



Original Research Article

Evaluation of Antimicrobial Activity of Crude Extracts of Seaweed *Sargassum johnstonii*

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ABSTRACT

Keywords

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The present study deals with the preliminary analysis for antimicrobial activity of seaweed *Sargassum johnstonii* collected from Porbandar coast, west coast of India. *Sargassum johnstonii* was extracted with two organic solvents such as methanol + ethyl acetate and hexane, while antimicrobial activity was studied against 7 pathogens. Results indicated high inhibition of the methanol + ethyl acetate extract of *S. johnstonii* against pathogens such as *Bacillus* sp. (11 mm), *Enterococcus faecalis* (11 mm), *Salmonella typhi* (13 mm), *E. coli* (12 mm), *Halobacterium salinarum* (14 m), *Staphylococcus epidermidis* (12 mm) and *Rhizobium* (13 mm), compared to hexane extract. Further screening for phyto-chemicals constituents of both extract showed positive results for the presence of alkaloid, carbohydrates, protein, and phenolic compounds. The considerable antimicrobial activity and the presence of phyto-chemicals indicated that *S. Johnstonii* can be considered as a source of active principles against pathogens.

Introduction

The coastal and marine environment offers very rich source of important compounds of structurally novel and biologically active metabolites (Anake and Pichan, 2004). Seaweeds considered as one of the rich source of biologically active metabolites (Ely *et al.*, 2004). Many phycocolloids obtained from marine algae such as alginate, carrageenan and agar have been used in medicine and pharmacy (Taskin *et al.*, 2001), some algal substances known to have bacteriostatic and bactericidal activity (Ely *et al.*, 2004). It has

been also reported that seaweeds contain high levels of minerals, vitamins, essential amino acids, indigestible carbohydrates and dietary fiber (Jimenez-Escrig and Goni, 1999).

Seaweeds from the west coast of India are well known for diversity and its antibacterial activities (Manilal *et al.*, 2009). In this context, the objective of this work was to evaluate the antimicrobial activity of *Sargassum johnstonii* (Phaeophyceae) against human pathogens and preliminary

analysis of phytochemicals for further applications.

Materials and Methods

Sample collection and identification

The plants of *Sargassum johnstonii* were collected from the Porbandar coast of India (Lat. 21° 35'. 02" N; Long. 69° 36'.98" E). The alga which attached exclusively on the intertidal rocky substratum was collected. The collected seaweed was initially washed thoroughly with filtered seawater and again with distilled water to remove extraneous materials. Species was identified using standard books and manuals (Silva *et al.*, 1996).

Preparation of seaweed powder

The fresh algal sample was air dried. The dried sample was ground using mixer grinder. The powdered sample was packed in plastic bags and kept in desiccators at room temperature for further use.

Preparation of seaweed extracts

Two gram of dried seaweed sample was taken in to test tube and 10 ml of solvents (n-hexane and methanol + ethyl acetate in 1: 1 ratio) was added and sample was soaked for 12 hours, and then shaken with the help of cyclomixer for 10 mins. The samples were centrifuged at 10,000 rpm for 10 mins at room temperature. Finally supernatant was collected and transferred to watch glass and all the solvents are evaporated using hot air oven at 45 °C. The extract was collected and used for further studies.

Bacterial assay

The two solvents extracts were tested for antimicrobial activity against the bacterial

pathogens such as *Bacillus* sp., *Enterococcus faecalis*, *Salmonella typhi*, *E. coli*, *Halobacterium salinarum*, *Staphylococcus epidermidis* and *Rhizobium* obtained from the Government Hospital, Rajkot.

Preparation of broth culture

The test bacterial pathogens were inoculated into the nutrient broth and incubated for 24 hours before start of the experiment.

