PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF CRUDE EXTRACT OF SARGASSUM *TENERRIMUM* J. AGARDH AGAINST POTENTIAL HUMAN PATHOGENS

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Abstract

The algal biomasses have potential to produce a variety of bioactive compounds against pathogenic microorganisms. The aim of this study was to evaluate the phytochemical analysis and antibacterial activity of brown algae *Sargassum tenerrimum* J. Agardh against fourteen gram positive and twenty two gram negative potential human pathogens. Qualitative phytochemical investigations indicated the extract of *Sargassum tenerrimum* contained alkaloids, steroids, glycosides, phenols and saponins. However, the antibacterial screening indicated that the extract was effective against three gram positive *Micrococcus leulies* ATCC 9341, *Streptococcus fecalis* and *Streptococcus pyogenes* and five gram negative *Pseudomonas aeroginosa, Pseudomonas aeroginosa ATCC, Proteus vulgaris, Serratia marcesens* and *Acinetobacter baumanii* bacteria. The range of zone of inhibition was 16mm to 19mm for gram positive (MIC 120-160 microgram/ml) and 15mm to 22mm for gram negative strains (MIC 150-220).

Introduction

Due to injudicious approach of employing antibiotics in treating clinical infections, the pathogenic bacteria have acquired resistance at an exorbitantly high rate since the advent of antibiotics (Mazela and Daviesb, 1999). Various drug resistance were developed for human and plant pathogenic microorganisms due to indiscriminate use of commercial antimicrobial drugs (Shafique *et al*., 2011). Sea weeds have been extensively explored for its antibacterial activities and in quest of bioactive compounds; the extracts are being subjected to purification techniques (Leary *et al*, 2009, Bhakuni and Silva, 1974, Rao *et al.*, 1988) and are considered as effective pharmaceutical candidates like antibiotics, antiviral agent, antifungal agents (Donia and Hamann,2003; Faulkner,2002 and Garg *et al*., 1992) anti-inflammatory products and antioxidants etc (Patra *et al.,* 2009 and Fleurence, (1999). According to Shameel and Tanka, (1992), sea weeds occupy a wide range of substrates as benthic to rocks or growing in water pools and are economically important. Seaweeds are used as food, feed and fertilizer in many parts of the world. According to Ito and Hori, (1989) seaweeds contain low calorie food, but rich in vitamins, minerals and dietary fibers and have been extensively explored for its antibacterial activities and in search of some antibacterial products. Moreover, these natural origin substances have infact very least chances of adverse reactions on human health rather than the chemical based synthesized pharmaceutical products.

The genus Sargassum has been studied for exploiting its potential as antimicrobial agents (Chiao-Wei *et al*., 2011). A literature survey in Pakistan revealed that however; there have been a number of studies on ecology, anatomy, taxonomy and distribution from the coast of Pakistan (Shameel 1987, Shameel and Tanaka, 1992) but no attention was paid on the antibacterial activities of seaweeds. The present study gives an account of the antibacterial potential of *Sargassum tenerrimum* against common potential human bacterial pathogens. It is hoped that this information may be useful as a part of integrated disease management based on improved resistance.

Materials and Methods

Sample Collection and extract preparation: *Sargassum tenerrimum* were collected from Buleji area during low tide in February. The collected samples were kept into plastic bags containing water to avoid discoloration and brought them in the Laboratory of Dendrochronology and Plant Ecology, Department of Botany, Federal

Urdu university-Karachi-Pakistan for further processing. The algal surface was washed with sterilized distilled water to remove surface contaminants such as small marine organisms, sand and other debris and were dried at 55°C for 48 h and ground into a fine powder. The powdered samples were later stored in the refrigerator at 4 C until used. Seaweed extracts were prepared, with some modifications, following Senevirathne *et al*. (2006). In brief, seaweeds were extracted with distilled water in the concentration of 5% in a shaking incubator at 25°C for 3 days. The extracts were filtered with Whatman's No. 1 filter paper and reextracted three times. Each extract was concentrated, evaporated and lyophilized to acquire a dry extract. The dry extracts were kept in desiccators until ready to be used. Both the extracts were stored in airtight glass containers sealed further with parafilm protected from sunlight till further work.

Screening of antibacterial activity: The test organisms for this study were isolated, identified, maintained and stored in the Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan. The antibacterial activity of *Sargassum tennerimum* against fourteen gram positive and twenty two gram negative bacteria were examined. All the bacterial isolates were checked for purity and maintained on nutrient agar at 4^0c in the refrigerator until required for use. Antibacterial activity of crude extract against pathogenic bacteria was determined by using agar-well method. Autoclaved Muller Hinton broth was used to freshen the bacterial culture, later wells were punched into Muller Hinton Agar and 10 microliters of culture were poured into the wells (Perez *et al*, 1990). All plates were incubated at 28$\pm$ 2°C for 24 -48 hours and after incubation diameter of zone of inhibition was measured.

