activity than n-hexane and ethyl acetate (Manilal *et al*., 2009; Rangaiaha *et al*., 2010). Methanol extract of *S. polycystum* similar to *S. glaucescens* showed more activity against bacterial and fungal strains (Kausalya and Narasimha, 2015). HPLC identification test is required to confirm the presence of the active components and molecular weights of the Methanolic and Hexanolic extracts of *S. glaucescens*. In the present study and in line with Marimuthu *et al*. (2012) the HPLC profile for *S. glaucescens* exhibited novel markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of impurity in ayurvedic drugs such as medicinal plant extracts (Marimuthu *et al*., 2012). The antibacterial and antifungal activity of the algae extract can be attributed to the various phytochemicals present in the *S. glaucescens.* Alkaloids, saponins and flavonoids component are found to be related with antimicrobial effects in different studies using algae extracts. Results of the present study demonstrated that methanol extract of *S. glaucesens* can be used as an alternative to antibiotics in gentamycin and fluconazole which is now largely used in human pathogenic infections.

The results of the present study on *S. glaucescens* using four various solution extracts against eight different human pathogens showed an important antimicrobial and antifungal activity. However, more investigation has to be done on separation, purification and detection of the active ingredients in order to recognize their antifungal and antifungal activity.

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References

Ahmad, I. and Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*, 74(**2**), 113-23.

Ahmad, I. and Aqil, F., 2007. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiological Research*, 162(**3**), 264-75.

Alizadeh, H., Rahnema, M., Semnani, S.N., Ajalli, M. and Branch, Z., 2014. Synergistic antifungal effects of quince leaf's extracts and silver nanoparticles on *Aspergillus niger*. *Journal of Applied Biological Sciences*, 8(**3**), 10-3.

Amin, A., Irfanullah, M., Hameed, A., Andaleeb, S. and Ayaz Khan, M., 2012. Determination of antibiotic resistance pattern in *Escherichia coli* strains isolated from animal faeces in a farm house. *Iranian Journal of Veterinary Research*, 13(**3**), 250-4.

Aqil, F., Ahmad, I. and Owais, M., 2006. Evaluation of anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity and synergy of some bioactive plant extracts. *Biotechnology Journal*, 1(**10**), 1093-102.

Ambreen, A., Hira, K., Ruqqia, A. and Sultana, V., 2012. Evaluation of biochemical component and antimicrobial activity of some seaweeeds occurring at Karachi coast. *Pakistan Journal of Botany*, 44(**5**), 1799-803.

Arikan, S., Lozano-Chiu, M., Paetznick, V. and Rex, J.H., 2001. In vitro susceptibility testing methods for caspofungin against *Aspergillus* and *Fusarium* isolates. *Antimicrob. Agents Chemother*, 45, 327-330

Barbosa, J.P., Pereira, R.C., Abrantes, J.L., dos Santos, C.C.C., Rebello, M.A., Frugulhetti, I.C.D.P.P. and Teixeira, V.L., 2004. *In vitro* antiviral diterpenes from the Brazilian brown alga Dictyota pfaffii. *Planta medica*, 70(**09**), 856-860.

Betoni, J.E.C., Mantovani, R.P., Barbosa, L.N. and Di Stasi, L.C., 2006. Fernandes Junior A. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Memorias do Instituto Oswaldo Cruz*, 101(**4**), 387-90.

Bhagavathy, S., Sumathi, P. and Bell, I.J.S., 2011. Green algae Chlorococcum humicola-a new source of bioactive compounds with antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine*, 1(**1**), S1-S7.

Bhaskar, PV., Grossart, HP., Bhosle, NB. and Simon, M. 2005. Production of macroaggregates from dissolved exopolymeric substances (EPS) of bacterial and diatom origin. *Fems Microbiology Ecology*, 53, 255-264.

Blair, J.M., Webber, M.A., Baylay, A.J,, Ogbolu, D.O. and Piddock, L.J., 2015. Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(**1**), 42-51.

Brkljaca, R. and Urban, S., 2014. Chemical profiling (HPLC-NMR & HPLC-MS), isolation, and identification of bioactive meroditerpenoids from the southern Australian marine brown alga *Sargassum paradoxum*. *Marine drugs,* 13**,** 102-127.

