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ANTIBACTERIAL ACTIVITY OF SEAWEED *HALIMEDA OPUNTIA* FROM THE COASTS OF SOUTH ANDAMAN

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ABSTRACT
Marine Seaweeds are utilized as a potential source of food, animal feed, drug discovery and fertilizers and also used for their bioactive potential. In the present study, extracts of *Halimeda opuntia* was evaluated for the antibacterial activity against two human pathogens *viz*., *Escherichia coli* and *Staphylococcus aureus*. Seaweed collected from the intertidal region of Kodiyaghat, South Andaman were extracted using four different organic solvents namely ethanol, methanol, hexane and chloroform and further screened for their antibacterial activities against the pathogenic bacterial strains by agar well diffusion method. It was observed that ethanol extract of *H. opuntia* inhibited the growth of bacterial strains than any other test extracts. The maximum zone of inhibition against *E. coli* and *S. aureus* was 21mm and 19mm using 70% ethanol extract and 19mm and 21mm using absolute ethanol, respectively. The results suggest that the *H. opuntia* as a good source of antimicrobial compounds.

KEY WORDS: Seaweeds; Andaman Sea; antimicrobial compound; bioactive substances; *Halimeda opuntia*.

INTRODUCTION

Seaweeds are marine plants of Cryptogams represented by four major classes such as Chlorophyta (Green algae), Phaeophyta (Brown algae), Rhodophyta (Red algae) and Cyanophyta (Blue-green algae)[14]. Being an important source for bioactive substances and phycocolloids, they are also rich in minerals, vitamins and trace elements and therefore termed as medical food of the 21stcentury[8]. However, most of the studies in India are mainly focus on their resource survey, distribution, taxonomy, ecology, physiology, phycocolloidal chemistry, pharmacological aspect and their culture [3,7,8,20].

An enormous amount of interest has been generated on seaweeds and their extracts for the bioactive compounds which has immense medicinal potential and food supplements[13]. The macro algae are reported to be a major source of various bioactive compounds with antibacterial[12], antiviral[5] and antitumor activities[10]. Many reports have also suggested that seaweeds are a good source of chemical compounds that produce a wide variety of biologically active secondary metabolites [1,2,6,11,16,18,19]. Many components of seaweed also have potential antimicrobial activity and they are now in the focus for natural product discovery. Furthermore, seaweed is a cheap and easily available raw material and with large biomass, which is not the case for most other potential source of marine natural products. It is being reported that seaweeds were found to possess active compounds against human bacterial pathogens, fish bacterial pathogens, leaf spot disease of plant and marine pathogenic microorganisms[2,3,7,8,9,20]. Bio-stimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Similarly, seaweeds also have some of the valuable medicinal components such as antibiotics, antioxidants, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations[6,7,16, 18,19,20]. Many of the potential seaweeds which show a significant level of bioactivity are widely distributed in the coastal regions of many continents[5,8,9].

In the present study, it was found that the crude extract of *H. opuntia* obtained from the intertidal regions of Kodiyaghat, South Andaman had antibacterial activities and inhibited the growth of *E. coli* and *S. aureus*. This study was aimed at isolating the extracts of seaweed with four different organic solvents *viz*., Ethanol, Methanol, Hexane and Chloroform and studying their antibacterial activity against pathogenic bacterial strains. A bioassay guided fractionation of the crude extracts was done using open column chromatography (Silica gel 60-120 mesh, Himedia) method and all the fractions [Distilled water (F1); 70% Ethanol (F2); and Absolute Ethanol (F3)] were tested for bioactivity potential .

MATERIALS & METHODS

Study Area

Andaman and Nicobar Islands are located in the Bay of Bengal forming a chain of 572 Islands & Islets extending for about 850 km between Lat. 6° - 14° N and Long. 92°-94° E. The Andaman group is the biggest and accounts for 6340 km^2 area and the intertidal region of the islands houses several seaweed species. Seaweed *Halimeda opuntia* (L) Lamouroux, is recognized by its calcified and flabellate segmented habit[4]. For the present study *Halimeda opuntia* was collected from the coast of Kodiyaghat, (Lat. 22°03.940' N, Long. 93° 09.017' E) South Andaman (Fig.1).

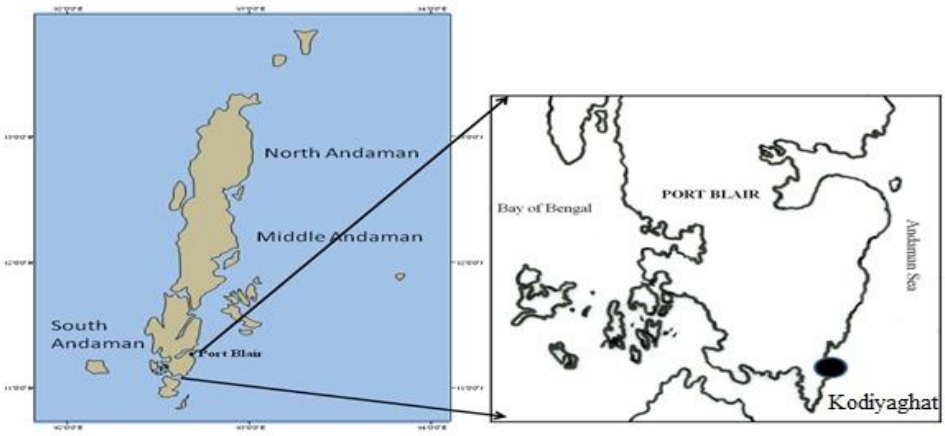

FIGURE 1. Map showing the study area along the South Andaman coast, A & N Islands, India.

