



ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

Microbicidal activity of crude extracts from *Sargassum wightii* against *Bacillus cereus*

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ABSTRACT

The present study was investigated to explore the antibacterial activity of four different solvent (petroleum ether, chloroform, acetone and ethanol) crude extracts of marine brown seaweed *Sargassum wightii*. Crude extracts were screened against human pathogen *Bacillus cereus*. The antibacterial efficiency was performed by agar well diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. The results revealed that the crude extract of petroleum ether showed prominent inhibiting activity against *B. cereus* and other crude extracts showed below detectable level. The highest microbicidal activity (zone of inhibition) 9.0 ± 0.32 mm was obtained at the concentration of 250 $\mu\text{g/ml}$ and the lowest activity was 3 ± 0.20 mm at 31.25 $\mu\text{g/ml}$ concentration. The MIC and MBC values were found to be 125 and 250 $\mu\text{g/ml}$ respectively. Results of this study suggested that the compounds present in the crude extracts of petroleum ether showed high activity against *B. cereus* and further studies are required to purify the active principles.

Key Words: Seaweed, antibacterial activity, pathogen, microbicide, solvents.

INTRODUCTION

World Health Organization (2004) reported that infectious diseases are a major cause of morbidity and mortality worldwide and bacterial infection causes high rate of mortality in human population (Farias *et al.*, 2000). The enhanced treatment of infectious diseases by microbicide has limitations because of its changing patterns of resistance in pathogens and side effects. These limitations demand for the identification of new antimicrobial compounds derived from natural sources especially from marine (Ballantine *et al.*, 1987).

Marine seaweeds are rich source of structurally novel and biologically active metabolites. Secondary and primary metabolites isolated from seaweeds are potentially active in the pharmaceutical industry against human and other diseases (Farias *et al.*, 2000). Most of the secondary metabolites of marine seaweeds such as flavonoids, terpenoids, alkaloids and phenolic compounds show wide range of anti-bacterial activities against the human pathogens (Thirumaran *et al.*, 2006) and recent research implies that polysaccharides like inulin, oligo-fructose, galacto oligosaccharides and lactulose can also act as potent prebiotic compounds against pathogenic microbes in humans.

The antimicrobial activity of seaweeds in the coastal area of Tamil Nadu is still needs to be explored. *Sargassum wightii* belongs to brown seaweeds and are widely found in south Indian coastal belt with remarkable biological activities.

Bacillus cereus is a Gram-positive, rod-shaped aerobic bacterium with endospore (Todar, 2008) and can multiply

quickly even at room temperature. *B. cereus* is responsible for a minority (2–5%) of food borne diseases (Todar, 2008; Davis, 2010), causing severe nausea, vomiting and diarrhea, aggressive than necrotizing fasciitis. Food poisoning is a major health problem, affecting both industrialized and developing countries.

Objective of the present study was to screen the crude solvent extracts of *S. wightii* against the food borne pathogen *B. cereus*.

MATERIALS AND METHODS

Antibacterial assays

Bacterial culture

B. cereus was collected from the Rajah Muthiah Medical College, Annamalai University, Tamil Nadu, India. Collected strain was grown in Trypticase soy-polymyxin broth under aerobic conditions at 37°C in a rotary shaker at 200 rpm to reach exponential growth.

Screening of antibacterial activity

Crude petroleum ether, chloroform, acetone and ethanol extracts of the seaweed *S. wightii* were obtained by sequential solvent extraction method using a soxhlet. The antibacterial activity of crude extracts was performed by agar plate well diffusion assay on Mueller Hinton Agar (Marudhupandi and AjithKumar, 2013). Mueller Hinton agar media was prepared, sterilized and poured into sterile Petri dishes. Wells of 6 mm diameter were made on the agar plates by using sterilized well cutter. Inoculum in exponential phase of growth, equivalent to a 0.5 McFarland standard was swabbed on to the surface of the agar. Crude extracts of the solvents (petroleum ether, chloroform, acetone and ethanol) were prepared and dispensed in to the wells in the concentrations ranging from 250, 125, 61.5 and 31.25 $\mu\text{g/ml}$. The plates were then incubated at 37°C for 24 h. The antibacterial activity of the crude extracts was determined by observing the diameter of zone of inhibition (in mm) on the plates.

