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Antibacterial Activity of Marine Algae against Clinically Relevant Bacterial Strains

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A B S T R A C T

Marine algae are considered to be one of the largest source for biogenic compounds. They produce a wide variety of chemically active metabolites that possess antibacterial, antifungal, anti algal, antifouling properties and are also finding importance in therapeutics. The aim of this study was to evaluate the antibacterial activity of marine algae against clinical pathogenic isolates. A total of four marine algae; two red and two brown algae were chosen and screened for their antibacterial activity. Solvent extraction were obtained from the seaweeds with three organic solvents (methanol, ethyl acetate and chloroform) and tested for antibacterial activity. Of the four algal extracts, the Methanolic extract of the red algae, *Portieria hornemannii* showed maximum inhibition activity against all the five clinical isolates while others showed a moderate activity. The results indicate that these algal extracts can further be analyzed and purified for relevant antibacterial compounds which can be used in therapeutics and other applications.

Introduction

Bacterial infections are the most common cause of diseases and might lead to adverse effects in humans. The emergence of new infection causing agents and antibiotic resistant bacterial strains has made the treatment more complicated and challenging. There is always a demand for new antibacterial agent to combat the challenges in treating the infections.

Marine algae, commonly referred as seaweed are gaining attraction for the synthesis of new anti-microbial agents.

Marine algae are currently being explored throughout the world as a novel source for biogenic compounds. Marine algae are considered to be one of the largest sources for biogenic compounds. They produce a wide variety of chemically active metabolites that possess antimicrobial, antifouling properties (Bhadury *et al*., 2004) and are also finding importance in therapeutics. The bioactive compounds from the marine algae are known to possess antibacterial, antifungal, anti algal, antiviral,

anti malarial, anticancer and antifouling properties.

Many studies on marine algae have shown that the seaweeds are a rich source of biologically active compounds (Omar *et al*., 2012; Pereira *et al*., 2002). Marine algae serve as an inexhaustible reservoir for bioactive compounds that are finding application in pharmaceutical, medicine, food industries and cosmetics. Seaweeds are also used as human food and cattle feed for its rich nutritional value and high content of dietary fibers.

Most of the secondary metabolites from seaweeds possess bacteriostatic and bactericidal properties. Also these metabolites show a broad spectrum of biological activity. The chemically active metabolites from seaweeds have shown a diverse phytochemical activity (Mtolera *et al*., 1996). The antibacterial activity of many seaweed extracts has been reported (Sarah Saleh *et al*., 2013; Kausalya and Narasimha Rao, 2015; Omar *et al*., 2012; Rajasulochana *et al*., 2009). Both the red and brown algae have shown significant antibacterial activity against pathogenic bacteria (Caccamese *et al*., 1985, Vallinayagam *et al*., 2009).

Though the marine algae are diverse and distributed abundantly, only a little has been explored and studied. The present study is aimed to evaluate the antibacterial potential of four marine algae in different solvent system and identify a novel species with antibacterial activities.

Materials and Methods

Sample collection and identification

Fresh seaweeds were collected in polythene bags containing seawater from the coastal line of Mandapam, Ramanathapuram district, India and transported to the laboratory. Then the algal materials were thoroughly washed in running tap water to remove the attached epiphytes and other marine debris. Finally it was washed with distilled water and allowed to dry under shade for 5 – 7 days, until the moisture was completely removed. The dried material was ground to a coarse powder using an electrical mixer and stored in air tight containers at room temperature. Totally four algal species were collected and the taxonomic identification was done by experts at Botanical survey of India (BSI), Coimbatore, India.

Algal extraction

The algal extraction was carried out in three different solvent systems with increasing polarity. Chloroform, ethyl acetate and methanol were used for the extraction process. Extraction was carried out by soaking 10g of the powdered sample in 100ml of respective solvents (1:10 – w/v) in a conical flask and kept on a shaker (120 rpm) at room temperature for 24 hrs. The mixture was filtered using Whatman No.I filter paper and re-extraction was done until the solvent becomes colorless. The filtrate thus obtained was allowed to concentrate and stored for further studies.

