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Research Article

Antibacterial activity of marine macroalgae *Padina gymnospora* and *Turbinaria conoides* collected from Mandapam Coast of Tamilnadu, India

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Abstract

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms' may be potential bioactive compounds of interest in the pharmaceutical industry. To date, many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new pharmaceuticals. In the present study, the macroalgae *Padina gymnospora* and *Turbinaria conoides* were collected from the sea shores of Mandapam(South east coast of Tamil Nadu India). The dried samples were prepared crude extract using five different solvents (methanol, ethanol, acetone, ethyl acetate, and chloroform). The extracts of collected seaweeds were tested against Gram positive and Gram negative human pathogenic bacteria by Disc diffusion method. The maximum zone of inhibition was observed in the methanol extract of *Padina gymnospora* against *Staphylococcus aureus* and minimum activity was observed in ethyl acetate extract *Turbinaria conoides* against *Escherichia coli*. The result of present study reveals that the *Padina gymnospora* may be a rich source of potential bioactive molecules which can be isolated and further screened for various biological activities.

Keywords: Antibacterial activity, *Padina gymnospora*, *Turbinaria conoides*, Disc diffusion Methods, MIC, Seaweeds.

1.Introduction

Seaweeds or marine macro algae are the renewable living resources which are also used as food, feed fertilizer in many part of the world. Seaweeds of nutritional interest as they contain low calorie food, but rich vitamins, minerals and dietary fibers (Ito and Hori, 1989).The several marine organisms produce bioactive metabolites in response to ecological pressures such as competition for space, maintenance of unfouled surfaces, deterrence of predation and the ability to successfully reproduce (Konig *et al.,* 1994).These bioactive compounds offer rich pharmacological potential (Lindequist and Schweder, 2001). Seaweeds are considered as source of bioactive compounds and produce a greater variety of secondary metabolites characterized by abroad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman *et al.,* 2003; Chanda *et al.,* 2010). Seaweeds or marine algae are potentially prolific source of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents (Kolanjinathan *et al*., 2014; Kolanjinathan and Saranraj, 2014). The present study was undertaken to investigate the antibacterial activities of solvent extract of seaweeds from Mandapam coast against 11 human pathogenic bacteria. The marine environments representing approximately half of global biodiversity are an enormous resource for new compounds.

2.Materials and Methods

2.1. Sample collection and preparation

The fresh algae samples of *Padina gymnospora* and *Turbinaria conoides* were collected from the Mandapam southeast coast of India. Then collected seaweed were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The collected seaweed ware then thoroughly washed with tap water followed by distilled water. After completely drying, the seaweed material (1.0 kg) was ground to a fine powder using Electrical blender. 40 g of powdered sea weeds were extracted successively with 200 ml of solvents (Methanol, Ethanol, Acetone, Ethyl acetate and Chloroform) in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

2.2. Disc preparation

6 mm diameters of disc were prepared from pretreated Whatman No.1. Filter paper. Then it's sterilized in the hot air oven at 160°C for 1 hour. The solvent extracts of *Padina gymnospora* and *Turbinaria conoides* (Methanol, Ethanol, Acetone, Ethyl acetate and Chloroform) were mixed with 1ml of Diethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent extracts of seaweeds at two different concentrations ranging of 5mg/ml and 10mg/ml to check their antibacterial activity. The paper discs which contain 5% DMSO were used as a blind control and the paper discs containing Ampicillin (10mg/disc) used as a positive control.

2.3. Collection of test bacterial cultures

Eleven different bacterial cultures Viz... *Staphylococcus aureus* (MTCC - 3160), *Streptococcus epidermis* (MTCC - 889), *Streptococcus pyogenes* (MTCC - 1926), *Bacillus cereus*(MTCC - 1427), *Proteus mirabilis*(MTCC - 1429), *Escherichia coli*(MTCC - 1195), *Pseudomonas aeruginosa*(MTCC - 7093), *Vibrio cholera* (MTCC - 3904), *Salmonella typhi*(MTCC - 3215), *Klebsiella pneumonia* (MTCC - 4032) and *Serratia marcescens*(MTCC - 2645) were obtained from MTCC, Chandigarh, India.

2.4. Determination of Antibacterial activity

2.4.1. Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards and then used for the determination of antibacterial activity.