Preparation of solvent extracts with *H. opuntia*

The collected algal sample was carried to the laboratory in polythene bag and washed thoroughly with sterile water for removing detritus and any epibionts associated with them. The cleaned sample was air dried in the dark on absorbent paper for 24 hrs at room temperature for removing the water content. Following, the dried samples were weighed separately to 50g dry weight and each sample was homogenized separately to fine powder with the help of clean motar and pestle. The powdered sample was soaked in pure solvents like ethanol, methanol, hexane and chloroform separately in the ratio of 1:4 for 24 hours at room temperature. After 24 hours, samples were filtered by using filter paper (GFC/Whatman) and centrifuged at 10,000 rpm for 15 minutes and supernatants were collected. Then the samples were concentrated by using the Rotary Evaporator (Buchi RII Rotavapour) at 35°C and stored in refrigerator for further analysis.

Bioassay of crude extract against bacterial pathogens

Autoclaved petriplates were poured with 15ml sterilized agar medium, (Mueller Hinton, Himedia), labeled and allowed to solidify. The micro-organisms inoculated were cultured 24 to 48 hrs in nutrient broth medium. Following, 100µl of pure test organisms were pipetted out using a sterile micopipette and spread on the agar medium in separate petriplates using sterilized L-shaped glass rod and allowed to dry for few minutes. Four wells of 5mm diameter were punched out with the help of sterile cork borer. Following, 100µl algal extracts (with respective solvents) were loaded separately in each well. Agar plates were maintained in triplicates for each algal extract and a control plate was also prepared for the comparative assessment of the activity. Plates were then kept at 37°C for 24 hours in a BOD incubator (Macro Scientific works Pvt. Ltd.). After 24 hours of incubation period, the zone of inhibition was measured in millimeters and tabulated.

Purification of active extract using column chromatography and its bioassay

On the basis of the results obtained from bioassay, ethanol extracts of *H. opuntia* showed highest activity against test bacteria. So, further purification of *H. opuntia* crude extract was carried by open column chromatography by Silica gel 60-120 mesh (Himedia) as the substrate, employing a step gradient solvent system, where the crude extracts were fractionated with Distilled water (F1); 20% Ethanol (F2); 70% Ethanol (F3) and Absolute Ethanol (F4). These four fractions were further concentrated by rotary evaporator and each fraction was checked for their antibacterial activity against *E. coli* and *S. aureus.*

RESULTS

Bioassay of crude extracts using bacterial strains

As shown in the Fig. 2 the zone of inhibition against two pathogens *E. coli* and *S. aureus* is well evident. The anti-bacterial activity of 70% ethanolic extract of *H. opuntia* against two pathogenic bacterial strains exhibited a prominent zone of inhibition (Table 1). The maximum zone of inhibition observed for *E. coli* was 21mm and *S. aureus* was 19mm, whereas the methanol and hexane extracts showed very little activity against *E. coli*. Moreover, distilled water showed no activity against the test bacterial strains.

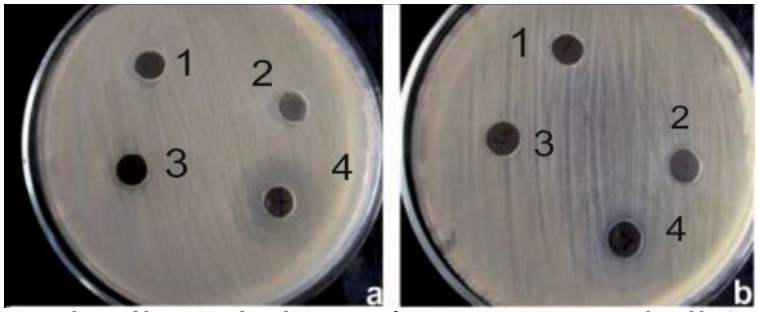

FIGURE 2: Inhibition zones obtained by 70% Ethanolic extract of *H. opuntia* against a: *E. coli* and b: *S. aureus*. Both a and b: 1: Distilled water; 2: 100 % Hexane; 3: 70 % Methanol and 4: 70 % Ethanol.