Antibacterial activity of the seaweeds

Clinical pathogens: Source and culture condition

Staphylococcus aureus (MTCC 96), *Escherichia coli* (MTCC 443), *Proteus vulgaris* (MTCC 426), *Klebsiella pneumonia* (MTCC 109) and *Bacillus subtilis* (MTCC 441) were chosen based on their clinical importance. The bacterial strains were obtained from the Microbial

Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial stock cultures were stored in a refrigerator at 4°C; subcultures were done and maintained on nutrient agar slants.

Antibacterial activity of the extracts in various solvents by agar well-diffusion assay:

For the antibacterial assay, 10mg of each algal extract was dissolved in 1ml of Dimethyl sulfoxide (DMSO) to form the test extracts. Fresh overnight broth culture of the clinical pathogens were prepared and inoculated onto Mueller Hinton agar plates by uniformly swabbing using a sterile cotton swab. Then five wells of 6mm diameter were made on the plates using a sterile cork borer. To each well 100µl of the test extracts obtained from chloroform, ethyl acetate and methanol were added. Dimethyl sulfoxide (DMSO) was used as negative control and the broad spectrum antibiotic Tetracycline was used as positive control. The plates were incubated for 24 hours at 37 °C. After incubation, the zones of inhibition were measured and the results were compared to determine the solvent system with maximum activity. The tests were done in triplicates for quality results.

Evaluation of antibacterial activity of the potent seaweed with various concentration of Methanol extracts

The crude Methanolic extract of the identified potential seaweed was dissolved in DMSO (10mg in 1ml) and antibacterial activity was determined. To the above procedure, varying concentration of crude Methanolic extract dissolved in DMSO was added as follows: 25µl, 50µl, 75µl, 100µl and 125µl. The plates were incubated and the zone of inhibition was recorded.

Results and Discussion

Seaweed identification

Of the four seaweeds, two seaweeds were identified as red algae belonging to Rhodophyta i) *Portieria hornemannii* ii) *Gracilaria corticata* and other two belonging to brown algae, Phaeophyta i) *Stoechospermum marginatum* ii) *Padina tetrastromatica*

Evaluation of the antibacterial activity of the algal extracts

The crude extract of all the four algal species showed significant antibacterial activity against the tested bacterial strain. Methanolic extracts of the seaweeds exhibited strong inhibition activities when compared to ethyl acetate and chloroform. Of the four algal species, the red algae *Portieria hornemannii* showed maximum inhibition activity against all the clinical pathogens. It was observed that the methanolic extract of *Portieria hornemannii* exhibited high inhibition against *Staphylococcus aureus* (24mm inhibition zone), *B*acillus *subtilis* (21mm), *Escherichia coli* (16mm), *Proteus vulgaris* (18mm) *Klebsiella pneumonia* (11mm). The ethyl acetate and chloroform extract also inhibited the test pathogens at moderate level (Table 1). The methanol extract of the other red algae, *Gracilaria corticata* exhibited a good antibacterial activity (Table 2). Similarly a moderate inhibition was observed in both the brown algae *Stoechospermum marginatum (Table 3) and Padina tetrastromatica (Table 4).* The ethyl acetate and chloroform extracts showed comparatively less inhibition than methanol extracts. Further various concentration of the methanoliic extract of *Portieria hornemannii* evoked strong inhibition as the concentration increased (Table 5).

