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Research Article

IN-VITRO ANTIBACTERIAL ACTIVITY OF SOME RED SEAWEED COLLECTED FROM THE MANDAPAM COASTAL AREAS OF TAMIL NADU, INDIA

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Abstract

The present study was aimed to screen the antimicrobial activity of some red seaweeds collected from the Mandapam coastal areas of Tamilnadu. Antibacterial activity was evaluated using disc diffusion technique in Mueller Hinton agar and broth dilution technique was carried out in Mueller-Hinton broth for evaluating Minimal Inhibitory concentration. Among the three red algae tested *Gracilaria corticata* exhibited maximum antibacterial activity against *Streptococcus pyogenes* and *Klebsiella pneumoniae*, followed by the *Gracilaria edulis* against *Staphylococcus aureus* and *Enterobacter aerogenes*. Methanol extract of all the algal species showed higher inhibitory concentration against all the tested pathogens. The results revealed that methanol seemed to be a good source material for the extraction of bioactive molecules from the selected marine algae especially from *G.corticata.*

Keywords: *Gracilaria corticata,* broth dilution technique, Methanol extract,antimicrobial activity.

Introduction

Marine environment is an exceptional reservoir of natural products containing bioactive compounds, which exhibit structural/ chemical features not found in terrestrial natural products (Carter 1996). Most of the marine organisms produce bioactive metabolites in response to ecological pressures, including competition for space, the fouling of the surface as well as they develops chemical strategy for defence to ensure their survival, and to synthesize extremely active molecules, since having to act as aqueous medium much diluted (Ireland *et al*., 2000). Seaweeds have a unique place in traditional medicine of maritime nation as vermifuges, aesthetics and antibiotics in the treatment of cough, wounds, gout, goiter, hypertension, veneral diseases, cancer and a variety of other sickness (Stein, 1984; South 1987).

Many species of algae able to produce a great variety of secondary metabolites, antibacterial and antiviral properties in the highly volatile fractions and great variety of halogenated alkanes, saturated and unsaturated ketones, aldehyde, alcohols, epoxides and halogenated derivatives of acetic and acrylic acids (Garg, 1993). Compounds with cytostatic, antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae (Lindequist and Schweder, 2001; Newman *et al*., 2003). In the present study red algal samples collected from the Mandapam coastal areas of Tamilnadu were screened for their antibacterial activity and the minimum inhibition concentration were also tested.

Materials and Methods

Collection and extraction of seaweeds

Algal samples such as *Gracilaria corticata, Gracilaria edulis* and *Hypnea musciformis,* were collected from the Mandapam coastal regions of Tamilnadu and cleaned of epiphytes and extraneous matter, brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried and ground

in an electric mixer. Powdered algal samples were extracted in Soxhlet apparatus using various organic solvents *viz.,* Methanol, acetone, chloroform, ethyl acetate and hexane. Crude extracts obtained were stored in refrigerator at 4^{o}C for future use.

Antibacterial Activity

Three different algal samples were tested against bacterial pathogens (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermidis, Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumoniae* and *Enterobacter aerogenes*) collected from Rajah Muthaiah Medical College, Annamalainagar, Tamil Nadu, India.

Antibacterial activity was screened using Disc diffusion technique of Bauer *et al*. (1966) in sterile Mueller-Hinton Agar plates. 20 µL of each algalnegative applied per sterile 6 mm diameter filter paper discs, inoculated and incubated at 37^{o}C for 24 hours. Organisms were plated in triplicates and the average values were tabulated. Ampicillin 5mg/ml was used as control and blank discs impregnated with solvent were used as positive and negative control. The results are expressed as mean ± SD.

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was tested by broth macro dilution method (Ericsson and Sherri, 1971). The extracts were dissolved in 5% DMSO to obtain 128mg/ml stock solutions. MIC was determined at the concentration of 64, 32, 16, 8, 4, 2 and 1 mg/ml for seaweeds extracts and 50µl of the test organism was transferred onto each tube containing Mueller-Hinton broth and incubated at 37^{o}C for 24 hours. The lowest concentration without any growth or turbidity after macroscopic evaluation was determined as minimum inhibitory concentration (MIC).