TABLE 1. Anti-bacterial activity of crude extracts of the *H. opuntia* against test organisms

S. No.	Crude Extract (µl)	Area of Inhibition (mm)	
		E. coli	*S. aureus*
1.	Distilled water	-	-
2.	100 % Hexane	+	-
3.	70 % Methanol	+	-
4.	70 % Ethanol	++ (21mm)	++ (19mm)

(−, No Activity; +, Activity; ++, Good Activity in mm)

Bioassay of fractionated crude extracts using bacterial strains

On bioassay of the fractions obtained after open column chromatography, F4 (Absolute ethanol) exhibited significant activity against two test micro-organisms (Table 2). The zone of inhibition is well observed as depicted in Fig.3. Maximum zone of inhibition observed for *E. coli* and *S. aureus* was 19mm and 21mm, respectively. The results suggest that the active compound is a hydrophobic substance which inhibits bacterial growth in the laboratory conditions.

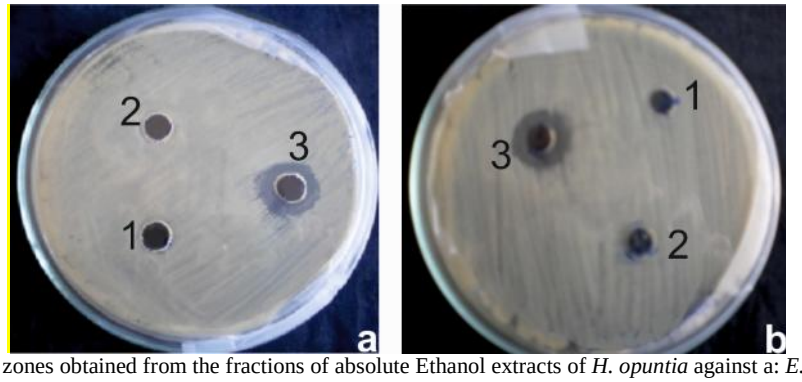

FIGURE 3: Inhibition zones obtained from the fractions of absolute Ethanol extracts of *H. opuntia* against a: *E. coli* and b: *S. aureus*. Both a and b: 1: Distilled water; 2: 70 % Ethanol and 3: Absolute ethanol.

TABLE 2. Antibacterial activity of fractions obtained from ethanol crude extract against test organisms.

S. No.	Fractions	Area of Inhibition (mm)	
		E. coli	*S. aureus*
1	Distilled water	-	-
2	70% Ethanol	-	-
3	Absolute ethanol	++ (19mm)	++ (21mm)

(−, No Activity; +, Activity; ++, Good Activity in mm)

DISCUSSION

Seaweeds are considered as a good source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. They can be used as a potential source for isolation of bioactive compounds of interest for pharmaceutical applications. In the present study, *Halimeda opuntia* from South Andaman was screened for the presence of antimicrobial compound, with the aim of identifying the potential seaweeds with antimicrobial abilities, which can inhibit the growth of pathogenic bacterial strains. It was found that *H. opuntia* contain some active molecules, which are inhibiting the growth of *E. coli* and *S. aureus* in the laboratory conditions, that make it useful as development of antimicrobial compounds in the future. As reported earlier, extracts of *H. opuntia* and *Sarconema filiforme* showed activity against human pathogens [17]. Also extracts of *H. opuntia* showed activity against six other species of microorganisms [12,18] which makes it a potential seaweed species having antibacterial compounds. The study suggests that the 70% ethanol extracts of *H. opuntia* exhibited considerable zone of inhibition against *E. coli* (21mm) and *S. aureus* (19mm) as compared to other solvents such as distilled water, 100% hexane and 70% methanol. This shows that 70% ethanol is an ideal solvent for the isolation of active anti-bacterial compounds and can be used for extracting the active antibacterial components from *H. opuntia.* Also, absolute ethanol fraction of *H. opuntia* obtained by column chromatography proved to be best extract which shows zone of inhibition against bacterial strains. The considerable zone of inhibition was observed against *E. coli* (19mm) and *S. aureus* (21mm). The antimicrobial properties in seaweeds have developed due to their inhibitory properties against diverse groups of microorganisms present in their natural environment. It may be both long term defense as well as rapid activation induced by environmental conditions. This antimicrobial activity of seaweeds may be influenced by several other factors as well such as the habitat and the season of algal collection, different growth stages of plant, experimental methods, type of pathogenic bacteria used for the antibacterial assay *etc* [1]. A good zone of inhibition by *H. opuntia* against *E. coli* and *S. aureus* in the present study projected the presence of active compounds against these

pathogenic bacteria. Further, purification and characterization of such biologically active compound will be an important contribution to the society in the era of increasing antibiotic resistance pathogens.

CONCLUSION

To the best of the knowledge, this is the baseline work carried over *H. opuntia* from South Andaman. The crude extract of seaweed with 70% ethanol and its fractionation with absolute ethanol showed considerable zone of inhibition against *E. coli* and *S. aureus*. From these preliminary investigations, it is indicated that the seaweeds are a potential source of bioactive compounds. However, a detailed study has to be done on active compounds to understand their bio prospects.

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