2.4.2. Disc diffusion method

The antibacterial activity of *Padina gymnospora* and *Turbinaria conoides* extracts were determined by Disc diffusion method proposed by Bauer *et al*. (1996). A bacterial suspension (number 0.5 in McFarland scale about 1.5×10^8 bacteria ml^{-1}) was spread on Mueller-Hinton (pH 7.4) agar using a cotton swab. The Mueller Hinton agar plates were prepared and inoculated with test bacterial organisms by spreading the bacterial inoculum on the surface of the media. The discs containing extracts (Methanol, Ethanol, Acetone, Ethyl acetate and Chloroform) at two different concentration (5mg/ml and 10mg/ml) was placed on the surface of the Mueller Hinton agar plates. The paper discs which contain 5% DMSO were used as a blind control and the paper discs containing Ampicillin (10mg/disc) act as a positive control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm). Each assay in these experiments was repeated three times for concordance.

2.4.3. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the *Padina gymnospora* and *Turbinaria conoides* extracts against bacterial isolates was tested in Mueller Hinton broth by Broth macro dilution method. The seaweeds extracts were dissolved in 5% DMSO to obtain 128 mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Mueller Hinton Broth for bacteria to get a concentration of 1, 2, 4, 8, 16, 32 and 64 mg/ml. Fifty µl of standardized suspension of the test organism and devoid of seaweeds extracts/FAME active principle. The culture tubes were incubated at 37^oC for 24 hours. The lowest concentrations which did not show any growth of tested organism after macroscopic valuation was determined as Minimum inhibitory concentration.

3.Results and Discussion

In the present study, antibacterial activity of five different solvents *viz*., methanol, Ethanol, Acetone, Ethyl acetate and chloroform extracts of *Padina gymnospora* was evaluated against pathogenic bacteria. Among five solvent extracts tested, the methanol extract showed the greatest inhibition diameters against Gram positive and Gram negative bacterial isolates. These results are in agreement with the observations of Vlachos *et al*. (1996), Gonzalez *et al*. (2001), Ozdemir *et al*. (2004), Karabay-Yavasoglu *et al*. (2007), Taskin *et al*. (2007) and Kandhasamy and Arunachalam(2008), who reported that extracts prepared with methanol showed the best activity. The results from the present study showed that the Gram positive bacteria are more susceptible than Gram negative bacteria on seaweeds extracts which was also supported from earlier works with different species of seaweeds indicating that the more susceptibility of Gram-positive bacteria to the algal extracts was due to the differences in their cell wall structure and their composition (Thiripurasundar *et al.,* 2008; Vanitha *et al*., 2003;Prakash *et al*.,2005; Selvi *et al.*,2001; Ozdemir *et al.,* 2004).

The methanol extract of *Padina gymnospora* (5.0 mg/ml)showed highest mean zone of inhibition (22±0.4mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Proteus mirabilis*(21±0.5mm), *Staphylococcus aureus* (21±0.3mm), *Streptococcus epidermis* (20±0.6mm) and *Bacillus cereus* (20±0.2mm). For Gram negative bacterium, the maximum zone of inhibition was recorded in methanol extract of *Padina gymnospora* against *Klebsiella pneumoniae* (21±0.5mm) followed by *Serratia marcescens* (21±0.3mm), *Salmonella typhi* (20±0.6mm), *Pseudomonas aeruginosa* (20±0.5mm), *Escherichia coli* (20±0.3mm) and *Vibrio cholerae* (15±0.4mm). The zone of inhibition obtained from the Hexane extract of seaweed *Padina gymnospora* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO control and the positive control Ampicillin showed zone of inhibition ranging from 17±0.8 mm to 24±0.8mm against the test bacterial pathogens (Table - 1). The Minimum inhibitory concentration (MIC) values of *Padina gymnospora* against bacteria was ranged between 1 to 64 mg/ml. The lowest MIC (1 mg/ml) value was recorded against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermis, Proteus mirabilis* and *Bacillus cereus*, *Klebsiella pneumonia* and *Serratia marcescens* (Table - 2).