Results

The antibacterial activity of organic extracts (hexane, acetone, chloroform, ethyl acetate and methanol) obtained from three seaweeds were tested against pathogenic bacteria was studied in comparison to the reference drug Ampicillin (5 mg/ml).

The antibacterial activity of marine seaweed crude extracts of *Gracilaria corticata* was investigated against bacteria and the results were given in the Table-1. The methanol crude extract of *Gracilaria corticata* (5.0 mg/ml) showed highest mean zone of inhibition (20 ± 0.4 mm) against the Gram positive cocci *Streptococcus pyogenes* and Gram negative bacteria *Klebsiella pneumoniae* (19 ± 0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermidis, Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes.*

The antibacterial activity of marine seaweed crude extracts of *Gracilaria edulis* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-2. The *Gracilaria edulis* methanol crude extract (5.0 mg/ml) showed maximum zone of inhibition (17 ± 0.4 mm) against the Gram positive cocci *Staphylococcus aureus* and Gram solventbacteria*Enterobacter*extracts*aerogenes* (15was±0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 20 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1.25 mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Klebsiella pneumonia.*

The antibacterial activity of marine seaweed crude extracts of *Hypnea musciformis* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-3. The methanol crude extract of *Hypnea musciformis* (5.0 mg/ml) showed highest mean zone of inhibition (16 ± 0.4 mm) against the Gram positive cocci *Streptococcus epidermidis* and Gram negative bacteria *Pseudomonas aeruginosa* (15 ± 0of.8mm)standardized.Nozoneofinhibitionwas seensuspensioninDMSOblind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 mg/ml) value of methanol crude extract was recorded against *Klebsiella pneumoniae*.

Discussion

Antibacterial assay of red, brown and green algae against the Gram positive and Gram negative bacteria has been established by so many researchers. Lavanya and Veerapan (2011) tested the *in vitro* antibacterial of selected seaweeds including various solvent extracts of *Gracilaria* sp. against human pathogens, in which methanol extracts showed significant activity and the other solvent extracts showed moderate antibacterial activity. The results of the present study also showed that methanol is the best solvent for extracting antimicrobial compounds. Taskin *et al.* (2007), Siddqiui *et al.* (1993) and Etahiri *et al.* (2003) mentioned that