Screening for antimicrobial activity

Antimicrobial activity of seaweed extract was tested by agar-well diffusion method. Petri dishes with 20' ml of Muller Hinton agar (Himedia) were prepared. Agar plates were surface inoculated uniformly from the overnight broth culture of the tested microorganisms. In all cases, the concentration was approximately 1.2×10^6 CFU/ml. Then 6 mm diameter well was cut in the centre of the MHA plates, 100 µl of *S. johnstonii* extract was added and plates were incubated for 24 hours at 37 °C. After incubation period the zone of inhibition were recorded included with well diameter. The solvents without seaweed extracts were used as a negative control for the test.

Phyto-chemical analysis

The phyto-chemical analysis of *S. johnstonii* was carried out to assess the qualitative determination of chemical compounds in crude extracts using standard precipitation and colouration methods. The phyto-chemical compounds such as carbohydrate, proteins, phenolic compounds and alkaloids were assessed by following standard procedures (Harborne, 1973; Brindha *et al.*, 1981; Lala, 1993). The results revealed the presence or absence of all specific compounds in the crude extracts.

Based on the colour intensity, the results were recorded as H (High), M (Moderate) and L (Low) as shown in Table-1.

Detection/determination of Alkaloids

50mg filtered solvent free extract was stirred with 2ml of diluted hydrochloric acid and filtered. To 1ml of filtrate, a drop of Mayer's reagent was added along the sides of test tube and then observed for a white creamy precipitate.

Detection of Carbohydrates

It was done as per Molish's test- 100mg extract was dissolved in 5ml of water and filtered. In to 2ml of filtrate, two drops of alcoholic solution of α -naphthol were added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the sides of the test tubes and allowed to stand and then observed for the formation of violet ring.

Test for Proteins

Millon's test- Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Detection of Phenolic compounds

50mg extract was dissolved in 5ml of D/W. a few drops of neutral ferric chloride solution was added and observed for a dark green colouration.

Results and Discussion

Antimicrobial activity of methanol + ethyl acetate extract of *S. johnstonii*

The antimicrobial activity of methanol +

ethyl acetate extract of *S. johnstonii* was shown in Fig. 1. The maximum inhibition zone in methanol + ethyl acetate extract of *S. johnstonii* was registered against *H. salinarum* with 14 mm followed by *S. typhi* and *Rhizobium* sp. with 13 mm, *E. coli*, *S. epidermidis* with 12 mm each, *Bacillus* sp. and *Enterococcus faecalis* with 11 mm.

Antimicrobial activity of N-hexane extract of *S. johnstonii*

The recorded antibacterial activity for N-hexane extracted *S. johnstonii* showed maximum inhibition against *H. salinarum* (14 mm) followed by *S. typhi* and *Rhizobium* sp. with 11 mm, *E. coli*, *S. epidermidis* with 10 mm each, *Bacillus* sp. and *Enterococcus faecalis* with 8 mm (Fig. 2).

Screening of phytochemicals

Preliminary phyto-chemical screening of methanol + ethyl acetate and N-hexane extracts of seaweed *S. johnstonii* confirmed presence of compounds such as carbohydrate, proteins, phenolic compounds and alkaloids (Table 1).

The seaweed extracted with two different solvents showed significant antimicrobial proficiency against human pathogens. It is understood that the presently reported constitution and concentration of phenolic compounds in *Sargassum johnstonii* can inhibit the growth of microbes as stated earlier by Vijayabaskar and Shiyamala (2011) for the antimicrobial activity of methanol extract of *Sargassum swartzii* and *Turbinaria ornata*. They reported that the methanol extract of the *S. swartzii* and *T. ornata* possessed superior activity against gram positive and negative pathogenic bacteria due to phenolic compounds present in the respective seaweed extracts. Alkaloids present in *S. johnstonii* are also the

responsible factor for high antimicrobial properties. Similarly Omulokoli *et al.* (1997) and Cowan (1999) earlier reported high antimicrobial activity against both Gram-positive and Gram-negative bacteria.