The methanol extract of *Turbinaria conoides*(5.0 mg/ml)showed highest mean zone of inhibition (20±0.4mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Proteus mirabilis*(19±0.5mm), *Staphylococcus aureus* (19±0.3mm), *Streptococcus epidermis* (18±0.6mm) and *Bacillus cereus* (18±0.2mm). For Gram negative bacterium, the maximum zone of inhibition was recorded in methanol extract of *Turbinaria conoides* against *Klebsiella pneumoniae* (19±0.5mm) followed by *Serratia marcescens* (19±0.3mm), *Salmonella typhi* (18±0.6mm), *Pseudomonas aeruginosa* (18±0.5mm), *Escherichia coli* (18±0.3mm) and *Vibrio cholerae* (13±0.4mm). The zone of inhibition obtained from the Hexane extract of seaweed *Turbinaria conoids* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO control and the positive control Ampicillin showed zone of inhibition ranging from 13±0.8 mm to 22±0.8mm against the test bacterial pathogens (Table - 3). The Minimum inhibitory concentration (MIC) values of *Turbinaria conoides* against bacteria were ranged between 1 to 64mg/ml. The lowest MIC (1 mg/ml) value was recorded against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermis, Proteus mirabilis* and *Bacillus cereus*, *Klebsiella pneumoniae* and *Serratia marcescens* (Table - 4).

SubbaRangaiah *et al*. (2010) showed that the seaweed extracts in different solvents exhibited different antimicrobial activities. In case of *Sargassum ilicifolium, Padina tetrastromatica,* of the various solvents used for seaweed extractions, maximum inhibition was noticed with ethanol extracts and minimum with chloroform crude extracts while in case of *Gracilaria corticata*, maximum inhibition was noticed with methanol and minimum with chloroform extracts. Antifungal activity of all the crude extractions of *Gracilaria corticata* showed maximum activity against *Rhizopus stolonifer*. The crude extracts exhibited mild activity against *Mucor racemosus* and *Rhizoctonia solani* and no activity against *Candida albicans*. The results of the present findings showed that the seaweed extract *Gracilaria edulis*has the inhibitory activity against *Candida albicans* but their research was in contrast with the present study

Table - 1: Antibacterial activity of solvent extracts of *Padina gymnospora*

Microorganisms	Zone of inhibition (mm) mg/ml										
	Methanol		Acetone		Ethyl acetate		Chloroform		Ethanol		Positive control*
	5	10	5	10	5	10	5	10	5	10	10µl
Staphylococcus aureus	17±0.5	21±0.3	14±0.3	17±0.3	13±0.5	17±0.4	13±0.5	16±0.4	14±0.3	16±0.6	20±0.5
Streptococcus pyogenes	17±0.3	22±0.4	12±0.5	15±0.5	13±0.4	16±0.3	12±0.3	15±0.5	13±0.2	16±0.5	22±0.3
Streptococcus epidermis	16±0.4	20±0.6	13±0.3	16±0.3	14±0.3	18±0.5	13±0.5	16±0.6	15±0.5	17±0.3	18±0.8
Proteus mirabilis	17±0.5	21±0.5	14±0.4	17±0.3	16±0.2	18±0.3	15±0.4	17±0.5	18±0.3	19±0.4	22±0.6
Bacillus cereus	16±0.4	20±0.2	13±0.3	16±0.8	16±0.6	19±0.4	16±0.5	18±0.3	17±0.6	19±0.5	23±0.5
Escherichia coli	17±0.5	20±0.3	13±0.6	15±0.6	18±0.5	20±0.6	15±0.3	17±0.3	18±0.5	19±0.3	21±0.3
Pseudomonas aeruginosa	17±0.3	20±0.5	13±0.2	15±0.3	16±0.3	19±0.3	15±0.6	18±0.4	18±0.3	19±0.3	22±0.7
Vibrio cholerae	15±0.3	17±0.4	12±0.3	11±0.4	13±0.4	15±0.4	12±0.5	14±0.6	13±0.5	16±0.4	20±0.5
Salmonella typhi	17±0.4	20±0.6	13±0.4	16±0.4	16±0.2	18±0.5	15±0.3	17±0.4	18±0.3	20±0.6	21±0.6
Klebsiella pneumonia	17±0.5	21±0.5	13±0.5	16±0.6	16±0.5	19±0.4	15±0.2	17±0.5	18±0.2	20±0.5	21±0.8
Serratia marcescens	17±0.6	21±0.3	14±0.3	16±0.7	17±0.6	19±0.5	15±0.4	18±0.4	18±0.3	20±0.6	21±0.4

± - Standard deviation, *Ampicillin

Table - 2: Minimum inhibitory concentration of solvent extracts of *Padina gymnospora*

