Research Article

ANTIBACTERIAL PROPERTIES OF *TURBINARIA CONOIDES* FROM GULF OF MANNAR COAST

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ABSTRACT

The antibacterial properties of chloroform, methanol, ethanol and water extracts of marine algae *Turbinaria conoides* (J.Agardh, *Ochrophyta*) from mandapam region of Gulf of Mannar Southeast coast of India were tested against Gram-positive strains such as *Bacillus subtilis* and *Streptococcus pyogenes;* Gram-negative strains like *Salmonella typhimurium and Proteus mirabilis*. The preliminary phytochemical analysis of extracts from *Turbinaria conoides* showed the presence of biologically active compounds namely alkaloids, flavonoids, steroids, terpinoids, cardiac glycosides and tannins. Results demonstrated that methanolic extract of *Turbinaria conoides* exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. It was observed that the extract of *Turbinaria conoides* recorded maximum activity against *Streptococcus pyogenes*.

Keywords: *Turbinaria conoides*, Antibacterial agents, Complex mixtures, agar well diffusion.

INTRODUCTION

Turbinaria conoides (J.Agardh, Class: *Phaeophyceae*, Order: *Fucales*, Family: *Sargassacea*) is an abundantly growing brown seaweed in coastal seashore of South India. Marine macro algae are rich in varied source of bioactive natural products and have been studied for its biocidal and pharmaceutical properties[1]. In recent years, there are several reports of macro algae derived compounds that have a wide range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, cytotoxic and anti-diabetic[2]. At present seaweeds constitute commercially important marine renewable resources which are providing valuable thoughts for the development of new drugs against cancer, microbial infections and inflammations[3]. Marine algae have been screened broadly to isolate life saving drugs or biologically active substances all over the world[4].

Bacterial infection cause high rate of mortality in human population and aquaculture organisms[5, 6]. Currently, the use of antibiotics increased significantly due to heavy infections and pathogenic bacteria becoming resistance to drugs[7]. Findings suggested that marine algae use targeted antimicrobial chemical defense strategies by eliciting secondary metabolites, which are important in ecological interactions between marine macro organisms and microorganisms[8]. However, many seaweeds and sponges suffer remarkably low levels of microbial infection, despite lacking cell-based immune systems. The extracts of many species of marine algae inhibit the growth of Gram-positive and Gram negative bacteria. There are studies on antimicrobial activity of marine algae[9, 10].

Even though marine algae from many coastal regions of India were investigated for antimicrobial activity, majority of marine algae from Gulf of Mannar, South east coast of Tamilnadu, India were left unexplored for bioactive substances. In the present study, we describe the antibacterial characteristics of chloroform, ethanol, methanol and water extracts of marine algae *Turbinaria conoides* obtained from the coast of Gulf of Mannar.

MATERIALS AND METHODS

Sample Collection

In the present study, *Turbinaria conoides* (J.Agardh) macro algae was collected from Mandapam coastal region (78.8°E, 90.17°N), in Gulf of Mannar, Tamilnadu, South India on low tide during December 2010 and immediately brought to the laboratory in polythene bags and washed several times with seawater to remove sand, mud and attached fauna. The algae were cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, algae were dried in shade at room temperature for one week. The dried algal material were homogenized to fine powder and further subjected to extraction.

Preparation of extracts

Powdered algal sample (500g) was taken and extracted successively with different solvents in the order of their polarity chloroform, ethanol, methanol and water using soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to get their corresponding residues. The algae extracts were further subjected for antimicrobial activity by agar well diffusion method.

Microorganisms Tested

The following strains of bacteria were used; *Bacillus subtilis* (MTCC 121), Streptococcus *pyogenes* (MTCC 1927), *Proteus mirabilis* (MTCC 1429) and *Salmonella typhimurium* (MTCC 98) were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures were

maintained in nutrient agar (High media, India) slants at 40°C and were sub-cultured before use. These bacteria are clinically important ones causing several infections and it is essential to overcome them through some active therapeutic agents.

Antibacterial Assay

The antibacterial activity of *Turbinaria conoides* was studied by cup-plate agar diffusion method[11]. The turbidity of each bacterial suspension was adjusted the optical density a 0.5 McFarland standard, resulting in a suspension containing 1.5 x 108 CFU/mL. Mueller- Hinton agar was prepared, inoculated with bacterial cultures, and transferred to sterile 15-cm diameter Petri dishes. The medium in the plate was allowed to set at room temperature for 10 minutes and solidify for 30 minutes. Three wells (6-mm inner diameter) were made in each plate. Stock solutions of the test residual extract were prepared at concentrations of 100, 300, and 500 mg/mL. One hundred microliters of each concentration was placed in the well with sterile pipettes. In each plate, 1 well was used for the control, standard, and test respectively. Chloramphenicol (100 µg/mL) was used as the standard, and the respective solvent was used as the control. The petri dishes were incubated for 16 h at 37°C and examined with regard to size of the zones of inhibition. Methanol was used as the control. The length of the inhibition zone was measured in millimeters from the edge of the well to the edge of the inhibition zone, and the results were tabulated.

