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Screening of Antibacterial and Antifungal Activity of Freshwater and Marine Algae as a Prominent Natural Antibiotic Available in Bangladesh

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ABSTRACT

In Bangladesh, algae have not been adequately explored for their potential as a source of bioactive substances. So, present work provides the eligibility of algae commonly found in Bangladesh as a prominent natural antibiotic against various pathogens. *In vitro* screening of organic solvent extracts (methanol, ethanol and chloroform) of ten fresh water and marine algae showed antimicrobial activity carried out by disc diffusion method against two gram positive, four gram negative bacteria and one fungus. Marine algal species performed better than fresh water algal species. Green algae are more potent than red and brown algae. Ethanolic extracts are more effective than methanolic and chloroform extracts. *Ulva lactuca* and *Chlorella* sp. revealed the best activity among other algal species in all solvent forms. *Spirogyra crassa* showed very poor antibacterial activity where its antifungal activity was moderate. *Escherichia coli* was more resistant bacteria in a comparison to others because it showed totally resistant against some algae. Highest zone of inhibition (26 mm) was recorded for chloroform extract of *Ulva lactuca* against *E. coli* where chloroform extract of *Dictyopteris membranacea* didn't show any microbial activity. At the end, it was clear that almost all extracts of all algal species revealed antimicrobial activity against all pathogens. These results give an indication of the presence of promising antimicrobial compounds in the algal species under studied. Further phytochemical studies are needed to elucidate these compounds structures and activity for use algae as an alternative natural antibiotic against synthetic conventional antibiotics.

Key words: Algae, antimicrobial activity, disc diffusion, zone of inhibition, resistant

INTRODUCTION

Infectious diseases are one of the main causes of high morbidity and mortality in human beings around the world, especially in developing countries (Waldvogel, 2004). The severity of the diseases have increased in recent years significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. Antibiotic resistance in bacteria and fungi is one of the major emerging health care

related problems in the world, it became a greater problem of giving treatment against resistant pathogenic bacteria (Sieradzki *et al*., 1999). Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives (Smith *et al*., 1994; Ireland, 1988). Aquatic organisms are a rich source of structurally novel and biologically active compounds (Ely *et al*., 2004). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry (Febles *et al*., 1995). Algae have wide span of ecosystems contributes to the innumerable chemical compounds that they are able to synthesize. A number of antimicrobial compounds have been identified in microalgae as well as macroalgae (De Marsac and Houmard, 1993). A large number of microalgal extracts and/or extracellular products have been proven antibacterial, antifungal, antiprotozoal and antiplasmodial activity (Mayer and Hamann, 2005; Cardozo *et al*., 2007). 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 (Cabrita *et al*., 2010) and marine macro-algae are considered as the actual producers of some bioactive compounds with high activity (Shimizu, 1996).

Oscillatoria sancta (Kützing ex Gomont) is a fresh water cyanobacterium belong to the class Cyanophyceae under Cyanobacteria phylum. *Oedogonium echinospermum*, another fresh water green algae is a member of Chlorophyceae (Phylum-Chlorophyta). *Spirogyra crassa* (L.) Kutz is a filamentous green alga which is common in fresh water habitats in Bangladesh which is the member of class Conjugatophyceae under phylum Charophyta. It is known that Spirogyra possesses antibacterial activity (Stefanov *et al*., 1999). *Nostoc* spp. is a genus of cyanobacteria (Cyanobacteria phylum) found in both marine and fresh water habitat. *Chlorella* is a genus of single cell green algae belonging to the Phylum-Chlorophyta and class Trebouxiophyceae. This is a fresh water/terrestrial species. *Acanthophora spicifera* is a species of marine red algae in the class Florideophyceae under the phylum Rhodophyta. *Sargassum vulgare* C. Agardh is a marine genus of brown (Phylum Ochrophyta and class Phaeophyceae) macroalgae (seaweed). *Ulva lactuca* Linnaeus, a green alga in the class Ulvophyceae (phylum Chlorophyta) is a marine species. It is known as sea lettuce has long been used as food and as a traditional medical remedy to treat some helminthic infections, fever, urinary diseases and dropsy (Kim *et al*., 2007). The antimicrobial activity of *Ulva lactuca* was reported to be caused by the acrylic acid commonly found in the algae (El Yamany, 2008). *Enteromorpha prolifera* is a marine green algae belongs to class to Ulvophyceae (Phylum Chlorophyta). *Dictyopteris membranacea* is a brown or yellow-green marine algae which belongs to the class Phaeophyceae (Phylum Ochrophyta) species. In this study, we amide to determine the antimicrobial activities of some marine and fresh water algae available in Bangladesh.