The present result was also supported by Praneeth *et al.* (2011) that the different phyto-chemical compounds that include steroids, alkaloids, terpenoids, glycosides,

phenols, flavonoids, amino acids and oils contains in brown seaweeds are liable for the antimicrobial efficacy against the human pathogens. The results clearly reveal the antibacterial nature of *S. johnstonii* and as a good source of phyto-chemicals. It is suggested that this seaweed can be exploited for the management of diseases caused by pathogenic bacteria in human.

Table.1 Presence of phytochemical constituents in methanol + ethyl acetate and n hexane extract of *S. johnstonii*.

Extract	Carbohydrate	Protein	Phenolic compounds	Alkaloids
Methanol + ethyl acetate	H	H	M	M
N-hexane	H	H	M	M

Fig.1 Antimicrobial activity of methanol + ethyl acetate extract of *S. Johnstonii*

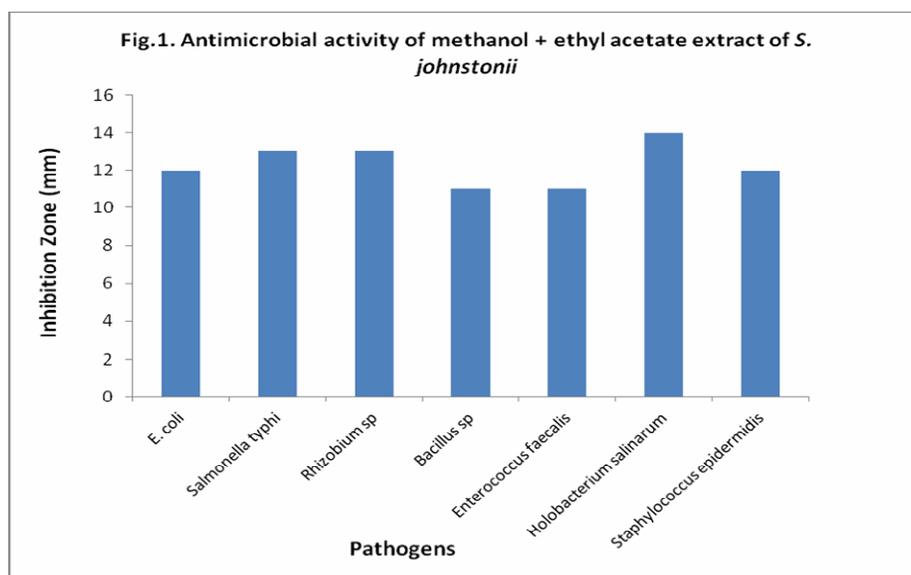
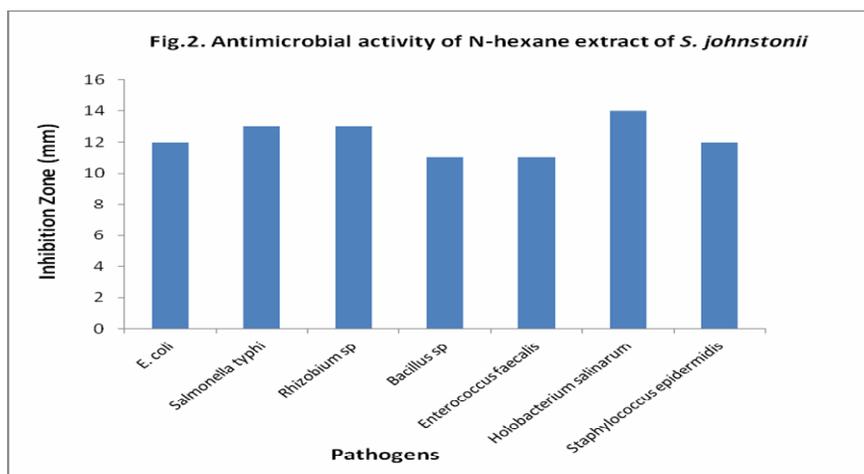


Fig.2 Antimicrobial activity of N- hexane extract of *S. johnstonii*



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