Determination of Minimum inhibitory concentration (MIC): MIC of crude extract was determined by Micro broth dilution method using 96-well microtitre plate (Samie *et al*., 2005). Stock solution of 100 mg/ml of crude extract was prepared in distilled water. Two fold serial dilutions of extracts was made in 100 µl broth and subsequently 10 µl of two hours old fresh culture matched with 0.5 Mac Farland index was added to each well. One well served as antibiotic control while other served as culture control. Microtitre plate was incubated for 24 hours at 37 ºC.

Pytochemical analysis of the extract: The qualitative pytochemical analyses were also determined for the determination of alkaloids, flavanoids, terpenoids, phenols, saponins, tannins and others (Brindha *et al*, 1977, Harbone, 1973 and Doughari *et al*, 2007)

Results and Discussion

Sargassum tennerimum belongs to brown algae, as reported in some studies that brown algae possess more potential to kill pathogenic microorganisms (Vallinayagam *et al*, 2009). The antibacterial activity of *Sargassum tennerimum* against fourteen gram positive and twenty two gram negative bacteria were examined. It was observed that the extract was effective against three gram positive *Micrococcus leutieus* ATCC 9341, *Streptococcus fecalis* and *Streptococcus pyogenes* and five gram negative *Pseudomonas aeroginosa, Pseudomonas aeroginosa ATCC, Proteus vulgaris, Serratia marcesens* and *Acinetobacter baumanii* bacteria (Table 1). The range of zone of inhibition was 16mm to 19mm for gram positive and 15mm to 22mm for gram negative strains. The maximum activity (22mm) was recorded against *Sarratia marceseus* and minimum (15mm) against *Streptococcus fecalis*. Vallinayagam *et al*., (2009) noted highest antibacterial activity in brown algae. Caccamese *et al*., (1985) also reported that brown algal extracts showed higher activity than the extracts of red algae. The results indicated that this species may have selective response mechanism. Against tested bacterial strains, as suggested by Vallinayagam *et al*., (2009).

Table 1. Antibacterial potential of *Sargassum tenerrimum* extract explored by Agar well diffusion method in terms of zone of inhibition (mm).

Extract / Bacteria	Inhibition zone (mm)
Gram positive bacteria	
Micrococcus leutus ATCC 9341	20
Streptococcus fecalis	19
Streptococcus pyogenes	21
Gram negative bacteria	
Proteus mirabilis	19
Pseudomonas aeroginosa	17
Pseudomonas aeroginosa ATCC	17
Proteus vulgaris	19
Serratia marcesens	15

*Gentamicin was used as an antibiotic control (Vaghasiya *et al*, 2009

Minimum Inhibitory Concentration were also determined by Microdilution method to search the therapeutic concentration which was found in the range for gram positive (MIC 120-160 microgram/ml) for gram negative strains (MIC 150-220) (Table 2). All the other bacterial strains were resistant to these extracts. The results depicted that the extract showed better antibacterial property for gram negative in vitro gram positive strains. *S. tennerimum* was effective against all tested bacterial strains both gram positive and gram negative. We did not get any activity against some of the bacterial pathogens tested probably due to the reason as we used crude extract and it might have some inhibitory substances that interfere the antibacterial activity range (Sastry and Rao, 1994).There is also a need to employ all possible techniques to search the biocactive compound that exhibits the antibacterial action. As far as qualitative photochemical analysis is concerned, the extract of *Sargassum tenerrimum* possessed alkaloids, steroids, tannins, glycosides, phenols and saponins (Table 3) as also mentioned in a study by Bhiagabhati *et al*., (2011) that reported the presence steroids, sapnonins, anthraquinones, alkaloids but did not get glycosides in *Sargassum muticum* extracts.

Table 2. Minimum Inhibititory Concentration (MIC) *Sargassum tenerrimum* extract in µg/ ml were determined by Microdilution method.

Extract / Bacteria	MIC (µg/ml)
Gram positive bacteria	
Micrococcus leutus ATCC 9341	120
Streptococcus fecalis	180
Streptococcus pyogenes	160
Gram negative bacteria	
Proteus mirabilis	150
Pseudomonas aeroginosa	160
Pseudomonas aeroginosa ATCC	220
Proteus vulgaris	200
Serratia marcesens	240

Table 3. Qualitative phytochemical activity of *Sargassum tenerrimum extract.*

Compounds	Presence/absence
Alkaloids	+
Anthroquinone	-
Phenolic compouds	+
Saponins	+
Steroids	+
Glycosides	+
Tannins	+

Note: + ve indicates presence, - ve indicates absence

In this study, primary screening of *Sargassum tenerrimum* J. Agardh was carried out in quest of exploring antibacterial potential of this algae, the very first time in Pakistan. Despite the growing interest about bioactive products of sea weeds, no consideration has been given towards the antibacterial activity of clinical pathogens. The results were quite good as significant activity was found against some potential human pathogens. Further and comprehensive analysis to find out the pure bioactive compound must be carried out to introduce it in the world of therapeutics to combat the load of infectious diseases.

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