MATERIALS AND METHODS

Sample collection: Algal materials were collected from the Karnafully River, Foy's lake and sea beach located in Chittagong in Bangladesh. Samples were stored in plastic bags and transported to the laboratory under iced conditions. The samples were initially washed thoroughly with sea water to remove sand and any adhering substance and then washed thoroughly with fresh water to remove salts and stored at -20°C until compound extraction. The algal species were identified based on the schemes reported in the literature (Guiry, 2015 Aleem, 1993; Coppejans *et al*., 2009). These cleaned fresh materials were air dried and then powdered with the help of a blender. Then the powdered samples were stored in a dark place and brought under to different extraction methods.

Extraction of selected algal species: The extraction of powdered algal samples was done using methanol, ethanol and chloroform. Aliquots of 25 g of the powdered algal samples were soaked in 250 mL of the solvents for 24 h. Later the soaked samples were homogenized in an electric blender along with the solvents at room temperature, filtered and concentrated under reduced pressure using a rotary evaporator. Each dried precipitate was re-dissolved in the corresponding solvents to give 50 mg mL^{-1} extracts and preserved at 5°C in airtight bottles until further use.

Determination of antimicrobial efficacy

Bacterial strains: The used bacterial test organisms were *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633) as gram positive species and *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia (*ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (ATCC 33459) as gram negative species grown in Mueller Hinton agar at 37°C for 24 h (Merck). Fungal species was unicellular *Candida albicans* (ATCC 60192) grown in potato dextrose agar (Hi Media) at ambient temperature for 72 h. The bacterial isolates were multidrug resistant determined by disc diffusion assay according to Iwalokun *et al*. (2004).

Antimicrobial assay: Antimicrobial activity was evaluated using the disc diffusion technique in petri dishes (NCCLS, 1993). Briefly, sterile filter paper discs, 6 mm in diameter were loaded with 25 µL of the different antibacterial compound extracts and were air dried. Discs containing standard concentration of ciprofloxacin for bacteria and amphotericin B for fungi were used as control. The discs were placed on Muller Hinton agar plates inoculated with each of the previously mentioned microorganisms. Plates were

incubated for 24 h at 37°C and the inhibition zones that formed around the discs were measured (mm diameter). All tests were performed in triplicate and the average clear zone greater than 10 mm were considered positive results (Lima-Filho *et al*., 2002).

RESULTS

The methanolic, ethanolic and chloroform extracts were evaluated for their potential bioactivity against six human bacterial pathogens and one fungus. Table 1 revealed that almost all of the algal species of different solvents showed their potency against microorganisms. Algal extracts prepared with ethanol and methanol had active principles than chloroform that could inhibit growth of the pathogenic bacteria which were tested. Ethanolic extract of *Oscillatoria sancta* ((Kützing ex Gomont) showed maximum antimicrobial activity against *E. coli* (16.1 mm) among other microorganisms where no antimicrobial activity was observed against *S. aureus* (0 mm) using chloroform extract. Extract of different solvents of *Oedogonium echinospermum* showed average antimicrobial activity except *Candida albicans*. All the bacterial isolates except *S. typhi* and *S. aureus* were resistant against *Spirogyra crassa* (L.) Kutz but extract of all solvents showed a better activity against *Candida albicans*. Ethanolic and methanolic extracts of *Acanthaphora spicifera* performed a good antimicrobial activity but chloroform extract was not good enough comparatively. *Escherichia coli* was resistant against *Sargassum vulgare* where all microbial isolates were sensitive. On the other hand