Cannell, R.J., 1993. Algae as a source of biologically active products. *Pesticide Science*, 39(**2**), 147-53.

Cox, S., Abu-Ghannam, N. and Gupta, S., 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*, 17, 205-220.

Das, S.W.A.R.N.A.M.O.N.I., Borah, M.U.K.U.N.D.A.M. and Ahmed, S.H.A.G.U.F.A., 2013. Antibacterial activity of the ethanolic extract of leaves of *Citrus maxima* (Burm.) Merr. on *Escherichia coli* and *Pseudomonas aeruginosa*. *Asian Journal of Pharmaceutical and Clinical Research,* 6(**Suppl 4**), 136-139.

Davis, T.A., Volesky, B. and Mucci, A., 2003. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*, 37(**18**), 4311-30.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N., 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97(**4**), 654-60.

Esmaeili, A., Saremnia, B. and Kalantari, M., 2015. Removal of mercury (II) from aqueous solutions by biosorption on the biomass of Sargassum glaucescens and Gracilaria corticata. *Arabian Journal of Chemistry*, 8(**4**), 506-11.

Fallah, S.H., Asgharpour, F., Naderian, Z. and Moulana, Z., 2013. Isolation and determination of antibiotic resistance patterns in non-typhoid *Salmonella* spp isolated from chicken. *International Journal of Enteric Pathogens*, 1(**1**), 17-21.

Franceschi, A., Tuccori, M., Bocci, G., Vannozzi, F., Di Paolo, A., Barbara, C., Lastella, M., Blandizzi, C. and Del Tacca, M., 2004. Drug therapeutic failures in emergency department patients: a university hospital experience. *Pharmacological Research*, 49(**1**), 85-91.

García-Ríos, V., Ríos-Leal, E., Robledo, D. and Freile-Pelegrin, Y., 2012. Polysaccharides composition from tropical brown seaweeds.

Phycological Research, 60(**4**), 305-15.

Ghaednia, B., Mehrabi, M.R., Mirbakhsh, M., Yeganeh, V., Hoseinkhezri, P., Garibi, G. and Ghaffar Jabbari, A., 2011. Effect of hot-water extract of brown seaweed Sargassum glaucescens via immersion route on immune responses of Fenneropenaeus indicus. *Iranian Journal of Fisheries Sciences*, 10(**4**), 616-630.

Ghalem, B.R. and Mohamed, B., 2008. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. *African Journal of Pharmacy and pharmacology*, 2(**10**), 211-5.

Heuer, H., Schmitt, H. and Smalla, K., 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology*, 14(**3**), 236-43.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M. and Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology*, 22(**6**), 253-61.

Ibtissam, C., Hassane, R., Jose, M., Francisco, D.S.J., Antonio, G.V.J., Hassan, B. and Mohamed, K., 2009. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. *African Journal of Biotechnology*, 8(**7**).

Joshi, B., Sah, G.P., Basnet, B.B., Bhatt, M.R., Sharma, D., Subedi, K., Janardhan, P. and Malla, R., 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *Journal of Microbiology and Antimicrobials*, 3(**1**), 1-7.

Kausalya, M. and Narasimha, G.M., 2015. Antimicrobial activity of marine algae. *Journal of Algal Biomass Utilization*, 6(**1**), 78- 87.

Kumar, V., Murugesan, S. and Bhuvaneswari, S., 2015. Phytochemical analysis of red alga *Champia parvula* (C. Agardh) collected from Mandapam coast of Tamil Nadu, India. *International Journal of Advances in Pharmaceutics*, 4(**3**), 15-20.

Mahianeh, A., Ghaednia, B., Mirbakhsh, M., Velayatzadeh, M., Mohammadi, E. and Jafari, M., 2014. The effects of brown alga, *Sargassum glaucescensas* (Agardeh, 1948) against selected bacterial, fungal and yeast pathogens of shrimp. *International Journal of Biosciences* (IJB), 5(**12**), 399-405.