RESULTS

Four different extracts of *Turbinaria conoides* were tested for their anti-bacterial activity against strains of Gram positive, Gram negative bacteria using agar well diffusion method. The result of antimicrobial activity against tested pathogens was tabulated (table 1).

Table 1: Antibacterial activity of crude extract of *Turbinaria conoides* (J.Agardh)

Test Sample	Concentration (µg /ml)	Pathogens used and Diameter of zone of inhibition (in mm)			
		Gram positive organisms		Gram negative organisms	
		Bacillus subtilis	*Streptococcus pyogenes*	*Salmonella typhimurium*	*Proteus mirabilis*
Chloroform extract	300	8	7	4	2
	500	12	13	7	3
Ethanol extract	300	9	11	3	-
	500	12	15	5	1
Methanol extract	300	11	10	2	-
	500	19	16	6	2
Water extract	300	3	5	-	-
	500	6	8	2	-
Chloramphenicol (Standard)	30	23	22	24	23

Values are means of three replicates recorded to the nearest whole millimeter.

The crude extracts of *Turbinaria conoides* in different solvents exhibited diverse antibacterial activities. The methanol extract of *Turbinaria conoides* was most active against all tested pathogens followed by chloroform and ethanol extracts, the minimum with water extracts. *Bacillus subtilis* growth was highly inhibited (11mm at 300 µg /ml and 19mm at 500 µg /ml) by the methanol extracts of *Turbinaria conoides* when compared with other extracts. The antimicrobial activity of *Turbinaria conoides* was observed in dose dependent manner for all four extracts. Highest antibacterial activity was exhibited in 500 µg /ml than 300 µg /ml dose level against all the tested pathogens.

DISCUSSION

Marine algae synthesize active constituents which are used in conventional and complementary medicine. Different varieties of marine algae were reported to have active ingredients that can cure diseases. Currently large proportions of population prefer to use remedies of natural origin for curing illness as these claimed to produce less side effects[12]. The present study was focused on *Turbinaria conoides* for the presence of phytochemical constitutes and antibacterial activity against Gram-positive and Gram-negative bacteria.

The evolution of antibiotic-resistant pathogenic bacterium has stimulated the search for alternative antibacterial agents from alternative sources including sources from the ocean. The powers of marine macro algae have been realized for thousands of years and its potential as producers of pharmaceutical products have been reviewed[13].

The results of the present study clearly showed that marine algal extracts showed antibacterial activity against tested pathogenic bacterial strains including antibiotic resistant strains. The effectiveness of the active compounds present in the methanol extracts of *Turbinaria conoides* causes the production of growth inhibition zones that appear as clear areas surrounding the wells. Antibacterial activity may be due to active components which are present in the extracts. Among tested pathogens, the methanol extracts of *Turbinaria conoides* showed maximum growth inhibition against Gram-positive bacteria especially *Staphylococcus aureus,* while chloroform extract and ethanol extract of *Turbinaria conoides* showed moderate activity of all Gram-positive and Gram-negative bacteria. Water extracts of *Turbinaria conoides* was showed mild activity against Gram-positive and Gram-negative pathogens. A dose depend increase in the antibacterial activity was

observed in all the extracts of *Turbinaria conoides* against the tested pathogens.

Gram-positive bacteria were more effectively controlled by the *Turbinaria conoides* extracts than Gram-negative bacteria. Similar observations indicating that the more susceptibility of Gram-positive bacteria to the algal extracts is due to the differences in their cell wall structure and the composition of the cell wall[14, 15]. The principle strength of the active components depends on the use of a suitable solvent to extract it[16]. Hornesy and Hide[17] reported that crude extracts of marine algal species showed inhibitory activity against pathogenic bacteria. But variation in antibacterial activity is due to the method of extraction, solvent used in extraction and season at which samples were collected. Previous reports on red algae exhibited high antibacterial activity[18- 20]; in contrast, green algae *Turbinaria conoides* were also effective against some selected microbial pathogens. Methanol extract of *Turbinaria conoides* has been reported for its strong antibacterial effect against Enterococci sp[21].

Results from this study revealed that the crude methanol extract of seaweeds contain certain constituents with antibacterial property which enables the extract to overcome the barrier in Gram-negative cell wall[22].

CONCLUSION

In addition, these results form an absolute basis for selection of the plant for additional phytochemical and pharmacological investigation. *Turbinaria conoides* is currently undergoing detailed investigations with objectives of isolating biologically active molecules along with the search for novel antimicrobial agents.

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