Table-1: Antibacterial activity of crude extracts of *Gracilaria corticata*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positivecontrol	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positivecontrol
Staphylococcus aureus	19±0.3	18±0.3	13±0.4	12±0.4	12±0.6	18±0.5	1	1	4	8	2	4
Streptococcus pyogenes	20±0.4	16±0.5	12±0.3	9±0.5	12±0.5	20±0.3	1	2	8	8	4	8
Streptococcus epidermidis	18±0.6	17±0.3	14±0.5	11±0.6	14±0.3	16±0.8	1	2	8	16	4	8
Bacillus subtilis	19±0.5	18±0.3	14±0.3	13±0.5	16±0.4	21±0.6	1	1	4	4	2	8
Bacillus cereus	18±0.2	15±0.8	15±0.4	14±0.3	16±0.5	19±0.5	1	1	4	8	2	8
Escherichia coli	18±0.3	14±0.6	16±0.6	13±0.3	16±0.3	19±0.3	2	2	8	8	4	4
Pseudomonas aeruginosa	18±0.5	14±0.3	15±0.3	14±0.4	15±0.3	20±0.7	2	4	8	16	8	4
Vibrio cholerae	13±0.4	10±0.4	10±0.4	10±0.6	12±0.4	18±0.5	4	8	32	64	16	16
Salmonella typhi	18±0.6	17±0.4	14±0.5	14±0.4	15±0.6	21±0.6	4	4	16	32	8	16
Klebsiella pneumoniae	19±0.5	17±0.6	14±0.4	11±0.5	16±0.5	22±0.8	1	2	4	8	2	8
Enterobacter aerogenes	19±0.3	17±0.7	15±0.5	14±0.4	16±0.6	19±0.4	1	1	4	4	2	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-2: Antibacterial activity of crude extracts of *Gracilaria edulis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positivecontrol	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positive control
Staphylococcus aureus	19±0.4	18±0.5	15±0.4	12±0.6	15±0.5	18±0.5	1	2	8	16	4	4
Streptococcus pyogenes	17±0.3	15±0.3	13±0.5	12±0.3	13±0.3	20±0.3	2	4	8	16	4	8
Streptococcus epidermidis	16±0.5	15±0.2	13±0.5	12±0.4	14±0.4	16±0.8	2	4	16	32	8	8
Bacillus subtilis	15±0.6	14±0.6	11±0.5	11±0.5	12±0.5	21±0.6	1	2	8	8	4	8
Bacillus cereus	16±0.4	15±0.5	12±0.3	11±0.2	14±0.3	19±0.5	1	2	8	16	4	8
Escherichia coli	15±0.6	14±0.4	12±0.7	11±0.6	13±0.5	19±0.3	2	4	16	16	8	4
Pseudomonas aeruginosa	14±0.4	14±0.6	13±0.5	12±0.3	13±0.3	20±0.7	4	4	16	32	8	4
Vibrio cholerae	13±0.4	12±0.3	11±0.2	11±0.5	12±0.4	18±0.5	8	16	32	32	32	16
Salmonella typhi	15±0.4	14±0.5	12±0.3	11±0.6	13±0.4	21±0.6	8	16	32	64	32	16
Klebsiella pneumoniae	16±0.2	15±0.5	13±0.5	12±0.5	14±0.3	22±0.8	1	2	8	16	4	8
Enterobacter aerogenes	17±0.5	15±0.3	12±0.6	11±0.3	14±0.3	19±0.4	2	4	16	32	8	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-3: Antibacterial activity of crude extracts of *Hypnea musciformis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positivecontrol	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positivecontrol
Staphylococcus aureus	16±0.2	14±0.3	12±0.2	12±0.3	14±0.3	14±0.5	4	8	16	32	8	4
Streptococcus pyogenes	15±0.3	15±0.6	12±0.5	11±0.3	13±0.6	20±0.3	2	4	8	16	4	8
Streptococcus epidermidis	16±0.4	15±0.3	13±0.4	11±0.6	14±0.5	16±0.8	2	4	16	32	8	8
Bacillus subtilis	14±0.5	13±0.4	12±0.3	10±0.5	12±0.4	21±0.6	2	4	8	16	8	8
Bacillus cereus	14±0.6	14±0.5	12±0.5	12±0.4	12±0.3	19±0.5	2	4	16	32	16	8
Escherichia coli	15±0.7	14±0.5	12±0.3	12±0.5	13±0.4	19±0.3	4	8	16	32	8	4
Pseudomonas aeruginosa	15±0.8	15±0.6	12±0.2	12±0.4	14±0.6	20±0.7	4	8	16	32	16	4
Vibrio cholerae	12±0.4	11±0.6	11±0.3	10±0.3	11±0.3	18±0.5	8	16	32	64	32	16
Salmonella typhi	12±0.6	13±0.3	11±0.2	12±0.6	12±0.3	21±0.6	8	16	32	64	32	16
Klebsiella pneumoniae	13±0.5	12±0.5	11±0.4	10±0.5	12±0.5	22±0.8	1	2	8	16	4	8
Enterobacter aerogenes	15±0.4	14±0.4	13±0.6	11±0.5	11±0.7	19±0.4	2	4	8	16	8	8

Mean ± SD, * positive control- Ampicillin (5 µg)

methanol extract of *Hypnea musciformis* exhibits strong antibacterial activity which is similar to the present investigation. Kim and Lee (2008) used methanolic extract to observe strong antibacterial activities. Cordeiro *et al*. (2006) showed successive extraction with acetone, methanol-toluene, ether and chloroform-methanol. Margret *et al*. (2008) reported that methanol extract of *Acanthophora spicifera* was active against Gram negative bacterial pathogens.

Subba *et al*. (2010) screened *G.corticata* which showed broad spectrum of antibacterial activity against both Gram positive and Gram negative bacteria, their findings were similar to the present study in case of both groups. In the present investigation, the methanol extracts showed strong antimicrobial activity against the tested bacterial strains when compared with Ampicillin as standard.

Conclusion

In this study, we demonstrated that the macro algal extracts exerted antibacterial activity against different human pathogenic bacteria and their growth was strongly inhibited at the concentration of 1 mg/ml to 8mg/ml. This study paves the way for isolation of various bioactive compounds from seaweeds useful against dreadful diseases caused by various microorganisms.

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