Table.1 Antibacterial activity of the crude extract of *Portieria hornemannii* in various solvents

S.No	Clinical Pathogens	Zone of Inhibition (mm)				
		Methanol extract	Ethyl acetate extract	Chloroform extract	Standard (Tetracycline)	Negative control (DMSO)
1	*Escherichia coli*	16	8	10	19	-
2	*Klebsiella pneumonia*	11	7	8	24	-
3	*Proteus vulgaris*	18	8	9	16	-
4	*Staphylococcus aureus*	24	10	8	19	-
5	Bacillus subtilis	21	11	7	15	-

Table.2 Antibacterial activity of the crude extract of *Gracilaria corticata* in various solvents

S.No	Clinical Pathogens	Zone of Inhibition (mm)				
		Methanol extract	Ethyl acetate extract	Chloroform extract	Positive control (Tetracycline)	Negative control (DMSO)
1	*Escherichia coli*	15	8	8	14	-
2	*Klebsiella pneumonia*	9	7	8	24	-
3	*Proteus vulgaris*	11	8	9	16	-
4	*Staphylococcus aureus*	15	9	8	15	-
5	Bacillus subtilis	13	11	7	14	-

Table.3 Antibacterial activity of the crude extract of *Stoechospermum marginatum* in various solvents

S.No	Clinical Pathogens	Zone of Inhibition (mm)				
		Methanol extract	Ethyl acetate extract	Chloroform extract	Positive control (Tetracycline)	Negative control (DMSO)
1	*Escherichia coli*	12	8	-	14	-
2	*Klebsiella pneumonia*	13	8	8	24	-
3	*Proteus vulgaris*	11	-	-	16	-
4	*Staphylococcus aureus*	14	-	-	15	-
5	*Bacillus subtilis*	13	10	7	14	-

Table.4 Antibacterial activity of the crude extract of *Padina tetrastromatica* in various solvents

S.No	Clinical Pathogens	Zone of Inhibition (mm)				
		Methanol extract	Ethyl acetate extract	Chloroform extract	Positive control (Tetracycline)	Negative control (DMSO)
1	*Escherichia coli*	12	-	8	14	-
2	*Klebsiella pneumonia*	11	7	-	24	-
3	*Proteus vulgaris*	8	-	-	16	-
4	*Staphylococcus aureus*	14	8	8	15	-
5	Bacillus subtilis	13	10	7	14	-

Table.5 Antibacterial activity of the potent seaweed, *Portieria hornemannii* with various concentration of Methanol extracts

S.No	Clinical Pathogens	Zone of Inhibition (mm)						
		25µl	50µl	75µl	100µl	125µl	Standard (Tetracycline)	Negative control (DMSO)
1	*Escherichia coli*	7	9	11	16	19	14	-
2	*Klebsiella pneumonia*	-	8	10	11	14	24	-
3	*Proteus vulgaris*	-	8	10	18	21	16	-
4	*Staphylococcus aureus*	-	11	18	24	27	15	-
5	Bacillus subtilis	8	13	7	21	23	14	-

In the present study, the ability of seaweeds to inhibit clinical pathogens has been studied and the results indicate that the antibacterial agents from the seaweed can be used in control of bacterial infections. The investigation shows that the marine red algae, *Portieria hornemannii* is a potential species with high inhibitory activity against the tested pathogens. Earlier report has also reported the antibacterial activity of *P.hornemannii* and *G.corticata* (Fatima *et al*., 2015 and P. Rajasulochana *et al*., 2009). The antibacterial activity of the brown algae is comparatively less than the red algae; previous studies have reported high inhibition activity of *Padina tetrastromatica* in chlorofofrm extract (Subba *et al., 2010).* Similarly a report states that the extract from *Stoechospermum marginatum has high bactericidal activity* (Shanmughapriya *et al*., 2008). Data obtained in the present study indicates that methanol was the most effective solvent for the extraction of the bioactive compounds from the seaweeds

with significant antibacterial activity. As observed from the results, the seaweeds can be used as a potential source for antibacterial agents.

The secondary metabolites of almost all the seaweeds are rich in bioactive compounds that possess antibacterial activity and significant phytochemical properties. The present study also shows the antibacterial potential of the seaweeds which are in agreement with the previous studies. In conclusion, extracts of four marine algae obtained from various solvents were tested for their antibacterial activity. Among, the extracts tested, the methanolic extract of *Portieria hornemannii* exhibited the highest activity, followed by other algal extracts. The results indicate that these algal extracts can further be analyzed and purified for relevant antibacterial compounds which can be used in pharmacological products, therapeutics and other applications.

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