Table 1: Antimicrobial Activity of different extracts of different algae against test-pathogens

	Inhibition of growth expressed as diameter of inhibition zone (mm)						
Algal species and extracts	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Bacillus subtillis*	*Klebsiella pneumonia*	*Candida albicans*
Oscillatoria sancta							
Methanol	12.4	13.3	8.2	10.2	12.2	16.0	12.0
Ethanol	16.1	14.2	10.0	10.4	14.3	13.3	16.0
Chloroform	10.0	0.0	7.4	8.0	9.8	10.0	12.0
Oedogonium echinospermum							
Methanol	1.0	12.0	12.4	12.0	12.0	14.0	0.0
Ethanol	12.2	12.0	13.0	11.8	13.0	12.4	0.0
Chloroform	12.1	11.0	12.0	12.4	13.5	12.0	0.0
Spirogyra crassa							
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	12.0
Ethanol	0.0	8.3	0.0	11.7	0.0	0.0	13.0
Chloroform	0.0	0.0	0.0	0.0	0.0	0.0	13.0
Acanthaphora spicifera							
Methanol	14.9	12.9	14.5	13.2	17.0	12.0	11.9
Ethanol	15.7	13.2	14.0	14.2	14.3	11.0	10.7
Chloroform	0.0	0.0	0.0	0.0	6.0	6.23	0.0
Sargassum vulgare							
Methanol	0.0	12.8	11.1	13.7	10.9	13.5	11.0
Ethanol	0.0	12.7	12.7	10.3	11.2	14.0	11.2
Chloroform	0.0	11.6	12.0	10.3	11.3	13.9	10.0
***Chlorella* sp.**							
Methanol	14.0	15.2	15.2	14.2	17.9	14.8	19.8
Ethanol	12.6	17.6	13.7	13.1	18.6	13.8	19.1
Chloroform	12.2	17.0	13.1	13.1	17.9	13.5	19.1
Ulva lactuca							
Methanol	18.0	10.1	13.0	12.3	11.6	14.8	13.6
Ethanol	22.0	12.0	13.0	13.0	12.7	15.2	13.0
Chloroform	26.0	17.2	23.0	16.0	17.4	19.9	17.7
***Nostoc* sp.**							
Methanol	13.3	18.3	13.9	14.1	17.0	19.0	0.0
Ethanol	14.3	14.2	11.8	13.5	12.4	20.3	0.0
Chloroform	12.0	12.2	10.0	10.0	11.1	12.3	0.0
Enteromorpha prolifera							
Methanol	11.3	10.0	12.2	11.0	10.8	10.0	13.0
Ethanol	12.5	11.0	13.1	13.0	12.8	12.0	15.0
Chloroform	12.0	12.3	13.1	11.0	11.6	12.7	16.1
Dictyopteris membranacea							
Methanol	0.0	14.9	12.8	13.4	12.3	16.1	0.0
Ethanol	0.0	13.8	11.4	10.9	11.0	13.7	10.2
Chloroform	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin and Amphotericin B	22.5	18.2	20.4	20.5	19.8	18.4	21.2

all kinds of solvent extract of *Chlorella* sp. inhibited the growth of all bacteria and fungi with significant inhibition zone diameters. *Ulva lactuca* Linnaeus has a moderate activity against bacteria and fungi but chloroform extract showed better activity than other solvents. All kinds of solvents extract of *Nostoc* sp. showed antimicrobial activity except *Candida albicans*. Extracts of *Enteromorpha prolifera* performed a better role to inhibit the growth of all kinds of tested microbes with a moderate zone of inhibition. Chloroform extract of *Dictyopteris membranacea* was ineffective against all kinds of bacteria and fungi whereas methanolic and ethanolic extract were effective except *E. coli*.