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Table 1: Diameter of Zone of inhibition (in mm) produced by the crude solvent extracts of *S. wightii* against *B. Cereus*.

Concentration of extract (µg/ml)	Diameter of zone of inhibition (in mm)			
	Petroleum ether	Chloroform	Acetone	Ethanol
31.25	8.0±0.20	-	-	-
62.5	10.4±0.10	-	-	-
125	12.2±0.20	-	-	-
250	14.0±0.32	-	-	-

Determination of Minimum Inhibitory Concentrations

To determine the minimum inhibitory concentration (MIC) of the crude extracts of *S. wightii* tube dilution technique was employed. This test was done to determine the lowest concentration of crude extracts that inhibit the growth of bacteria. A loop full of exponential phase bacterial culture corresponding to 0.5 Macfarlands opacity was inoculated in to nutrient broth with different concentration of extracts ranging from 125, 62.5, 31.25 and 15.625 µg/ml. The tubes were then incubated at 37°C for 24 h. Turbidity was observed after the incubation period. MIC was defined as the lowest concentration of crude extract that completely inhibited the visible growth of the test microorganisms.

Minimum bactericidal concentration

To determine minimum bactericidal concentration (MBC), one loop full of the sample corresponding to MIC and the next higher concentrations were streaked individually on nutrient agar and incubated at 37°C for 18h. The lowest concentration of extract that showed no bacterial growth on agar plate represents the MBC value of the particular extract.

Statistical analysis

The experiments were conducted in triplicates and the results were expressed as mean ± S.E.M. Statistical analysis were performed with Sigma plot® 12.5 software, Systat, USA.

RESULTS AND DISCUSSION

Seaweeds serve as an important source of bioactive natural substances (Metzger *et al.*, 2002). *S. wightii* is one of the marine brown seaweed species, with tremendous biological applications and are known to be rich in sulphated polysaccharides content and these sulphated polysaccharides were found to possess wide range pharmacological and biomedical properties (Güven *et al.*, 1999), especially antimicrobial activity against human pathogens like *B. cereus*.

Antibacterial activity of the crude solvent extracts was performed against the bacterial pathogen *B. cereus* by agar well diffusion method. The maximum antibacterial activity of 14.0 ± 0.32 mm was found to be exhibited by the crude petroleum ether extract and other crude extracts of chloroform, acetone and ethanol exhibited no detectable activity against *B. cereus*.

The minimum inhibitory concentration and minimum bactericidal concentration of crude petroleum ether extract was 62.5 µg/ml and 125 µg/ml respectively against *B. cereus*. The minimum bactericidal concentration was showed as 31.25 µg/ml against *B. cereus*.

Antibacterial activity of brown seaweeds against Gram positive bacteria has been extensively studied by Kolanjinathan and Stella (2009). The extraction methods, solvents and seasonal variation play an important role in the microbicidal activities (Kandhasamy and Arunachalam,

2008). Kim and Lee (2008) used methanolic extracts of *Sargassum* sp. which showed strong antibacterial activities against Methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Rajasekar *et al.* (2014) indicated that acetone was the best solution for extracting the effective antimicrobial materials from *Sargassum myricystum*, *Turbinaria conoides*, and *Gracilaria edulis*; whereas, Karthikaidevi *et al.* (2009) used seven different solvents including chloroform and ethanol for extraction of antibacterial substances from *Ulva reticulata* and *Halimeda tuna*.

In this study, four extracts of different solvent extracts of *S. wightii* were screened for the antibacterial activity against the human pathogen *B. cereus*. The results showed that, petroleum ether extract of *S. wightii* showed high activity against *B. cereus* compared to the other extract and the MIC and MBC value was found to be 62.5 and 125 µg/ml, respectively against *B. cereus*. The compounds present in the petroleum ether extract which exhibits high antibacterial activity against *B. cereus* should be purified and characterized completely to explore its potential application as food preservative.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of CMS college of Science and Commerce and Dr. NGP Arts and Science College, Coimbatore, Tamil Nadu, India for their facilities.

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