Manilal, A., Sujith, S., Selvin, J., Shakir, C. and Seghal Kiran, G., 2009. Antibacterial activity of *Falkenbergia hillebrandii* (Born) from the Indian coast against human pathogens. Phyton (Buenos Aires). 161 P.

Marimuthu, J., Essakimuthu, P., Narayanan, J., Anantham, B., Tharmaraj, R.J.J.M. and Arumugam, S., 2012. Phytochemical characterization of brown seaweed *Sargassum wightii*. *Asian Pacific Journal of Tropical Disease*, 2, S109-S13.

May-Lin, B.Y. and Ching-Lee, W., 2013. Seasonal growth rate of Sargassum species at Teluk Kemang, Port Dickson, Malaysia. *Journal of Applied Phycology*, 25(**3**), 805-14.

Michalak, I. and Chojnacka, K., 2015. Algae as production systems of bioactive compounds. *Engineering in Life Sciences*, 15(**2**), 160-76.

Moellering, R.C., 2011. Discovering new antimicrobial agents. *International Journal of Antimicrobial Agents*, 37(**1**), 2-9.

Moreau, J., Pesando, D., Bernard, P., Caram, B. and Pionnat, J., 1988. Seasonal variations in the production of antifungal substances by some dictyotales (brown algae) from the French Mediterranean coast. *Hydrobiologia*, 162(**2**), 157-62.

Nascimento, G.G., Locatelli, J., Freitas, P.C. and Silva, G.L., 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(**4**), 247-56.

Noormohammadi, Z., Ghasemzadeh Baraki, S., Sheidai, M., Rafiee, F. and Gharanjik, B.M., 2011. Morphological diversity of Sargassum species of Iran. *Gene Conserve*, 10(**39**), 1-22.

Payghami, N., Jamili, S., Rustaiyan, A., Saeidnia, S., Nikan, M. and Gohari, A.R., 2014. Alpha-amylase inhibitory activity and sterol composition of the marine algae, Sargassum glaucescens. *Pharmacognosy Research*, 7(**4**), 314.

Plaza, M., Cifuentes, A. and Ibáñez, E., 2008. In the search of new functional food ingredients from algae. *Trends in Food Science and Technology*, 19(**1**), 31-9.

Pulz, O. and Gross, W., 2004. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, 65(**6**), 635-48.

Rangaiaha, G.S., Lakshmi, P. and Sruthikeerthia, K., 2010. The antimicrobial activity of the crude extracts of Chlorophycean seaweeds *Ulva*, *Caulerpa* and *Spongomorpha* spp. against clinical and phytopathogens. *Drug Invent Today*, 2, 311-314.

Salama, H.M. and Marraiki, N., 2010. Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L.(Polygonaceae), naturally growing in Egypt. *Saudi Journal* *of Biological Sciences*, 17(**1**), 57-63.

Samanta, I., Joardar, S.N., Das, P.K., Sar, T.K., Bandyopadhyay, S., Dutta, T.K. and Sarkar, U., 2014. Prevalence and antibiotic resistance profiles of Salmonella serotypes isolated from backyard poultry flocks in West Bengal, India. *Journal of Applied Poultry Research*, 23(**3**), 536-545.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Latha, L.Y., 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(**1**).

Savoia, D., 2012. Plant-derived antimicrobial compounds: Alternatives to antibiotics. *Future Microbiology*, 7(**8**), 979-90.

Srivastava, N., Saurav, K., Mohanasrinivasan, V., Kannabiran, K. and Singh, M., 2010. Antibacterial potential of macroalgae collected from the Madappam Coast, India. *British Journal of Pharmacology and Toxicology*, 1(**2**), 72-6.

Tassou, C. and Nychas, G., 1995. Antimicrobial activity of the essential oil of Mastic fum on gram positive and gram negative bacteria in broth and model food systems. *International Biodeterioration and Biodegradation*, 36, 411-20.

Teas, J., 1982. The dietary intake of Laminaria, a brown seaweed, and breast cancer prevention. *Nutrition and Cancer*, 4(**3**), 217-22.

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

Wright, G.D., 2007. The antibiotic resistome: The nexus of chemical and genetic diversity. *Nature Reviews Microbiology*, 5(**3**), 175-86.

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