DISCUSSION

Marine algal species showed better result than fresh water algal species. Green algae showed more efficacy than red and brown algae. Ethanolic extract performed better than methanolic and chloroform extracts. Methanolic extract more effective than chloroform. From the results of the present study it is clear that, organic solvents always have higher efficiency in extracting anti-bacterial compounds as extractant and chloroform as a solvent proved to be best suited for the extraction of the antibacterial constituent(s) from the algae. Ethanol extraction has been reported to result with higher antibacterial activity than methanolic extract while few other reports indicated chloroform as better extractant than ethanol and methanol (Rajasulochana *et al*., 2009). The antimicrobial activity definitely indicates the presence of antibacterial substances in algae which triggers and stimulates against pathogens. These results are in agreement with those reported earlier for extracting antibacterial substances such as hydroquinones, sesterpenoids, phenols, brominated phenols and polyphenols from species of Chlorophyceae, Phaeophyceae and Rhodophyceae (Faulkner, 2002). Active antibacterial extracts from different brown algae have been found to be made up of saturated and unsaturated fatty acids with a predominance of myristic, palmitic, oleic and eicosapentaenoic acids (Bazes *et al*., 2009). So, the antibacterial activities of the algae tested could be attributed to the type and amount of free fatty acids which have a role in the overall defense against the studied pathogenic gram positive and gram negative bacteria (Benkendorff *et al.*, 2005). The activity of *Ulva lactuca* Linnaeus against microbes indicates the presence of the fats and fatty acids may play an important role in the formation of many other bioactive secondary metabolites since some fatty acids have been shown to possess antibacterial activities (Barbosa *et al*., 2007; Oh *et al*., 2008). The results of antibiogram of *Oscillatoria sancta* (Kützing ex Gomont) were compatible with the study of Prakash *et al*. (2011) and Rao *et al*. (2007). In addition, *Chlorella* had clear antibacterial activity against bacteria and fungi especially *B. subtilis* and *S. aureus* in agreement with Ordog *et al*. (2004) and Ghasemi *et al*. (2004). *Nostoc* sp. was the most effective algal species against bacteria with great zone of inhibitions. These results agree with Kulik (1995) where they reported the effectiveness of *Nostoc* as an algal species. *Nostoc* sp. methanol extract affected on *S. aureus*, *B. subtilis* and *K. pneumonia* these results in agreement with Ostensvik *et al*. (1998). *Chlorella* extract gave the highest inhibition zone against *C. albicans* in agreement with Ghasemi *et al*. (2004) and Abedin and Taha (2008). The methanolic extracts of *O. echinospermum* exhibited the antibacterial activity against only one bacterium *S. typhi*. The results found from *Acanthaphora spicifera* were so similar with the research study of Pandian *et al*. (2011).

Another significant result of the present study is the less efficiency of the extracts of *Sargassum vulgare* C. Agardh. It did not show any antibacterial activity against *E. coil*. No growth inhibition of the bacteria was also reported by Ballantine *et al*. (1987) when the hexane extracts of the same algae were tested. No antimicrobial activity of *Spirogyra crassa* (L.) Kutz was observed against most of the bacteria except *S. aureus* and *S. typhi* which is so relevant with the result found form the study of Ivanova *et al*. (2011).

The algal extracts such as *Enteromorpha prolifera* and *Dictyopteris membranacea* (Stackhouse) Batters were active against gram positive and gram negative bacteria (Del Val *et al*., 2001). The inactivity of *Dictyopteris membranacea* against *E. coli* was quite similar with the result of Tuney *et al*. (2006) where activity against other microbes showed the same result studied by Alghazeer *et al*. (2013).

CONCLUSION

This study concludes that the extracts of algae showed biopharmaceutical potentiality. However whether such extracts will act as effective therapeutic agents remain to be investigated, the identification of the bioactive compounds and study of mechanisms of actions are necessary prior to application.

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