Microorganisms	Minimum inhibitory concentration (mg/ml)					
	Chloroform	Methanol	Acetone	Ethyl acetate	Ethanol	Positive Control*
Staphylococcus aureus	8	1	1	4	2	4
Streptococcuspyogenes	8	1	2	8	4	8
Streptococcus epidermis	16	1	2	8	4	8
Proteus mirabilis	4	1	1	4	2	8
Bacillus cereus	8	1	1	4	2	8
Escherichia coli	8	2	2	8	4	4
Pseudomonas aeruginosa	16	2	4	8	8	4
Vibrio cholerae	64	4	8	32	16	16
Salmonella typhi	32	4	4	16	8	16
Klebsiellapneumoniae	8	1	2	4	2	8
Serratia marcescens	5	1	1	4	2	8

*Ampicillin

Table - 3: Antibacterial activity of solvent extracts of *Turbinaria conoides*

Zone of inhibition (mm) mg/ml											
Microorganisms	**Methanol**		**Chloroform**		**Ethyl acetate**		**Acetone**		**Ethanol**		**Positive control***
	5	**10**	**5**	**10**	**5**	**10**	**5**	**10**	**5**	**10**	**10µl**
Staphylococcus aureus	15±0.5	19±0.3	12±0.3	15±0.3	11±0.5	15±0.4	11±0.5	14±0.4	12±0.3	14±0.6	18±0.5
Streptococcu spyogenes	15±0.3	20±0.4	10±0.5	13±0.5	11±0.4	14±0.3	10±0.3	13±0.5	11±0.2	14±0.5	20±0.3
Streptococcus epidermis	14±0.4	18±0.6	11±0.3	14±0.3	12±0.3	16±0.5	11±0.5	14±0.6	13±0.5	15±0.3	15±0.8
Proteus mirabilis	15±0.5	19±0.5	12±0.4	15±0.3	14±0.2	16±0.3	13±0.4	15±0.5	16±0.3	17±0.4	20±0.6
Bacillus cereus	14±0.4	18±0.2	11±0.3	14±0.8	14±0.6	17±0.4	14±0.5	16±0.3	15±0.6	17±0.5	21±0.5
Escherichia coli	16±0.5	18±0.3	11±0.6	13±0.6	16±0.5	18±0.6	13±0.3	15±0.3	16±0.5	17±0.3	19±0.3
Pseudomonas aeruginosa	16±0.3	18±0.5	11±0.2	13±0.3	14±0.3	17±0.3	13±0.6	16±0.4	16±0.3	17±0.3	20±0.7
Vibrio cholerae	13±0.3	15±0.4	10±0.3	9±0.4	11±0.4	13±0.4	10±0.5	12±0.6	11±0.5	14±0.4	18±0.5
Salmonella typhi	15±0.4	18±0.6	11±0.4	14±0.4	14±0.2	16±0.5	13±0.3	15±0.4	16±0.3	18±0.6	19±0.6
Klebsiella pneumonia	15±0.5	19±0.5	11±0.5	14±0.6	14±0.5	17±0.4	13±0.2	15±0.5	16±0.2	18±0.5	19±0.8
Serratia marcescens	15±0.6	19±0.3	12±0.3	14±0.7	15±0.6	17±0.5	13±0.4	16±0.4	16±0.3	18±0.6	19±0.4

± - Standard deviation, *Ampicillin

Table - 4: Minimum inhibitory concentration of solvent extracts of *Turbinaria conoides*

Minimum inhibitory concentration (mg/ml)						
Microorganisms	**Chloroform**	**Methanol**	**Acetone**	**Ethyl acetate**	**Ethanol**	**Positive Control***
Staphylococcus aureus	8	1	1	4	2	4
Streptococcu spyogenes	8	1	2	8	4	8
Streptococcus epidermis	16	1	2	8	4	8
Proteus mirabilis	4	1	1	4	2	8
Bacillus cereus	8	1	1	4	2	8
Escherichia coli	8	2	2	8	4	4
Pseudomonas aeruginosa	16	2	4	8	8	4
Vibrio cholerae	64	4	8	32	16	16
Salmonella typhi	32	4	4	16	8	16
Klebsiella pneumonia	8	1	2	4	2	8
Serratia marcescens	4	1	1	4	2	8

*Ampicillin

because their study did not showed inhibitory activity against *Candida albicans* (De-Campos *et al*.,1998;SanthanamShanmughapriya *et al*.,2008). Margret *et al*. (2008) reported that methanol extract of *Acanthophora spicifera* was active against Gram negative bacterial pathogen *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli.*

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